P029

Synergic effect of deferoxamine combined with fluconazole against fluconazole-resistant *Candida* Spp. through inhibited Cek1 MAPK signaling pathway

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: The opportunistic fungal infections represent an increasing threat to humans with the increase of immunocompromised patients, in which *Candida albicans* is the most common fungal pathogen. Though fluconazole (FCA) is still the first line choice to treat *C. albicans* infections, several limitations such as an increase in drug resistance compromised its clinical application. This study proposes a combination therapy of deferoxamine (DFO) and FCA to overcome *C. albicans* resistance.

Methods: Checkerboard microdilution assay was used to determine the minimum inhibitory concentration (MIC) of DFO used alone and in combination with FCA against FCA-resistant *Candida* Spp. Spot assay and time-kill curves were used to investigate the cell viability and dynamic inhibitory effect. Hyphal formation was performed to investigate the underlying mechanism of DFO. Then, a murine model of cutaneous candidiasis was established to explore the in vivo synergistic activity of DFO and FCA.

Results: DFO combined with FCA showed synergistic antifungal activity against FCA-resistant *C. albicans*, with a fractional inhibitory concentration index (FICI) of 0.25. Moreover, DFO combined with FCA significantly inhibited the activity of *C. glabrata* cells, which is naturally insensitive to antifungal drugs. The spot assay and time-kill curve assay indicated that DFO can turn the fungistasis activity of FCA into fungicidal activity. Hyphal formation study showed the inhibition of hyphal induction of *C. albicans*. DFO combined with FCA also significantly inhibited the expression of Cek1 MAPK signaling pathway-related genes (CEK1 and CPH1) and adhesion-related genes (ALS1). *In vivo* data showed DFO combined with FCA significantly reduced the pustule, CFU numbers, and inflammatory cell infiltration of skin tissue.

Conclusion: Our results suggest that DFO combined with FCA inhibited the transformation of yeast-hyphae through Cek1 MAPK signaling pathway, resulting in reduced infectivity and resistance of *C. albicans in vitro* and *in vivo*, which may provide a new option for the treatment of cutaneous candidiasis.

P030

Highly slow-release antifungal wound dressing for chronic dermatophytosis

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: The aim of this research was to make a biocompatible and affordable nanofibrous wound dressing that is able to release terbinafine at the site of chronic superficial fungal infection over time.

Methods: Polymer solution (10%) of poly (caprolactone) (PCL) was prepared in hexafluoroisopropanol (HFIP). Terbinafine hydrochloride (TFH) was added equal to 5% of PCL weight for drug-loaded samples. Electrospinning was performed with a 27-G-needle equipped syringe at a distance of 15 cm which the injection flow rate of the solution was 0.2 ml/h and a 30 kV voltage was applied. The measurements of drug release were performed with HPLC. Antifungal tests were done on three different fungal species and MTT assay was done based on ISO-10993 on 24 h L929 cells. The drug release was monitored for 144 h in a human body simulated system (incubation at 37°C, shaking by 30 rpm, and passing the drug through a wet Whatman filter into PBS).

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Results: The mean diameter of fibers was obtained at 1262 nм for PCL nanofibers without TFH and 249 nм for PCL nanofibers with TFH. The drug loading for PCL was 83.4%. Antifungal activity of PCL fibers was examined against a dermatophyte (*Trichophyton mentagrophytes*), a saprophyte (*Aspergillus fumigatus*), and a yeast (*Candida albicans*). The terbinafine hydrochloride-contained electrospun PCL fibers inhibited the growth of *T. Mentagrophytes* and *A. Fumigatus* but did not inhibit *C. albicans* growth. None of the samples showed cytotoxic effects after 24 h and 2 weeks.

Conclusions: The diameter of PCL nanofibers with TFH apparently decreased by fives times ($P > .05$). PCL nanofibers successfully inhibited two important fungal species while no toxicity was observed in MTT assay for its extraction of 2 weeks. They were able to release TFH slowly over time which makes them suitable for the treatment of chronic superficial fungal infections.

P031

Post-antifungal effect of the combination of a nidulafungin with amphotericin B and fluconazole against fluconazole-susceptible and resistant *Candida albicans*

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: Invasive Candidiasis is a life-threatening condition that kills a large number of immunocompromised patients each year around the world. We used post-antifungal effect studies to analyze the activities of anidulafungin, as a clinically crucial antifungal drug, amphotericin B, and fluconazole (alone and in combinations) against FLC-susceptible and -resistant Candida *albicans* isolates obtained from the cancer patients.

Methods: We tested the phenomenon of post-antifungal effects (PAFEs) of fluconazole (FLC), amphotericin B (AMB), anidulafungin (AFG), and combinations of FLC + AFG, AFG + AMB, and FLC + AMB against 17 *C. albicans* isolates obtained from the oral cavity of cancer patients. Isolates that had not been exposed to antifungals, served as a control group. Colony counts were performed at 0, 2, 4, 6, and 24 h after a brief (1 h) antifungal exposure.

Results: The FLC had no detectable post-antifungal effect independent of antifungal concentration and resembled drug-free FLC (control). When all AMB and AFG were compared with FLC, significant variations in the post-antifungal effect were observed. Combining AFG and AMB with FLC resulted in effective activity compared to FLC alone. Combination regimens were rated as indifferent in general. Interestingly, low dosages of the AFG displayed increasing fungistatic action as it neared a fungistatic endpoint against *C. albicans* isolates ($n = 17$).

Conclusion: Our findings suggest that brief exposure to AFG, in combination with FLC and AMB, at low concentrations of the medicines utilized, could be effective in the evaluation and optimization of new dosage regimens to manage candidiasis. However, future research will look at the clinical utility of our findings.

P032

Efficacy of novel azole compounds (ATTAF-1 and ATTAF-2) against *Candida albicans* in a murine model of invasive Candidiasis

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Poster session 1, September 21, 2022, 12:30 PM - 1:30 PM

Objectives: *Candida albicans* is the most common cause of nosocomial bloodstream infections and are associated with substantial morbidity and mortality in immunocompromised individuals. However, limited therapeutic approaches against invasive candidiasis are available. The rise in antifungal resistance highlights the urgent need to develop new therapeutic options and novel treatment strategies to combat later infections. A novel compound Aryl-1, 2, 4-triazol-3- ylthio, fluconazole alcohol-derived analogs (ATTAFs), has newly developed with potent *in vitro* activity against *Candida* species, including fluconazole-resistant isolates. The objective of this study was to further evaluate the in vivo effectiveness in a murine model of invasive candidiasis due to *C. albicans*.

Methods: Treatment with ATTAF-1 and ATTAF-2 significantly increased the survival of infected mice compared to the control group (5% DMSO plus inoculum).

Results: The antifungal action of ATTAF-1 and ATTAF-2 and their median survival time provided no evidence of a difference versus fluconazole. Although there was an obvious fungal load (mean log CFU/g of tissue) decrease by ATTAF-1 and ATTAF-2 in the kidney, spleen, and liver of the treated mice in comparison with the control group and not similar to each other in regard of the dose, fluconazole showed a significant decrease in the number of fungal loads, similar to the group treated with ATTAF-1 and ATTAF-2. Nevertheless, the results of this study indicate that the use of ATTAF-1 and ATTAF-2 as a therapeutic agent can exert significant *in vitro* and *in vivo* antifungal effects against *C. albicans*, increasing animal survival and significantly decreasing fungal loads.

Conclusion: Although we have identified two new compounds, ATTAF-1 and ATTAF-2, as novel promising Candidates for the treatment of *Candida* infection, more studies of ATTAF-1 and ATTAF-2 activity and their action mechanisms in animal models are warranted to enhance our understanding and establish their efficacy.

P033

Genomic epidemiology of the antifungal-resistant dermatophytosis epidemic, India, 2017-2019

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Objective: The epidemic of antifungal-resistant dermatophytosis in India has been reported. These infections are associated with severe morbidity, resistance to oral itraconazole and terbinafine, and the widespread misuse of topical steroids. Trichophyton indotineae has emerged as the predominant causative agent. In this study, we investigated 162 dermatophytosis infections from eight Indian states using genomic sequencing. The primary objective was to determine whether a clonal outbreak strain is responsible for the current epidemic.

Methods: A total of 161 *T. indotineae* and one *T. rubrum* isolates from skin scrapings collected from India in 2017-2019 and previously reported were sent to the U.S. Centers for Disease Control and Prevention (CDC) for genomic analysis. After species identification, genomic DNA was extracted and sequenced using Illumina NovaSeq. Single-nucleotide (SNP) analysis was performed using the portable workflow MycoSNP (v0.21). Briefly, MycoSNP prepared the reference genome, performed pre-processing, aligned sample reads to the reference using the BWA (v 0.7.17) alignment algorithm, and called variants using GATK (v4.1.4.1). High-quality SNPs were used for constructing phylogenetic trees using neighbor-joining (NJ) and maximum likelihood (ML) methods. Further, to understand if infections are genetically clustered by state or region, multi-dimensional scaling (MDS) was applied using the ML tree in R.

Result: SNP analysis identified 1259 450 variant sites which were used to construct an NJ and ML tree. The tree topology from both NJ and ML methods showed consensus. All 161 *T. indotineae* isolates from India clustered together forming a large, well-supported clade. SNP differences between the samples varied from 0-160 SNPs. Historical isolates available at CDC were included as controls and clustered over 40 000 SNPs from the clade comprising isolates from India. The MDS plot revealed that isolates did not cluster by state or region.

Conclusion: Antifungal-resistant dermatophytosis is an emerging threat with cases of chronic, recurrent infection reported from several countries including India. Additionally, the rapid spread of infections involves person-to-person spread. Our results suggest that a clonal outbreak of a *T. indotineae* strain is circulating in multiple states in India. Current plans are to expand the geographic scope of the study by including over 10 countries from Europe, Middle East, and the Americas. This work will allow the public health community to better understand the emergence and transmission of antifungal-resistant dermatophytosis worldwide.

P034

Non-conventional alternatives to prevent *Candida auris* infections

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Objectives: *Candida auris* represents a particular organism associated with multiple nosocomial infections and high mortality rates. Appropriate infection control measures play a major role in controlling the spread and multiplication of this pathogen. Unfortunately, there are very few data available on the effectiveness of disinfectants against *C. auris*. Chlorine-based products appear to be the most effective for inanimate surface disinfection, while iodine-based products are more suitable for skin antisepsis. *C. auris* has been demonstrated to survive on dry and moist surfaces for up to 14 days, so environmental cleaning to eliminate a source of nosocomial infections is a challenge. Thus, searching for new effective fungicidal agents is still a hot topic nowadays. Non-thermal plasma-activated water (PAW) has recently emerged as a powerful antimicrobial agent, but no data about its effectiveness on *C. auris* are available. The aim of our study was to assess the possibility of using PAW as a fungicidal agent against *C. auris* planktonic cells.

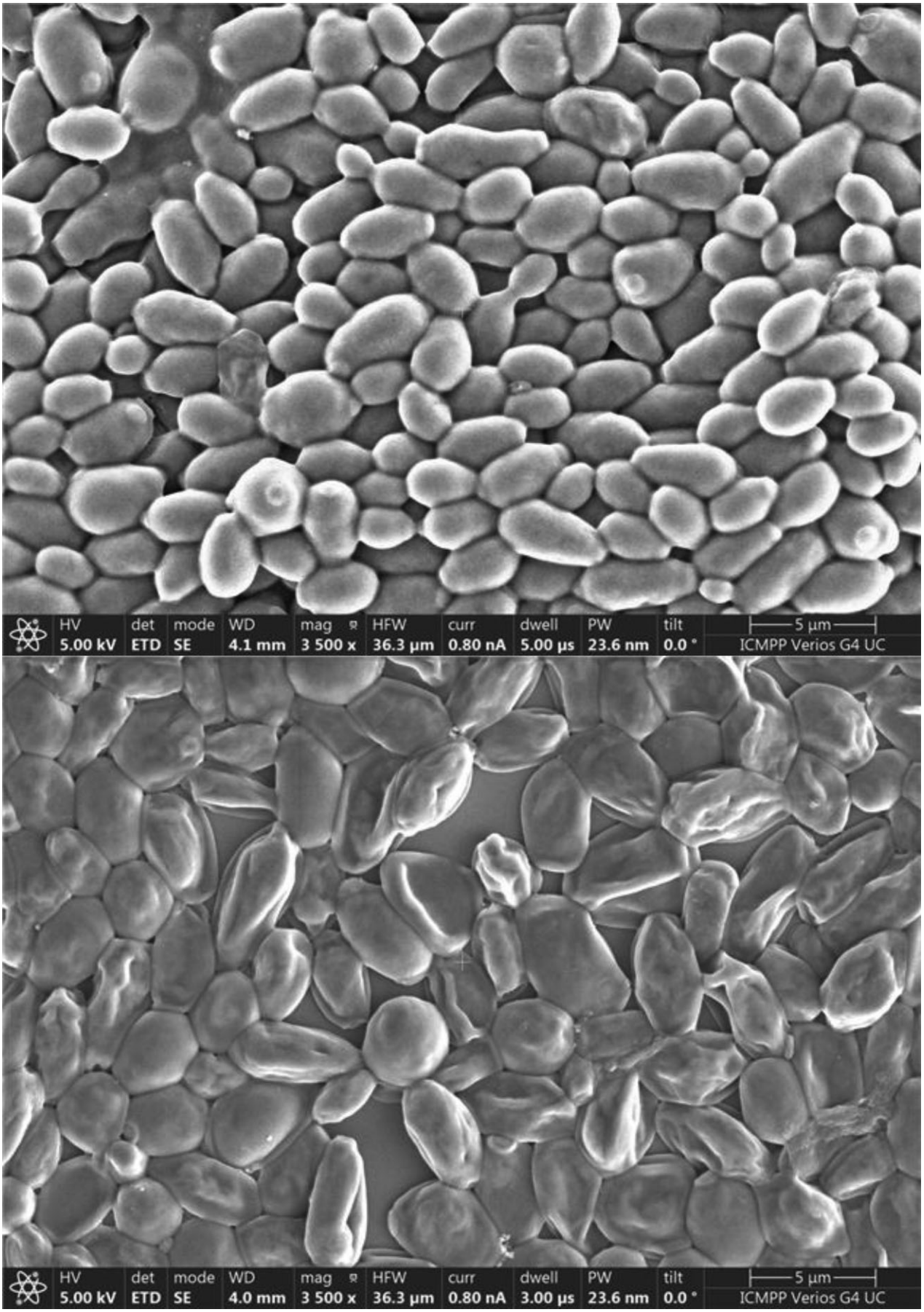
Methods: PAW was prepared using distilled water and a GlidArc reactor as previously described[1]. The final parameters of PAW were as follows: conductivity 446 ± 25 μS/cm, pH 2.78 ± 0.12, redox potential (ORP) + 1.06 V, NO2 192 ± 10 mg/L, NO3 1550 ± 95 mg/L, H2O2 2.6 ± 0.12 mg/L, and O3 1.08 ± 0.07 mg/L.

A type strain of *C. auris* (CBS 10913) was used in this study. Suspensions of yeast cells (107 CFU/ml) were prepared from overnight cultures and subsequently treated with PAW in a ratio of 1:10 for different periods of time (1, 3, 5, 7, 10, 15, and 20 minutes). Precise volumes of the mixtures were further inoculated on Sabouraud Dextrose Agar plates and Bact/Alert FA Plus bottles (bioMerieux, France) in order to evaluate the reduction of yeast burden after each contact period. In addition, some instrumental analysis (IA) methods were used in order to assess the impact of PAW treatment on yeast cell structure: Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectrometry (FT-IR), and Dynamic Light Scattering (DLS). All tests were performed in triplicates.

Results: A reduction higher than 5 log10 of viable yeast was achieved in 3 minutes. The sterilization level (i.e., >6 log10 reduction) was achieved after 5 minutes for the tested strain. IA clearly objectified the morphological changes in the treated yeasts compared with the untreated ones (Figs. 1 and 2).

Conclusion: Our research has successfully demonstrated the fungicidal effect of PAW against *C. auris*, opening a new field of research in the area of disinfectants.

1. Valentin Nastasa, Aurelian-Sorin Pasca, Razvan-Nicolae Malancus, Andra-Cristina Bostanaru, Luminita-Iuliana Ailincai, Elena-Laura Ursu, Ana-Lavinia Vasiliu, Bogdan Minea, Eugen Hnatiuc, Mihai Mares, [2021] - Toxicity Assessment of Long-Term Exposure to Non-Thermal Plasma Activated Water in Mice, Int. J. Mol. Sci., 22(21), 11 534; https://doi.org/10.3390/ijms222111534.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