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Research Article

Efficacy of Modulated Dielectric Barrier Discharge Atmospheric Nonthermal Plasma Air Treatment System against *Candida auris*

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Abstract

Background: *Candida auris* is an emerging fungus currently considered a serious global health threat. CDC has deemed *Candida auris* an "urgent antimicrobial resistant threat as it is often resistant to multiple antifungal drugs, spreads easily in healthcare facilities, and can cause severe infections with high death rates". *Candida auris* is difficult to identify with standard laboratory methods, exacerbating persistent outbreaks attributed to surface contamination [1].

This study examines the efficacy of modulated dielectric barrier discharge atmospheric nonthermal plasma air treatment against *Candida auris* in air and on surfaces.

Methods: Log reductions were recorded over time with modulated dielectric barrier discharge atmospheric nonthermal plasma air treatment of aerosolized *Candida auris*. *Candida auris* inoculated stainless steel, floor-tile, and plastic coupons were measured in a test room 22' x 12' x 10'.

Results: At 2 (two) minutes of treatment in all samples, no *Candida auris* was detected (at limits of detection). Log reductions up to 4.52 were observed at 30-seconds of treatment.

Discussion: **"**No-touch", automated enhancements such as modulated dielectric barrier discharge nonthermal plasma air treatment can reduce reliance on human operators and so have the potential to improve disinfection outcomes and the success of terminal disinfection [2] when used with current protocols in healthcare environments. Disinfection with modulated dielectric barrier discharge-generated nonthermal plasma demonstrated reductions to non-detectable levels in 2 minutes of aerosolized *Candida auris* and inoculations on common surfaces.

Conclusion: The efficacy of modulated dielectric barrier discharge nonthermal plasma air treatment to destroy *Candida auris* shown in this study suggests it is a highly effective enhancement to *Candida auris* mitigation in healthcare environments.

Keywords: *Dielectric Barrier Discharge; Nonthermal Plasma; Cold Plasma; Candida auris; Infection Prevention; No-Touch Disinfection*

Abbreviations

DBD: Dielectric Barrier Discharge; MDBD: Modulated Dielectric Barrier Discharge; ACP: Atmospheric Cold Plasma; *C. auris: Candida auris*

Introduction

Candida auris (C. auris) is currently reported to cause severe illness in hospitalized patients in several countries [3]. *C. auris* is resistant to commonly used antifungal drugs and can enter the bloodstream and spread throughout the body, causing serious invasive infection. Generally, not a threat to healthy people, it can colonize on skin and spread to high-touch surfaces. Patients with invasive medical devices such as intravenous and urinary catheters, and feeding and breathing tubes, are often at increased risk for developing *C. auris* infections [4].

This study was performed to determine the efficacy of atmospheric nonthermal plasma (NTP) generated by a modulated dielectric barrier discharge (MDBD) air treatment system to mitigate *C. auris* fungus in the air and on surfaces. The study measured log reductions of aerosolized *C. auris* and log reductions on stainless steel (S), floor tile (F), and plastic (P) coupons inoculated with *C. auris*.

A 4.35-Log reduction was observed at 30-seconds of treatment of aerosolized *C. auris*. A 4.48, 4.50, and 4.52-Log reduction was observed at 30-seconds on S, F, and P coupons, respectively. At 2 (two) minutes of treatment in all samples, no *C. auris* was detected (at limits of detection).

Materials and Methods

The MDBD air treatment unit was placed in the same location within a test room of a similar size to a hospital patient room, with system output being as evenly distributed as possible. The system was run as provided. Smoke testing was completed to determine areas where air in the test room was still or not being mixed. A small oscillating mixing fan approximately 50-CFM was placed in a specific location to ensure gentle but adequate mixing of air in the room to prevent "dead" spaces as confirmed by final smoke tests.

Air sensors (O3-units) were placed in the same location as the treatment system test. One Aeroqual 930 was placed in the worst-case location: the southeast corner of the room labeled SE(2). Two additional Aeroqual sensors were placed in locations CE(3), central east, and SW(4), southwest corner. An additional ECO A-22 sensor was placed in location NE(5), northeast. The final sensor, a handheld Aeroqual 300, was placed in location NE(1), northeast; is also used to "spot" check other locations.

To show efficacy on surfaces, 1,500 - 2,000, 4-cm round/square S, F, and P coupons were inoculated with bacteria. To show air efficacy within the treated room, aerosol testing was performed with *C. auris*.

Surface treatment

C. auris CDC B11903 was acquired from American Type Culture Collection (ATCC, Manassas, VA., USA) and maintained at 8°C on slants of tryptic soy agar (TSA, Hardy Diagnostics, Santa Maria, CA., USA). Cultured in tryptic soy broth (TSB, Hardy Diagnostics) at 26°C.

Every 24-h, cultures were transferred to TSB by loop inoculation. Cells (approximately $1x10^8$ to $1x10^9$ CFU/ml) from a 24-h static culture incubated at 26°C were used to inoculate a substantial number > 2,000, 4-cm round/square S, F, and P. The inoculum's suspension was enumerated by surface plating in duplicate samples on TSA and Bile Esculin Azide Agar after serial dilution in 0.1% peptone solution. Plates were incubated for 24-h at 26°C.

A 100-µl droplet from the initial surrogate suspension was used to inoculate the external surface of coupons S, L, and P with the final inoculum level of approximately 7-log CFU/4-cm. Inoculated samples were dried for 2-h at 22°C before NTP treatment. The 2-h drying allows the inoculated cells to attach to the surface host.

A substantial number, > 1,800, 4-cm, S, L, and P inoculated coupons were randomly placed at different locations and heights, in a room of a similar size to a hospital room, or Drs/Dentists office. Samples were randomly harvested from various room locations after exposure times at standard-maintained temperatures of 70 - 76°F and 45 - 77%-RH. After treatment, all samples were enumerated by

surface plating. The log-reduction of the inoculum was evaluated with and without consideration of resuscitation of injured cells after NTP treatment.

For positive control, three (3) 4-cm coupons were inoculated with the surrogate's cells and not exposed to NTP treatment. Three (3) negative controls, in which a set of 4-cm coupons were inoculated with a 100-µl droplet of sterile water and dried for 2-h. One (1) negative control was treated with NTP and the other was not subjected to NTP treatment. Each treatment sample was prepared in triplicate.

After the NTP treatment, each coupon was immediately removed, transferred into a 400-ml stomacher bag (Fisher Scientific Inc., PA., USA), and combined with 50-ml sterile 0.1% peptone solution, then mixed with an AES Easy Mix Stomacher (AES Laboratories, Princeton, NJ., USA) for 2-m at normal speed. Wash fluid was serially diluted for enumeration. A centrifugation method was used to recover low populations of NTP-injured bacteria.

S, F, and P coupons were inoculated with 0.1-mL aliquot of standardized suspension of the challenge microorganism and allowed to dry for 60 ± 5-minutes.

Exposure Period: The disinfection device was sterilized using isopropyl alcohol before testing. After drying, the coupons were aseptically placed inoculum side down onto the floor disinfection device using sterilized forceps.

Three (3) coupons were tested. Dilutions were plated via the pour plate method in duplicate on Sabouraud Dextrose Agar with Letheen and incubated for 5 to 7 days at 25 ± 2°C. After incubation, colonies were counted, and data were recorded. The geometric mean was calculated from the duplicated plates and log and percent reduction were calculated using the positive control counts.

Air treatment

Candida auris was acquired from American Type Culture Collection (ATCC, Manassas, VA., USA) and maintained at 8°C on slants of tryptic soy agar (TSA, Hardy Diagnostics, Santa Maria, CA., USA). Cultured in tryptic soy broth (TSB, Hardy Diagnostics) at 26°C. Every 24-h, the cultures were transferred to TSB by loop inoculation.

Cells (approximately $1x10^8$ to $1x10^9$-CFU/ml) from a 24-h static culture incubated at 26°C were used. *C. auris* (cultures) were centrifuged for 20-m at 5,000-rpm in sterile 15-mL conical tubes, growth media was removed, and the cells were resuspended in sterile PBS buffer for aerosolization.

Aliquots of these suspensions were enumerated on tryptic soy agar plates (Hardy Diagnostics, Santa Maria, CA., USA) for viable counts and stock concentration calculation. Test working stock was grown in sufficient volume to satisfy use quantities for all tests conducted using the same culture stock material.

Stock cultures were serially diluted and plated in triplicate (multiple serial dilutions) using a standard spread plate assay technique onto tryptic soy agar plates. The plated cultures were incubated for 24-hours and enumerated and recorded.

Stock culture was atomized (26-micron droplets) via model CF40K50T atomizer (Sonaer®, Farmingdale, NY). The atomized inoculum was introduced to the suction side of the Catalyst Reactor Unit near the intake aperture of the unit, the fan speed was set to full and the array voltage was set at 40. Infectivity media, 15-ml, was poured into each sterilized disposable plastic Petri dish 100 x 15-mm (Fisher Scientific) and randomly arranged to be removed at the scheduled time intervals.

Controls

To accurately assess the treatment system during aerosol testing, room control trials were performed with cultures over 4-to-6-hour periods without the respective treatment system in operation. This is to characterize the surrogate challenge aerosol for particle size distribution, aerosol delivery/collection efficiency, and viable concentration over time. Control testing was performed to provide

baseline comparative data in order to assess the actual reduction for the various treatment system challenge testing and verify that viable bioaerosol concentrations persisted above the required concentrations over the entire controlled test period.

Testing protocol

Surface treatments

Coupons S, F, and P were inoculated in triplicate, placed randomly in the room, and harvested at specific time periods.

Time (minutes): 0, 0.5, 1, 2, 4, 6, 8, 12, 24.

Air treatment

Controlled droplet size produced by a three (3) jet CH Technologies collision nebulizer. Inoculum is introduced to the nebulizer and dispersed as a fungal aerosol.

Time (minutes) 0, 0.5, 1, 2, 4, 6, 8, 12, 24.

Results

Time	*Candida auris*			Reduction
	Stainless Steel			
	CFU	Log	SD	
0.0	69,000,000	7.84	0.4	-
0.5	2,300	3.36	0.2	4.48
1	70	1.85	0.2	5.99
2	< 1	0.00	0.2	7.84
4	< 1	0.00	0.3	7.84
6	< 1	0.00	0.1	7.84
8	< 1	0.00	0.1	7.84
12	< 1	0.00	0.1	7.84
24	< 1	0.00	0.1	7.84

Table 1: *CFU reduction table C. auris on stainless steel.*

Time	*Candida auris*			Reduction
	Floor Tile			
	CFU	Log	SD	
0.0	69,000,000	7.84	0.3	-
0.5	2,200	3.34	0.2	4.50
1	93	1.97	0.2	5.87
2	< 1	0.00	0.2	7.84
4	< 1	0.00	0.1	7.84
6	< 1	0.00	0.1	7.84
8	< 1	0.00	0.1	7.84
12	< 1	0.00	0.1	7.84
24	< 1	0.00	0.1	7.84

Table 2: *CFU reduction table C. auris on floor tile.*

Time	*Candida auris*			Reduction
	Plastic			
	CFU	Log	SD	
0.0	69,000,000	7.84	0.3	-
0.5	2,100	3.32	0.2	4.52
1.0	85	1.93	0.3	5.91
2.0	< 1	0.00	0.2	7.84
4.0	< 1	0.00	0.2	7.84
6.0	< 1	0.00	0.1	7.84
8.0	< 1	0.00	0.1	7.84
12.0	< 1	0.00	0.1	7.84
24.0	< 1	0.00	0.1	7.84

Table 3: *CFU reduction table C. auris on plastic.*

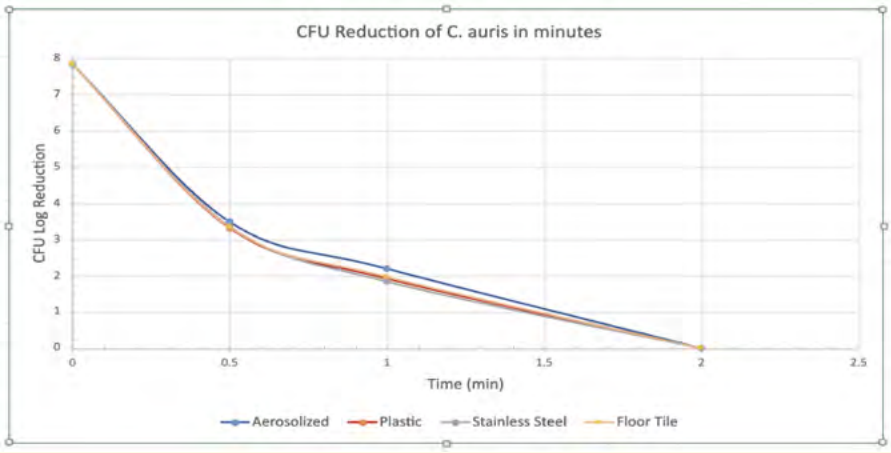

Figure 1: *C. auris reduction in minutes.*

Discussion

Disinfection with MDBD-generated NTP demonstrated a statistically significant reduction in aerosolized *C. auris* as well as inoculations on stainless steel, floor tile, and plastic coupons. These experiments represent a controlled, *in vitro* study of an automated MDBD NTP system. *C. auris* culture was used to quantify the reduction of the *C. auris* populations. Reductions of *C. auris* during treatment with NTP were 4.35-Log of aerosolized, 4.48-Log on stainless steel, 4.50-Log on floor tile, and 4.52-Log on plastic. All inoculations of *C. auris* were undetectable at 2 (two) minutes of treatment with NTP.

It should be noted that while chlorine-based disinfectants have similar efficacy as shown in this study [5], it is especially challenging to disinfect the air and all surfaces in a healthcare environment with manual chlorine-based or other disinfectants. The ability of NTP to reach everywhere air reaches could be considered of great benefit to support the reduction of surface and airborne pathogens in a healthcare environment.

Scales for CFU

The author would like to include reference to Scales for CFU created by Daniel Y. C. Fung [6], Food Science Kansas State University. These surface and air Quality Targets are important information for the protection of humans in healthcare environments. Just knowing the number of CFUs on or in an agar medium has very little meaning unless the number is related to per volume of per area of a surface (cm square), or per volume of air (cubic meter). These quality targets are as follows.

Target air quality:

- < 100 cfu/m^3 is considered clean and acceptable.
- 100 to 300 cfu/m^3 is marginal.
- > 300 cfu/m^3 is not acceptable and needs corrective action.

Target contact surface quality:

- < 5 cfu/cm^2 is considered clean and acceptable.
- 5 to 10 cfu/cm^2 is considered marginal.
- > 10 cfu/cm^2 is considered not acceptable and needs corrective action.

Conclusion

Conventional disinfection methods are limited by operator precision of application and contact time of disinfecting agents. Continuous "no-touch" disinfection systems remove or reduce reliance on operators and so may improve the efficacy of disinfection protocols. The efficacy of MDBD in destroying *C. auris* shown in this study suggests it is a highly effective enhancement to *C. auris* mitigation in healthcare environments.

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Conflict of Interest

The author declares the following potential conflicts of interest concerning the research, authorship, and publication of this study on the efficacy of MDBD technology in eliminating *C. auris*.

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