Reducing Pathogenicity of *Candida auris* Through Biofilm Disruption

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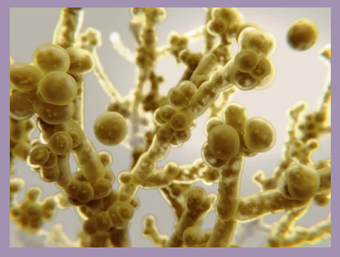
Abstract

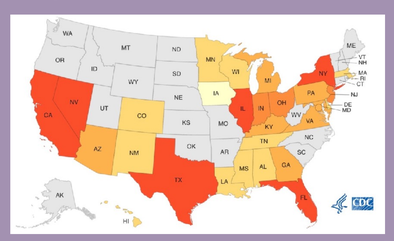
Candida auris is an emerging multi-drug resistant hospital acquired pathogen. *Candida* species are the third most common cause of healthcare related bloodstream infections, with a mortality rate of 30-60%. Candida albicans was previously thought to be the most pathogenic species of its genus. However, new studies have shown that *C. auris* produces biofilms at 10-fold greater burden than *C. albicans* on cutaneous surfaces and exhibits a unique stress resistance profile allowing it to adapt and survive in the skin niche more effectively than other *Candida* spp. Prior work has shown that both filastatin and taurolidine can inhibit *C. auris* biofilms. In a clinical setting *C. auris* has shown a strong resistance to fluconazole due to the biofilm the organism produces. In this study porcine skin cultures were used to test the efficacy of these drugs in inhibiting biofilm formation and possibly cutaneous infection. Biofilm production, as well as infection severity, was assessed through a drug resistance assay, flow cytometry and electron microscopy. Furthermore, susceptibility testing was performed using broth dilutions to evaluate if taurolidine and filastatin increase the efficacy of fluconazole. We expected that the use of an intermediary substance which weakens the biofilm, combined with an antimicrobial, would kill the fungus more effectively than an antibiotic agent alone. We found the combination of taurolidine, filistatin and fluconazole to be an effective treatment against *Candida auris* infection.

Introduction

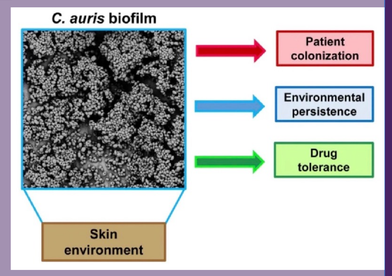
Candida species and other fungal pathogens contribute to a staggering toll of at least 13 million infections and 1.5 million deaths worldwide annually. Normally, *Candida* resides as part of the commensal flora in the gastrointestinal tract, vagina, mouth and on the skin surface without issue. However, when it proliferates uncontrollably or breaches deep into the body, it can lead to infections such as invasive candidiasis. In immunocompromised individuals, these infections escalate rapidly, resulting in severe complications such as wound infections and candidemia, with mortality rates soaring to nearly 60%.

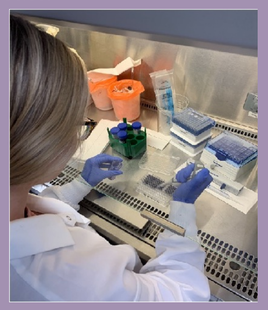


Candida auris, an emerging nosocomial pathogen, poses a growing concern in critical care medicine, particularly with the increasing number of immunocompromised patients. While the precise mechanism of its efficient patient-to-patient transmission remains unclear, there is speculation that its ability to form high-burden biofilms may play a significant role.



Previously, *Candida albicans* was considered the most pathogenic species within its genus. However, recent studies reveal that *C. auris* forms biofilms at a 10-fold greater burden on cutaneous surfaces compared to *C. albicans*. Moreover, *C. auris* demonstrates a distinct stress profile, enabling it to adapt and survive more effectively than other *Candida* species.





90% of *C. auris* isolates are resistant to fluconazole, the primary drug of choice for *Candida* species, underscoring the pressing need for additional research. Filastatin and taurolidine show promise in addressing *Candida auris* infections due to their targeted mechanisms, broad-spectrum activity, and potential synergy with existing antifungal drugs, warranting further investigation and clinical development.

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Results

Drug Resistance Assay

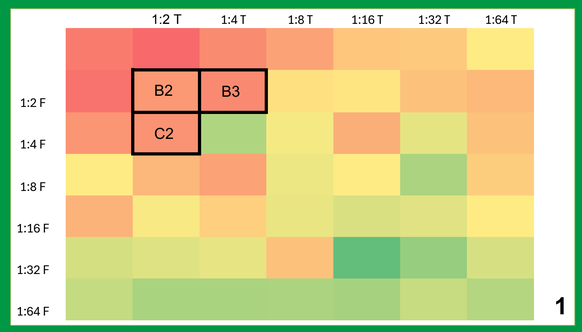
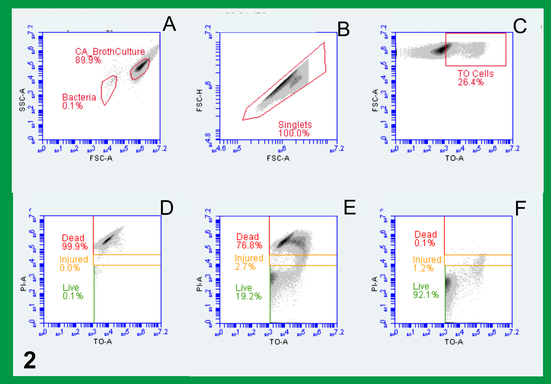
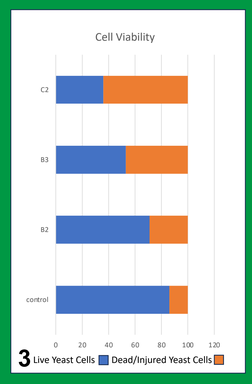


Figure 1 shows the biofilm production of *C. auris* after treatment with varying drug combinations. A darker red color indicates a lower biofilm concentration, while green indicates a higher biofilm concentration. Taurolidine is indicated by 'T' and filastatin is indicated by 'F.'. The three most effective concentrations were identified as wells B2, B3 and C2 which are outlined in black. B2 contained a 1:10,000 dilution of T and a 1:20,000 dilution of F. B3 contained a 1:20,000 dilution of T and a 1:20,000 dilution of F. C2 contained a 1:10,000 dilution of T and a 1:40,000 dilution of F. All 3 wells contained 90µl of inoculated yeast broth and 10µl of each respective drug dilution.

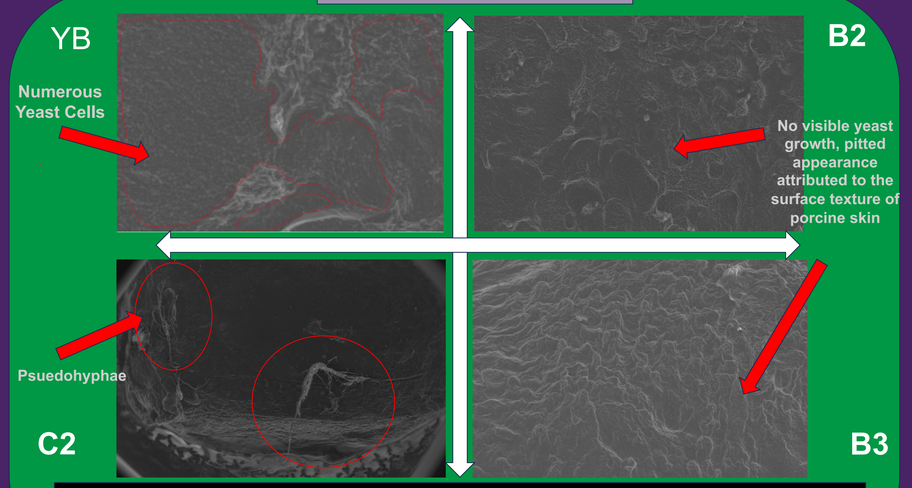
Live/Dead Analysis





Figures 2A-E illustrate the gating strategy used to identify the live and dead cells in flow cytometry. A)Yeast B)Yeast with TO stain C) Singlets TO D) Live/Dead/Injured TOPI heat treated E) Live/Dead/Injured TOPI Ethanol treated F) Live/Dead/Injured. Figure 3 shows the percentage of living vs. dead/injured yeast cells after each of the drug treatments.

Visualization of Biofilm Formation



The images above display porcine skin samples inoculated with a yeast control and the three most effective concentrations as defined in figure 1. All images were captured using a scanning electron microscope. This illustrates the effectiveness of the drug combinations in preventing the formation of biofilm in a skin infection model.

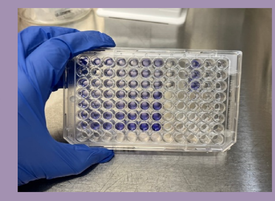
Methods

Drug Resistance Assay

YPD broth inoculated with *C.auris* at an OD of 0.5 McFarland → Taurolidine and filastatin prepared and serially diluted

Drug dilutions and yeast are pipetted into 96 well plate and incubated for 24 hrs at 37°C → Fixation and Staining: Methanol > Crystal Violet > PBS > Acetic Acid

Transfer 100 µl of solution to new well plate and read absorbance at 540 nm



Live/Dead Analysis

Flow Cytometry was used to assess the ratio of living vs. dead fungal cells in the 3 most effective yeast/drug dilutions.

Visualization of Biofilm Formation

12mm punches of pig skin incubated overnight in FBS and DMEM → Yeast and 3 most effective drug concentrations added and re-incubated → Biofilm formation analyzed on pig skin using scanning electron microscope

Discussion

The results of this study indicate that taurolidine and filastatin, in combination with fluconazole, have the potential to be an effective treatment for human infections caused by *Candida auris.* Chi squared analysis of our flow cytometry results indicated significant difference between the yeast control and the drug treatments. Further research must be conducted to better understand the effects of the drug combinations on human cells before the treatment can be implemented clinically. Additionally, our research showed that the drugs had a minimal effect on bacterial growth, which could suggest that this treatment may eliminate *C. auris* without damaging the normal flora of the skin. Further research is also needed to understand how different strains of *C.auris* respond to the combinations of drugs used in this experiment. Some results of this study could have been skewed by an episode of bacterial contamination that occurred. However, after this contamination all reagents, broths, and drugs were sterilized before the experiment was continued.

Special Thanks

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