In vitro Evaluation of Biofilm Disrupting Agents Against *Candida auris* and Other *Candida* species

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ABSTRACT

Background: *C. auris* has become a globally emerging fungal pathogen, frequently reported to be multi-drug resistant. In addition, it is frequently recovered from hospital environments and has been associated with recurrent fungal infections. Chlorhexidine (CHD) has been shown to be effective, but it has been associated with anaphylaxis reactions. Biofilm disrupting agents (BDAs) in the forms of topical gel (BlastX), wound wash solution (TorrentX), and surface disinfectants (NSSD) are novel agents with a broad spectrum of antimicrobial activity. BDAs have been used in the management of chronic wounds and to sterilize environmental surfaces. The goal of this study was to evaluate BDA technology against *C. auris* and other *Candida* spp and compare them to CHD.

Materials/methods: We evaluated the efficacy of various BDAs [topical gel, wound wash solution, and surface disinfectants] and CHD against *C. auris* isolates 0381 and 0386, *C. albicans* 90028 and *C. glabrata* 200918 by agar plate zone inhibition and time kill assays. The effectiveness of the microbicides was evaluated based on the magnitude of the inhibition zone and the reduction of CFU, respectively, compared to the drug-free control.

Results: All three BDAs and CHD inhibited *C. auris* growth effectively in a concentration dependent manner. In addition, CHD and the BDAs all showed excellent antimicrobial activity against pregrown *C. auris* cells. BDAs were highly effective against both *C. auris* isolates, whereas CHD was only moderately effective against *C. auris* 0386, suggesting possible emergence of resistance/tolerance to CHD in *C. auris* species. A comparative analysis of the efficacy of the BDAs and CHD against *C. auris and C. albicans* by kill curve studies showed up to 99.999% killing at conventional concentrations for these agents

Conclusions: All three BDAs and CHD have excellent activity against different *Candida* species, including *C. auris*. In addition, certain isolates of *C. auris* showed increased resistance/tolerance to CHD, but not to the BDAs. The fungicidal activity of these novel agents will be valuable in eradicating surface colonization of *Candida* spp, including *C. auris,* and possibly decrease the spread of this *Candida* spp. Further environmental studies are warranted.

BACKGROUND AND SIGNIFICANCE

Candida auris is an emerging multidrug-resistant fungal pathogen that presents a serious global health problem. *C. auris* infections have been reported worldwide in Europe, Asia, Africa, North and South America. The infection largely originates from the healthcare facilities and can be transmitted from a person to person. *C. auris* is often misidentified by standard laboratory techniques leading to inappropriate treatment and management. It has been known to cause outbreaks in healthcare settings.

OBJECTIVE

The primary objective of this study is to evaluate the efficacy of three novel biofilm-disrupting agents (BDAs) formulated in topical gel (BlastX), wound wash (TorrentX), and a surface disinfectant (NSSD) and compare to chlorhexidine (CHD) on *C. auris, C. albicans, and C. glabrata* monomicrobial biofilms *and* on polymicrobial biofilms composed of either *C. auris* or *C. albicans/S. aureus.*

MATERIALS AND METHODS

Microorganisms: *Candida auris* AR0381, AR0386 (Mycology Section, Center for Disease Control), *Candida albicans* 90028 and *Candida glabrata 200918* (American Type Culture Collection) were used in this study.

Agents Evaluated: Three biofilm disrupting agents (BDAs): Topical gel, wound wash, a surface disinfectant and Chlorhexidine Solution (CHD).

Sabouraud's Dextrose (SD) agar zone inhibition assay: The effect of the BDAs and CHD were investigated by zone inhibition assay. The details of each experiment are described in Figure 1 legend.

Time kill study: Overnight cultures of *Candida* spp. were grown in SD broth, the cells were washed and resuspended in appropriate medium and exposed to various concentrations of BDAs and CHD for 24 h. The extent of killing in the presence of various concentrations of the microbicidal agent is determined by CFU assay. For details see legends to Figs. 5 and 6.

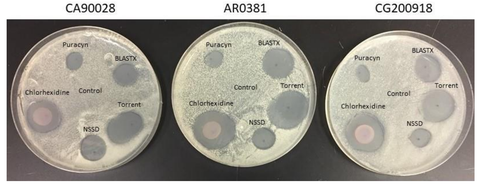

Figure 1. Typical photographic images showing the effects of various experimental (BDAs: BlastX, TorrentX and NSSD) and leading commercially available (CHD) microbicidal agents on the growth and biofilm formation of *Candida albicans* 90028, *Candida auris* AR0381 and *Candida glabrata* 200918 using Sabouraud's Dextrose (SD) agar plate inhibition assay. Briefly, SD agar plates were seeded with various *Candida* cells (1 x 10^5 cells/plate) and the plates were air-dried under a Biosafety Cabinet to remove excess amount of water. A uniform amount (20 µl) of the full-strength microbicidal agents (except BLASTX , 50%) were applied strategically to the SD agar plates as shown. The plates were incubated at 37°C for 48 h in a plastic sleeve for microbial growth to obtain a clear zone of inhibition. Equal volume of sterile saline was used as control.

RESULTS

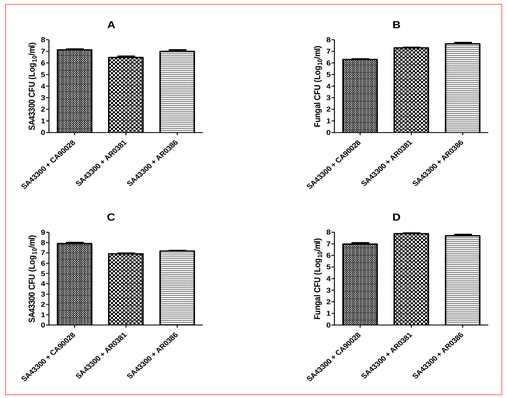

Figure 2. *Candida* and *Staphylococcus aureus* polymicrobial biofilm development in 50% fetal calf serum. Dual species *Candida* and *S. aureus* polymicrobial biofilms were developed by incubating 0.2 ml cell suspension (1 x 10^7 cells each/ml) in 50% fetal calf serum in 96-well TC plates for either 24 h (Panels A and B) or 48 h (Panels C and D) at 37°C. The biofilms were washed with sterile distilled water two times, resuspended in 0.1 ml sterile distilled water, diluted and then performed CFU assay. **RESULTS: Both 24 h and 48 h incubation in 50% fetal calf serum produced good sustainable polymicrobial biofilm. However, the CFU counts for the 48 h biofilms were approximately a $\log_{10}$ higher than that obtained for 24 h biofilm.**

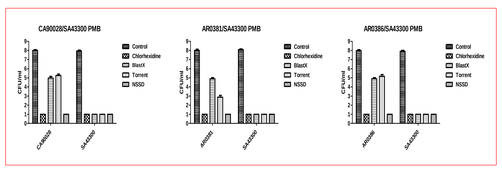

Figure 5. Antibiofilm activities of commercial (CHD) and experimental (BDAs) microbicides **determined in 96-well TC plates**. Dual species polymicrobial biofilms of *C. albicans* 90028, *C. auris* AR0381 and *C. auris* AR0386 with *S. aureus* 43300 were developed as previously described (Larkin et al AAC 61: e02396-16, 2017) in 96-well TC plates for 24 h at 37°C. The biofilms were washed with sterile distilled water two times and then treated with various microbicidal agents prepared in SD broth at a **concentration of 50%** for 24 h at 37°C. The microbicide-treated biofilms were washed with sterile distilled water three times, resuspended in 0.1 ml sterile water and the fungal and bacterial CFUs were determined. **RESULTS: All microbicidal agents completely killed biofilm associated microbial cells except BlastX and Torrent which yielded only ≥ 99.9% killing of the fungal cells.**

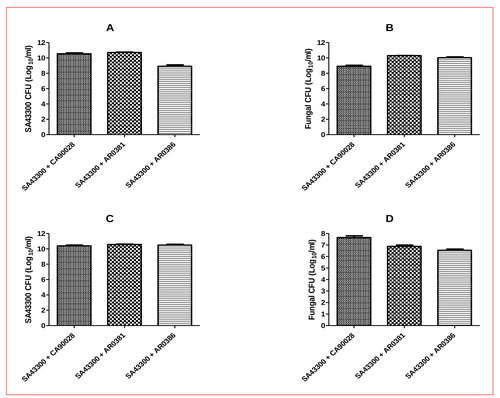

Figure 3. **Twenty-four-hour** *Candida* and *Staphylococcus aureus* polymicrobial biofilm. Dual species polymicrobial biofilms of *C. albicans* 90028, *C. auris* AR0381 and *C. auris* AR0386 with *S. aureus* 43300 were developed as previously described (Larkin *et al* AAC 61: e02396-16, 2017). Briefly, 0.1 ml suspensions of *Candida* (1 x 10^7 cells/ml) were incubated in 50% fetal calf serum in 96-well flat bottom TC plates at 37°C for 90 min for cell adhesion. After cell adhesion, the plates were gently shaken on a gyratory shaker for 5 min and the unbound cells were removed with a pipette. The attached *Candida* cells were then incubated with 0.2 ml SA43300 cell suspension (1 x 10^7 cells/ml) in SD broth (Panels A and B) or 50% SD broth + 50% BHI broth (Panels C and D) for 24 h for biofilm development. The biofilms were washed with sterile distilled water two times and then the bacterial and the fungal CFUs were determined. **RESULTS: The Larkin *et al* procedure produced more robust sustainable polymicrobial biofilms. Use of SD broth alone and 50% SD + 50% BHI yielded similar numbers of CFUs for both the fungal and bacterial species.**

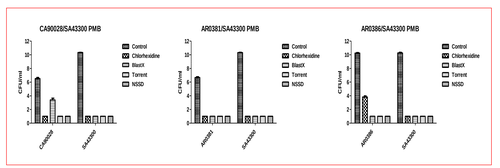

Figure 6. Antibiofilm activities of commercial (CHD) and experimental (BDAs) microbicidal agents **determined in 24-well TC plates**. Dual species polymicrobial biofilms of *C. albicans* 90028, *C. auris* AR0381 and *C. auris* AR0386 with *S. aureus* 43300 were developed as previously described (Larkin et al AAC 61: e02396-16, 2017) in 24-well TC plates for 24 h at 37°C. The biofilms were washed with sterile distilled water two times and then treated (24 h at 37°C) with **full-strength microbicidal agents** except BlastX 50% prepared in SD broth. The microbicide-treated biofilms were washed three times with sterile distilled water, resuspended in 1 ml sterile water and the fungal and bacterial CFUs were determined. **RESULTS:** The 24-well TC plate format produced more robust biofilm. All microbicidal agents completely killed biofilm associated microbial cells except BlastX and chlorhexidine which yielded only ≥ 99.99% killing of CA90028 and AR0386 cells, respectively.

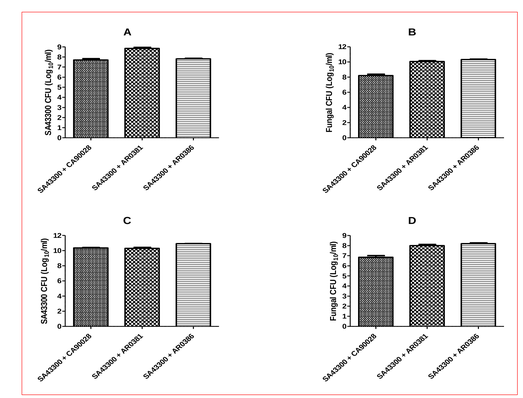

Figure 4. **Forty-eight-hour** *Candida* and *Staphylococcus aureus* polymicrobial biofilm. Dual species polymicrobial biofilms of *C. albicans* 90028, *C. auris* AR0381 and *C. auris* AR0386 with *S. aureus* 43300 were developed as previously described (Larkin *et al* AAC 61: e02396-16, 2017). Briefly, 0.1 ml suspensions of *Candida* (1 x 10^7 cells/ml) were incubated in 50% fetal calf serum in 96-well flat bottom TC plates at 37°C for 90 min for cell adhesion. After cell adhesion, the plates were gently shaken on a gyratory shaker for 5 min and the unbound cells were removed with a pipette. The attached *Candida* cells were then incubated with 0.2 ml SA43300 cell suspension (1 x 10^7 cells/ml) in SD broth (Panels A and B) or 50% SD broth + 50% BHI broth (Panels C and D) for 48 h for biofilm development. The biofilms were washed with sterile distilled water two times and then the bacterial and the fungal CFUs were determined. **RESULTS: Overall, both 24 h and 48 h *Candida/S. aureus* polymicrobial biofilms were morphologically similar and yielded similar numbers of CFUs.**

SUMMARY AND CONCLUSIONS

1. We investigated the microbicidal activity of three different biofilm disrupting agents [BDAs], a topical gel, a wound wash solution, and a topical disinfectant and compared them against chlorhexidine (CHD) against *C. auris, C. albicans, and C. glabrata* by SD agar inhibition and kill curves experiments.

2. All three BDAs and CHD inhibited the growth of *C. auris* AR0381 and AR0386 in a concentration dependent manner. However, CHD was less effective against AR0386.

3. The kill curve experiments show that both the BDAs and CHD completely killed *C. auris* at a low concentration.

4. All three BDAs showed excellent activity against monomicrobial and polymicrobial biofilms of *C. auris/S. aureus* polymicrobial biofilms.

Conclusions: The use of these novel BDAs with excellent antimicrobial and antifungal activity make them very valuable in eradicating surface and wound colonization of *Candida* spp, including the MDR- *C. auris*, and thus possibly decrease the spread of this *Candida* spp.

ACKNOWLEDGMENTS

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