Developing Ultrasound-Responsive Microbubbles for Treating Topical Candida Auris Biofilm Infections

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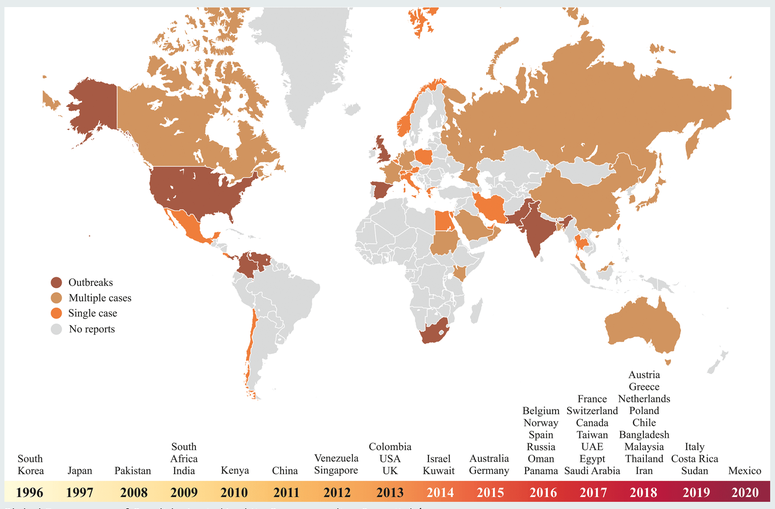
With an incidence of 13.0 to 20.3 episodes per thousand patient days, nosocomial infections present a significant area of concern due to their increased socio-economic burden, prolonged hospital stays, long-term disability and mortality.[1] In the last two decades, *Candida auris* has been an alarming global public health threat due to its swift and far-reaching emergence. After its discovery in 2009, the fungal pathogen has spread simultaneously to all corners of the globe. It has been reported across 23 countries, across five continents, from Venezuela to Japan[2]. More concerningly, it has demonstrated multiple drug resistant capabilities to azoles, echinocandins, polyenes antifungals and standard disinfection procedures utilising ammonium or cationic surface activation agents[4,5]. *C. auris'* ability to form biofilms has further bolstered its resistance to antifungals and resulted in higher minimum inhibitory concentrations (MICs) and sessile MICs (sMICs), increasing its difficulty in eradication from biotic and abiotic surfaces[2,4,6]. We propose to address this challenge by developing an ultrasound activated microbubble delivery mechanism to deliver antifungal drugs to wounds and skins, while utilising ultrasound for remote activation and purely mechanical effects.

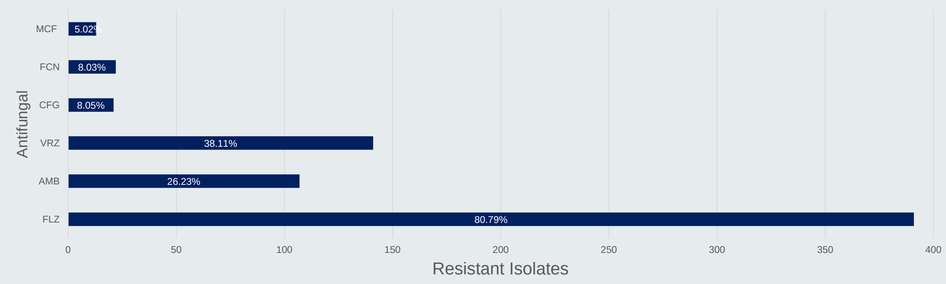
Emergence *of C. auris*



Global Emergence of Candida Auris (And Its Corresponding Severity) [4]

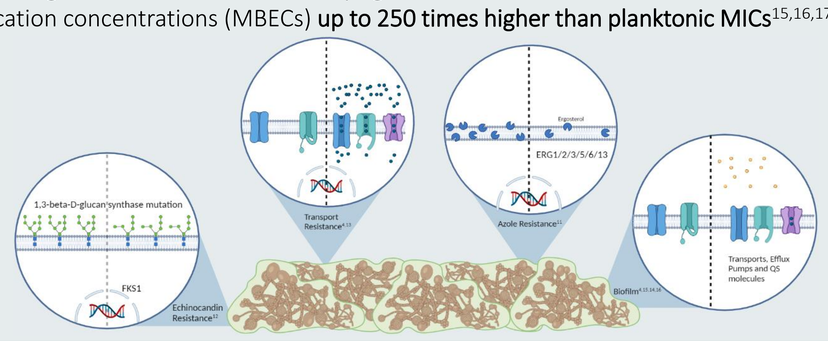
- First reported in 2009 in Japan in the ear discharge of a 70F patient, with subsequent reported cases of septicaemia in 2011.
- Significant outbreaks appearing in Colombia, India, Pakistan, United States, Venezuela, South Africa and European Union [2,4,6,7]
- Identified four genetically distinct clades arising in South Asia (I), East Asia (II), Africa (III) and South America (IV) with a fifth clade been reported in 2018 in Iran (V) [8]

Drug Resistance

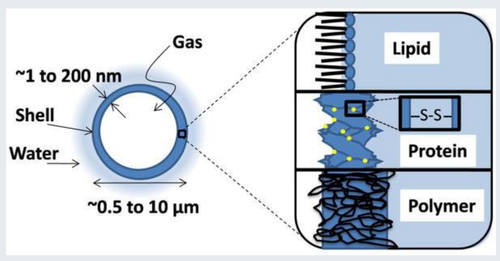


Antifungal Resistance Rates of *C. auris* strains (modified)[8]

- 41% of isolates are multi-drug resistant with 4% being pan-drug resistant[9]
- **All antifungals exhibited reduced activity against *C. auris* biofilms** with minimum biofilm eradication concentrations (MBECs) **up to 250 times higher than planktonic MICs**[15,16,17].



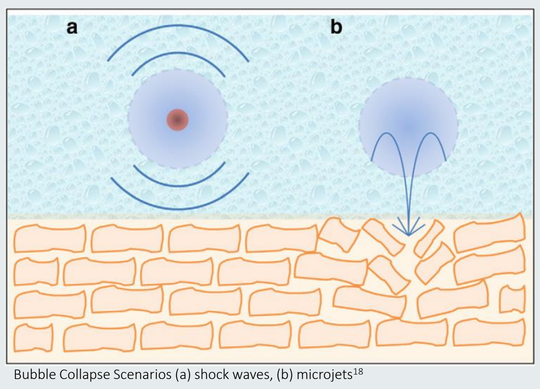
Ultrasound Microbubble Delivery



Typical Microbubble Characteristics with Shell Compositions[19]

- A surfactant, polymer, protein, or phospholipid shell encapsulates a gaseous core.
- Ability to be activated and regulated by adjusting ultrasound frequency, acoustic pressure, duration, and pulse repetition frequency.

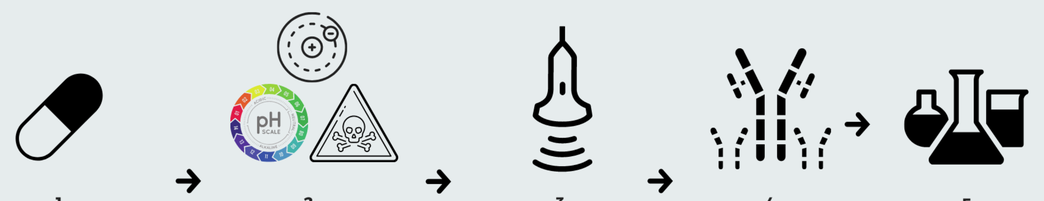
- US causes volumetric oscillation of MBs, resulting in flows that enhance wall shear stress.
- Adjustment of acoustic pressure results in stable and inertial cavitation, allowing further permeabilization and formation of transmembrane pores.



Bubble Collapse Scenarios (a) shock waves, (b) microjets[18]

Experimental Design

Developing drug profiles for microbubble drug loading



1 EXPLORATION
Exploration of antifungals, biological gases, essential oils, antimicrobial agents, antiparasitic drugs and enzymes

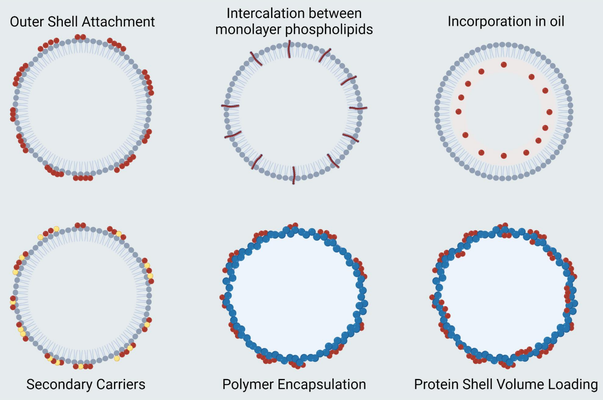
2 CHARACTERIZATION
Characterization via chemical properties (e.g. pH, polarity, stability, toxicity)

3 ADAPTABILITY
Identify pharmacokinetics, pharmacodynamics for suitability under ultrasound sonication

4 TARGETING
Explore appropriate passive or active molecular targeting

5 DRUG LOADING
Development of drug loading and administering mechanism for each class of treatments determined

Development of ultrasound responsive microbubbles loaded with antifungals for topical application



Microbubble Structures and Drug Localizations[20]

06 Microbubble Fabrication
Mechanical Agitation, Emulsification, Probe-type sonication, Spray drying

07 Fabrication of a medium or coupon

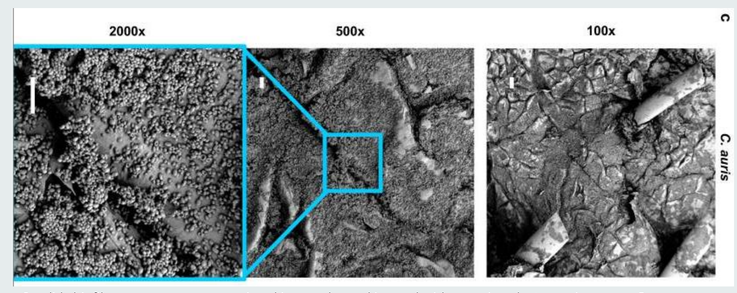
08 Detection of Microbubble Compound Release
High Performance Liquid Chromatography, High Speed Imaging, Active/Passive Cavitation Detection

Cultivation of *C. auris* Biofilms

09 Determining Growth Media
RPMI, Sabouraud's dextrose agar, Yeast Nitrogen Base

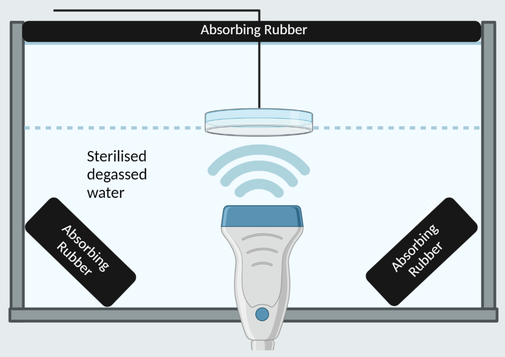
10 Determining Experimental Settings
Titanium Plates, Glass Beads, Glass/Plastic Wells, Petri Dishes

11 Duration of Incubation
24 or 48 hours at 37C



Candida biofilms were grown on porcine skin samples and imaged with scanning electron microscopy. Bars of 100µm, 20µm, and 10µm for ×100, ×500, and ×2,000 magnification, respectively.[21]

Determination of topical US-MB application on *C. auris* biofilms with ultrasound on abiotic media



Possible experimental set up utilising an abiotic surface without a medium[22]

12 Experimental Set Up
Set up of Ultrasound transducer and biofilm must be able to sustain sonication with increased stability and avoid ultrasound reflection

13 Activity of Medium on biofilm Formation
Measurement via Optical Density, Colony Forming Units, Confocal Laser Scanning Microscopy, Fluorescence Labelling, Biofilm Staining

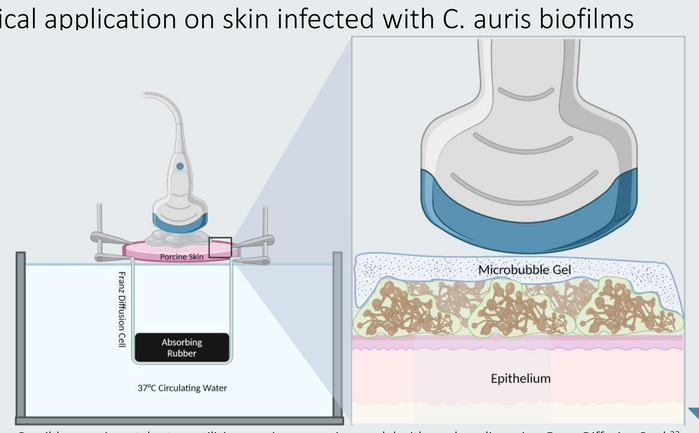
14 Quantification of Treatment Efficacy
MIC and MBEC quantification via EUCAST, CLSI standards

Determining efficacy and safety of US-MB topical application on skin infected with C. auris biofilms

15 In vivo US Stimulation on porcine or murine model

16 Activity of Medium on Biofilm Formation and Skin
Determination of biofilm size reduction, viable cells post treatment, penetration depth and concentration of particles

17 Determination of Growth Inhibition
Measurement of colonies post treatment



Possible experimental set up utilising porcine or murine model with a gel medium via a Franz Diffusion Pool [23]

Work Package 1
Work Package 2
Work Package 3
Work Package 4
Work Package 5

Hypothesis

We hypothesise that ultrasound microbubble delivery (US-MB) will increase permeability to drugs by cavitation and microstreaming effects while causing significant physical disruption of the biofilm to provide synergistic antifungal effect and biofilm shedding.

An Ideal Particle?

Efficacious antifungal treatment that is low to moderate in toxicity, able to achieve MIC50 and MIC90 with high structural integrity to withstand stable cavitation while allowing targeted therapy on the microbubble shell. A microbubble shell between 500 nm and 1 µm is optimal for drug loading. Ultrasound parameters should range from duty cycle of 10% to 50%, no displacement, with acoustic pressure in the range of 0.2 – 1.0 MPa.

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