The Relationship Between Extracellular Vesicle and Amphotericin B Resistance in Candida auris

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Abstract

Background: *Candida auris* (predominantly Clade I in Hong Kong) is a critical multidrug-resistant threat with variable Amphotericin B susceptibility. Beyond *ERG6* mutations, extracellular vesicles (EVs) may be implicated in adaptive resistance.

Objective: This study investigates EVs and AmB resistance in clinical *C. auris* from the Hong Kong Hospital Authority (Kowloon West Cluster).

Methodology: Following YeastOne baseline MIC profiling, EVs from an AmB-sensitive isolate were ultracentrifugation-isolated, density-purified, and supplemented (0.1–5 µg) into AmB assays.

Conclusion: Exogenous EVs significantly elevated AmB resistance in sensitive and resistant strains dose-dependently, which is similar to farnesol's phenotypic effects. Future RNA-Seq will map gene ontology changes to resolve these pathways.

Background

First isolated in 2009, Candida auris has rapidly become a major global health threat, classified as an "urgent threat" by the CDC and a "critical priority" by the WHO. It spans five distinct geographic clades, with a sixth recently proposed. While historical misidentification as C. haemulonii was common, the adoption of MALDI-TOF mass spectrometry has largely enabled the rapid, accurate diagnostics required for hospital outbreak containment (Du et al., 2020; Kim et al., 2024).

The pathogen is highly resilient, surviving common disinfectants like sodium hypochlorite to form persistent environmental reservoirs. This fuels nosocomial outbreaks with alarming mortality rates between 29% and 62%. C. auris exhibits a severe multidrug-resistant (MDR) profile; fluconazole resistance is near-universal (87–100%), and resistance to the second-line drug Amphotericin B has surged up to 61% in localized outbreaks (Kim et al., 2024; Pallotta et al., 2023).

Amphotericin B resistance involves multifaceted mechanisms. Traditional pathways rely on ergosterol biosynthesis mutations(notably in ERG6), which reduce drug binding affinity (Rybak et al., 2022). However, recent evidence reveals that Extracellular Vesicles (EVs) also play a pivotal role; variations in EV concentrations correlate with antifungal sensitivity results, suggesting they bolster resistance by sequestering the drug (Amatuzzi et al., 2022).

Objective

Investigate the relationship between extracellular vesicle (EV) and amphotericin B resistance in Candida auris.

Methodology

Isolate selection : Clinical isolates from Hong Kong Hospital Authority, Kowloon West Cluster

1. Antifungal sensitivity (M.I.C.)
   Candida auris antifungal susceptibility testing will be conducted using Thermo Fisher YeastOne, following standard protocol. The following antifungal agents will be included:
   - Fluconazole
   - Itraconazole
   - Voriconazole
   - Posaconazole
   - 5-Flucytosine
   - Amphotericin B
   - Caspofungin
   - Micafungin
   - Anidulagfungin

   The interpretation of Amphotericin B MIC breakpoints (≥ 2 µg/mL) will follow the recommendations provided in the CDC guidelines.

2. Extraction and purification of extracellular vesicles (EVs)
   - **Culture & Harvesting:** Amphotericin B sensitive *Candida auris* isolate was cultured on SDAC plates for 48 hours at 35°C and harvested into 25 mL of sterile PBS.
   - **Crude EV Isolation:** Cellular debris was pelleted via centrifugation at 4,300×g for 20 minutes, followed by supernatant filtration through a 0.45 µM membrane. The filtrate was subjected to ultracentrifugation at 100,000×g for 90 minutes; the resulting pellet was washed twice and resuspended in 200 µL of PBS.
   - **Density Gradient Purification:** Extracellular vesicles (EVs) were purified utilizing an iodixanol density gradient at 100,000×g for 16 hours.
   - **Characterization:** Fractions F3 to F8 were collected, washed at 160,000×g, and subsequently analysed for size distribution and yield metrics.

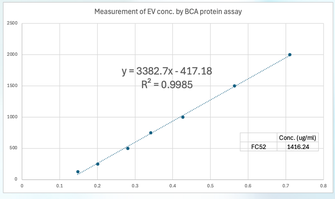
Layer	% iodixanol	Density (g/mL)	Dilution factor of 50% iodixanol	Volume of solution used (µL)					
				1 tube (total = 1100)		2 tube (total = 2200)		4 tube (total = 4400)	
				50% iodixanol	PBS	50% iodixanol	PBS	50% iodixanol	PBS
F1	0	1.031	/	0	1100	0	2200	0	4400
F2	6	1.059	3 in 25	132	968	264	1936	528	3872
F3	10	1.078	5 in 25 / 1 in 5	220	880	440	1760	880	3520
F4	14	1.098	7 in 25	308	792	616	1584	1232	3168
F5	16	1.107	8 in 25	352	748	704	1496	1408	2992
F6	20	1.127	10 in 25 / 2 in 5	440	660	880	1320	1760	2640
F7	24	1.146	12 in 25	528	572	1056	1144	2112	2288
F8	30	1.175	15 in 25 / 3 in 5	660	440	1320	880	2640	1760
F9	34	1.194	17 in 25	748	352	1496	704	2992	1408
F10	40	1.223	20 in 25 / 4 in 5	880	220	1760	440	3520	880

3. Effect of EV on Candida auris
   The biological functionality of Candida auris EVs will be characterized by incorporating discrete concentration gradients (0.1–5 µg) directly into Amphotericin B susceptibility assays. Variations in the Minimum Inhibitory Concentration (MIC) will be monitored across a panel of sensitive and resistant isolates.

   By comparing these profiles to baseline MIC determinations established without EVs addition, the relationship between EVs concentration and the modulation of antifungal efficacy will be precisely quantified.

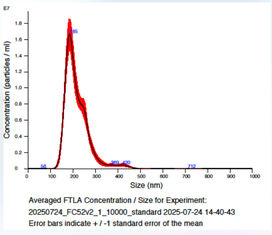
Results

1. Physical Characterization of Purified Extracellular Vesicles
   - Concentration measured by Thermo Scientific Pierce BCA Protein Assay kit



   - Size measured by nanoparticle tracking analysis

Mean: 215.4 +/- 3.4 nm
Mode: 194.6 +/- 9.4 nm
SD: 52.9 +/- 3.1 nm



2. Effect of EVs on Antifungal MICs

	Original MIC	MIC after treated with 5 ug EV
Amphotericin B Sensitive isolate	1 ug/mL	32 ug/mL
Amphotericin B Resistant isolate	2 ug/mL	32 ug/mL

Amphotericin B Sensitive isolate

		32	16	8	4	2	1	0.5	0.25	0.125	0.0625
EV conc. (ug)	0	NG	NG	NG	NG	NG	NG	G	G	G	G
	0.1	NG	NG	NG	NG	NG	NG	G	G	G	G
	1	NG	NG	NG	NG	NG	NG	G	G	G	G
	3	NG	NG	NG	G	G	G	G	G	G	G
	5	NG	G	G	G	G	G	G	G	G	G

Amphotericin B Resistant isolate

		32	16	8	4	2	1	0.5	0.25	0.125	0.0625
EV conc. (ug)	0	NG	NG	NG	NG	NG	G	G	G	G	G
	0.1	NG	NG	NG	NG	NG	G	G	G	G	G
	1	NG	NG	NG	G	G	G	G	G	G	G
	3	NG	NG	G	G	G	G	G	G	G	G
	5	NG	G	G	G	G	G	G	G	G	G

Discussion

Shivarathri et al. have established that Amphotericin B resistance in Candida auris relies on genomic and structural remodelling, specifically resulting in cell wall thickening and reduced cell membrane permeability(Shivarathri et al., 2022). While existing literature notes that the quorum-sensing molecule farnesol alters gene ontology (GO) to actively drive polyene tolerance(Jakab et al., 2024) , our study demonstrates that extracellular vesicles (EVs) significantly elevate resistance in a parallel fashion.

Consequently, we hypothesize that C. auris-derived EVs function similarly to farnesol, acting as biological vectors that modulate cellular signalling and genetic pathways to trigger these downstream structural adaptations. To validate this hypothesis and elucidate the underlying regulatory mechanisms, transcriptome sequencing (RNA-Seq) will be performed to compare the GO profiles between EV-treated and non-treated isolates.

Conclusion

Resistance Modulation: Extracellular vesicles (EVs) derived from Amphotericin B-sensitive Candida Auris isolates significantly enhance polyene resistance in both sensitive and resistant recipient strains.

Dose-Dependent Efficacy: The induction of phenotypic drug tolerance is concentration-dependent, with elevated EVs concentrations driving a progressively greater resistance effect.

Future Directions: Because these behavioural shifts suggest that EVs actively modulate cellular signalling and survival pathways, transcriptome sequencing (RNA-Seq) will be executed to investigate the impact of EV exposure on C. auris gene expression and map the precise regulatory networks governing the defence mechanism.

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