P012

Antibiofilm activity of staphylococcal peptide derivative(s) against *Candida auris* biofilms *in vitro* and in an animal model of catheter-associated infection

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Objectives: *Candida auris* has emerged as a major multidrug-resistant nosocomial pathogen worldwide. The organism exhibits a persistent, colonizing phenotype, usually associated with biofilm formation on hospital surfaces, medical equipment, and indwelling medical devices. Biofilm formation by *C. auris* can further aggravate the infection acquisition and outcome, owing to the intrinsic resistance of biofilms to disinfectants, antiseptics, and antifungal drugs. The present study aimed to evaluate the preventive and therapeutic efficacy of select peptide derivative(s) from staphylococci against *C. auris* biofilms *in vitro* and in a mouse model of *C. auris* catheter-associated infection.

Methods: Three potentially antimicrobial, staphylococcal alpha-helical amphipathic peptides (19-23 amino acids) were evaluated for antifungal and antibiofilm activity against clinical isolates of *C. auris*. The antifungal activity against *C. auris* planktonic cells was tested by broth microdilution assay according to the method of the Clinical and Laboratory Standards Institute. Biofilm assays were performed in 96 well, flat-bottomed microtiter plates in RPMI-1640, and the effect of the test agents on biofilm formation (MBPC, minimum biofilm preventive concentration) as well as pre-formed biofilms (MBIC, minimum biofilm inhibitory concentration) was determined by subjecting to a two-fold dilution range of the test agents (0.03-64 μM) for 24 h at 37°C, followed by XTT dye reduction test. Field-Emission Scanning Electron Microscopy was performed to observe the effect of peptides on biofilm morphology. The cytotoxicity of these compounds was elucidated on HeLa, HEK-293, and Raw 264.7 murine macrophages by MTT reduction test. The synergistic effect of the selected peptide with representative antifungal drugs belonging to three different classes (amphotericin B, caspofungin, and voriconazole) was tested by fractional inhibitory concentration assays. *In vivo* activity of the selected peptide was determined in a murine model of subcutaneous *C. auris* catheter-associated infection, alone and in combination with amphotericin B and caspofungin.

Results: Based on the antimicrobial activity, antibiofilm activity, and cytotoxicity data, the 19 amino acid, alpha helical staphylococcal peptide derivative (charge, +3; hydrophobicity, 0.634, and hydrophobic moment, 0.623) exhibited promising activity against *C. auris* biofilms. The peptide was particularly effective in preventing *C. auris* biofilm formation, with a median MBPC50 of 1 μM. It demonstrated synergistic activity with amphotericin B (FIC index, 0.3) as well as caspofungin (FIC index, 0.18), and an additive effect with voriconazole (FIC index, 0.71). When combined with 0.125 μM of the peptide derivative, nearly 4-to-8-fold lower amount of the drug was required to achieve results comparable to the drug-only controls. Furthermore, nearly 99% reduction in biofilm formation was noted at clinically-achievable trough levels of amphotericin B or caspofungin in combination with this peptide derivative. Similar results were noted in vivo, with nearly 99% reduction in biofilm formation of *C. auris* in catheter lumen using combination therapy with 0.125 μM of the peptide.

Conclusion: The present study demonstrates that a 19 amino acid, alpha helical staphylococcal peptide derivative exhibits promising antibiofilm activity against *C. auris*, particularly in preventing biofilm formation, *in vitro* and in a murine model of subcutaneous catheter-associated infection.

P013

Green synthesis of silver nanoparticles using *Trillium govanianum* and its antifungal potential against *Candida auris*

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Objective: This study aimed to evaluate the antifungal and anti-biofilm activities of silver nanoparticles synthesized using *Trillium govanianum*, a trans-Himalayan medicinal plant extract, against multidrug-resistant *Candida auris*.

Methods: Silver nanoparticles (AgNPs) were synthesized by adopting a green approach. Optimization and characterization of TG-AgNPs were carried out using UV-VIS spectroscopy, TEM, and FTIR. Minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and antibiofilm activity of the crude extract, as well as TG-nanoparticles, were evaluated. Furthermore, their effect on cell morphology and cell permeability was also analyzed using FESEM.

Results: Crude extracts of roots of *T. govanianum* exerted remarkable antifungal activity against multiple strains of *Candida auris*. The incorporation of silver nanoparticles to plant extract significantly improved the antifungal and antibiofilm activities against the tested strains in a synergistic manner.

Conclusion: Silver nanoparticles synthesized using Trillium govanianum extract provide eco-friendly, biocompatible nanostructures with antifungal activity and have the potential for use as a therapeutic/nutraceutical agent.

P014

Prevalence and antifungal susceptibility of *Wickerhamomyces anomalus* in a tertiary care center

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Objectives: The spectrum of yeast species causing fungemia has been expanding with emergence of many unusual pathogenic species. One such species is *Wickerhamomyces anomalus* which has been recognized as an important cause of nosocomial fungemia in neonates and pediatric patients. We evaluated antifungal susceptibility and the burden of antifungal resistance in *W. anomalus*.

Methods: Species identification of the isolates was performed using MALDI-TOF MS. Antifungal susceptibility testing was done according to the CLSI broth microdilution method following the M27- A3 protocol. *C. parapsilosis* ATCC22019 and *C. krusei* ATCC6258 were used as quality control strains. The lowest azole or echinocandin concentration inhibiting 50% visible growth and for amphotericin B, 100% growth-inhibition was taken as minimum inhibitory concentration (MIC). As species-specific breakpoints for resistance are not available for *W. anomalus*, the susceptibility data was interpreted using CLSI breakpoints (M60 1st edition) as surrogate markers of resistance.

Results: A total of 633 *W. anomalous* isolated over a 5-year study period from January 2016, through December 2019 at our center, were evaluated for susceptibility to amphotericin B, fluconazole, voriconazole, caspofungin, anidulafungin, and micafungin. The majority of *W. anomalus* was isolated from neonates (57.1%) followed by other pediatric patients (40.1%). Antifungal therapy was administered in 21% of the patients with fluconazole as the commonest antifungal agent (67.3%) followed by amphotericin B, 31.2%, and voriconazole (1.4%). For amphotericin B, 99.6% (602/604) of the isolates exhibited MIC ≤ 2 mg/l. For fluconazole, 81% of the isolates were susceptible while 19% of the isolates exhibited reduced susceptibility of which 3% were resistant, and 16% exhibited susceptible dose-dependent phenotype. Of all the fluconazole-resistant isolates, two (0.3%) isolates were cross-resistant to voriconazole. Among echinocandins, the resistant rate of 2.8%, 1.2%, and 1.6% wase noted in caspofungin, anidulafungin, and micafungin, respectively.

Conclusions: *Wickerhamomyces anomalus* exhibited intrinsic susceptibility to all the antifungal agents evaluated. A substantial resistance to fluconazole was noted in this species while resistance to echinocandins was low.

P015

A study to demonstrate heteroresistance and tolerance to azoles in *Candida tropicalis*

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Objective: *Candida tropicalis* rarely exhibits a trailing growth phenomenon in *in vitro* antifungal susceptibility testing (AFST) of azoles. This phenomenon is considered as 'sensitive' as per current CLSI guidelines. This phenomenon has been linked to treatment failure and such isolates could either be heteroresistant (HR) or tolerant, which is known with bacteria. HR subtype (<1%) of an isogenic susceptible cell population grows at drug concentrations at least 8 times higher than the minimum inhibitory concentration (MIC). Tolerant cells (5%-90% of the population) endure antimicrobial treatment several times above MIC. Characterizing these isolates could help relate their clinical susceptibility profiles to the treatment outcome. The aim of the study was to differentiate heteroresistance and tolerance from true azole resistance in *C. tropicalis* clinical isolates.

Methods: A total of 247 clinical isolates of *C. tropicalis* collected over a period of 7 months (September 2021 through March 2022) from PGIMER, Chandigarh were included in the study. Identification was done by MALDI-TOF MS and antifungal susceptibility testing to fluconazole was performed by CLSI broth microdilution method (M27-A3). Isolates exhibiting trailing growth phenomena were considered for further characterization. Cells from the trailing growth of each isolate were sub-cultured onto yeast extract peptone dextrose (YPD) agar to observe for phenotypic variations with and without fluconazole. The area under the population analysis (PAP) curve (AUC) was performed to determine the degree of heteroresistance. Yeast cells within the range of 102-105 cells/ml were spot inoculated in six replicates each onto a gradient of fluconazole (0.125-256 μg/ml) in YPD agar and incubated at 35°C for 5 days to determine the viable colony forming units (CFU).

Results: Out of the 225 fluconazole susceptible *C. tropicalis* isolates, 10 (4.4%) were found to exhibit a trailing growth phenomenon. A dose-response relationship showed a multimodal population distribution in these isolates with varying degrees of heteroresistance as demonstrated by area under curve that ranged from 171-1331. The heterogeneous subpopulations sampled from the growth at the highest fluconazole concentration (64 μg/ml) exhibited a similar MIC (± one 2-fold difference) as that of the parent isolate when tested individually. The heterogeneity range that determines a fold difference breakpoint of the isolates varied from 16 to >256.

Conclusion: An isogenic population of cells under the effect of fluconazole could give rise to phenotypically different subpopulations. With repeated exposure to the azole drugs, these seemingly susceptible isolates can emerge as fully resistant population. Clinically, the implications include relapse, treatment failure, and persistent chronic infection, owing to which this phenomenon needs attention. Current CLSI guidelines do not provide any criteria to separately classify these isolates from the susceptible and resistant varieties. Hence a definitive cut-off is warranted to identify the HR and tolerant subtypes. The AUC-PAP method could be refined further for discriminating heteroresistance from true resistance.

P016

Selected trans-Himalayan medicinal plants and their nano-particles express potent antifungal and antibiofilm activity against *Candida auris* and *Candida glabarata*

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Normal microbiota turned opportunistic pathogen *Candida glabrata* and pathogenic *Candida auris* are becoming major health concerns globally. Antimicrobial resistance is growing gradually now, even the newer antibiotics alone are not able to completely irradicate the resistant microorganisms. This dire situation reflects the need to have an alternative regime, especially with a diverse range of applicability.

Nanoparticles synthesized from medicinal plants are emerging as potent antifungal agents to battle against fungal pathogens with unique antimicrobial properties. Secondary metabolites obtained from the herbs like alkaloids, carbohydrates, esters, flavonoids, glycosides, lignin, phenol, steroids, tannin, etc are robust therapeutic and immune-modulator compounds. The selected herbs (*Aconitum heterophyllum, Allium ursinum, Paris polyphylla, Podophyllum hexandrum, Rheum austral*, and *Selenium vaginatum*) in this study have a diversity of activity expression and had multiple medicinal usages traditionally.

Extract of medicinal plants was produced and used as a reducing and stabilizing agent for silver nanoparticles preparation. After the synthesis of nano-particles antimicrobial activity was determined qualitatively and quantitatively. Biofilm activity and metabolic activity of the fungal cells after treatment with extracts and their nanoparticles were determined by crystal violet and XTT assay. Nanoparticles were characterized using UV-Vis, FTIR, and TEM.

Extracts from the medicinal plants show potent antimicrobial activity, MIC ranging from 56 μg/ml to 625 μg/ml while their nanoparticles show activity in the range of 9.75 μg/ml to 80 μg/ml. The nanoparticles prepared were in the size range of 5-70 nM. Nanoparticles inhibited biofilm formation till MIC/64 with P <.001 and significantly (P <.01) eradiated the 24 h established biofilm at 64*MIC till 16*MIC.

Uses of nanoparticles in the microbiological application are widely reported and have shown robust activity against almost all the organisms of the microbial world. Antifungal use in developing countries is uncontrolled and unregulated hence resistance to antimicrobial agents is increasing. These factors along with many others elevate the chances of nosocomial infections and globally *C. glabrata* accounts for 8%-10% of nosocomial candidiasis. Nanoparticles are more effective agents to fill the void created by the chemical-based drugs that are getting least effective in the treatment procedure as the drug resistance pattern in *Candida* is increasing dramatically. Plant-based reductants are good stabilizing agents also; they are environment friendly, non-hazardous, and less toxic. Less energy consumption and moderate condition preparation make green synthesis of nanoparticles most desirable process to proceed. Adding to this, if reductant has antimicrobial activity, it can enhance the potency of prepared nanoparticles.

P017

Echinocandin resistance mechanism in *Candida tropicalis* and *Candida glabrata*

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Objective: *Candida tropicalis* and Candida *glabrata* account for 41.6% and 7.08% of total *Candida*emia cases in India. Echinocandins are the first-line treatment option for these infections. Resistance to Echinocandins is rare with *Candida* sp. However, in recent years, has been noted across many centers. We determined the mechanism of echinocandin resistance in *C. tropicalis* and *C. glabrata*.

Methods: *C. tropicalis* and *C. glabrata* isolated from *Candida*emia patients over a period of 3 years (August 2016-July 2019), identified by MALDI-TOF-MS were used in this study. Antifungal susceptibility testing was done following CLSI broth microdilution reference method (M 27A). FKS1 gene was sequenced using species-specific primers for the presence of any mutation. To determine any changes in the cell wall chitin and glucan contents, expression fold changes of chitin synthase (CHS1, CHS2, and CHS3), and glucan synthase genes upon caspofungin treatment were determined using real-time qPCR. These findings were correlated with cell wall chitin and glucan content determined by flowcytometry.

Results: A total of 3558 *Candida* species were isolated from patients of all age groups at our hospital. *C. tropicalis* was the predominant agent (34%), while the prevalence of *C. glabrata* was 6%. A total of 17 (8.5%) *C. glabrata* and 3 (0.25%) *C. tropicalis* exhibited reduced susceptibility to echinocandins. All these isolates carried a wild-type FKS genes.

In *C. tropicalis*, inducible expression of Chs1, Chs2 and Chs3 genes were comparable among susceptible and resistant isolates1.8 (0.4-2.8) vs. 2.5 (0.9-6.6), P = .247]; [0.7 (0.3-1.8) vs. 0.7 (0.2-1.6), P = .793]; [1.3 (0.14-4.8) vs. 1.1(0.48-1.7), P = .522], respectively. In concordance with gene expression, there was no significant difference in cell wall chitin contents among resistant and susceptible [14.37 (6.5-24.8) vs 16.28 (6.0-24.7), P = .114] *C. tropicalis* isolates. In contrast in resistant isolates of *C. glabrata*, caspofungin treatment resulted in significantly higher induction of chitin synthase genes compared to susceptible isolates; Chs1 [2.34 (0.24-9.71) vs. 1.56 (0.55-4.5) (P = .007)], CHS2 [1.59 (0.33-8.0) vs. (2.3 (0.69-6.15), P = .0006], and CHS3 gene [3.8 (0.13-12.73) vs. 1.9 (0.56-7.16), P <.0001]. Flowcytometric data in terms of chitin content, correlated well with expression changes as staining index was significantly higher in resistant compared to susceptible isolates [320 (198-535) vs. 164 (5.34-254.10) P = .0001]. Glucan synthase expression was comparable in susceptible and resistant isolates of *C. tropicalis* [3.47 (1.57-7.63) vs. 4.41 (0.41-17.51), (P = .518)]. However, glucan synthase gene expression was found significantly increased in resistant *C. glabrata* isolates compared to susceptible isolates; 3.10 (1.02-16.45) vs. 1.61 (0.13-7.67), P <.001.

Conclusion: We evaluated the role of cell wall components in echinocandin resistance in isolates with reduced susceptibility to echinocandins but lacking an FKS1 mutation. While chitin was induced at higher levels in *C. glabrata*, a similar finding was not observed in *C. tropicalis*. This warrants further studies to elucidate the role of fungal cell wall polymers in resistance.