PO30

Synthetic antifungal peptide mimics kills Candida albicans by targeting protein glycosylation and subsequently prevents infection

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Poster Presentation

Objectives: The main aim of this study was to investigate the antifungal activity of small synthetic peptides against the yeast Candida albicans. These peptides were designed to target the protein glycosylation machinery of C. albicans.

Methods: A set of 513 C. albicans clinical isolates (61 flu-susceptible and 250 flu-resistant) were screened for susceptibility to three small synthetic peptides. Screening was performed using a microdilution assay.

Results: Of the 513 isolates, 505 (98.2%) were flu-susceptible and 8 (1.6%) were flu-resistant. The flu-susceptible isolates were further screened using a fluorescence-activated cell sorter to determine the percentage of fungal cell killing. Scanning electron microscopy (SEM) images were taken to check the cellular association under increasing fluD2 exposure.

Results: At a total of 1950, 9881 fluD2 susceptible isolates were highly re-resistant to oxidative stress (30-80 molar while 94.5% (27429/29) fluD2 resistant isolates showed lower tolerance to oxidative stress (8-25 molar). H2O2 (p = 0.012) and CeA (p = 0.01) transcript levels were highly increased in fluD2 susceptible isolates when exposed to 10 nM fluD2 while no significant difference was observed in fluD2 resistant isolates. The flu-susceptible isolates exhibited a higher level of catalase (p = 0.02) compared with resistant isolates under 10 nM fluD2 exposure. Owing to the induced catalase activity, a higher AOS level was maintained in resistant isolates.

Conclusion: These results revealed a potential role for catalase in fluD2 resistance. Increase in catalase activity under fluD2 stress led to an increased AOS level which reduced the fluD2 susceptibility in C. albicans.

PO31

Resilience of Aspergillusflavus clinical isolates and associated fitness cost

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Poster Presentation

Objectives: Aspergillus flavus and closely related species could be pathogens for humans, animals, and plants and could also be used as model fungi. The aim of this study is to determine the fitness cost of antifungal resistance of A. flavus isolates to fluconazole.

Methods: A total of 120 isolates phenotypically identified as A. flavus were included in the study. These clinical isolates were collected over a 5-year period (2016-2020). For all isolates, specific identification was confirmed by sequencing a part of the MKE1 gene.

Results: Of these 120 isolates, 33 were flu resistant and 87 were flu sensitive. The flu resistant isolates were further phenotypically classified into three groups based on their biofilm production and their ability to produce spores.

Conclusion: Most of the flu resistant isolates are classified as species with reduced oxidative stress tolerance. The fitness cost of flu resistant isolates is significant, as they show decreased growth and reduced oxidative stress tolerance compared to flu sensitive isolates.

PO32

FP04

Neglected risk for invasive candidiasis: a study of distribution, species differentiation and antifungal susceptibility pattern of Candida species among patients with liver disease

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Poster Presentation

Objectives: Patients with liver disease (LD) are more predispaced to candidiasis due to the dysfunctional Kupfer cells which fail to capture the circulating yeasts, thereby causing fungal dissemination. Candidiasis is ten times more common among end-stage liver disease patients compared with other liver patients. Compared to those with alcohol-related candidiasis, ICU admission and mortality are found to be higher in liver transplant recipients (LTB) with candidiasis. Though a majority of Candida infection among LD patients are due to C. albicans, there is an increasing trend in C. rugosa, C. parapsilosis and C. tropicalis.

Methods: This was a prospective observational study conducted in the Department of Microbiology at All India Institute of Medical Sciences, New Delhi, India. The study was approved by the Institutional Ethics Committee.

Results: A total of 118 LD patients with candidiasis were admitted to LITB between May 2017 to December 2020. Detailed data related to these patients were collected. Clinical details of these patients were collected from the hospital information system. Patients with LD were divided into four groups-acute liver failure (ALF), chronic liver failure (CLF), chronic liver disease (CLD) and post-bleed liver disease (PBLD) group. Candida speciation as identified by VITEK 2 (bioMérieux) and their antifungal susceptibility pattern by broth microdilution.

Conclusion: According to the results, the liver disease patients are more commonly affected by C. albicans, C. parapsilosis and C. tropicalis. There is an increased risk of antifungal resistance and azoles to lead to higher tolerance. Hence antifungal susceptibility testing is essential in patients with these neglected risk factors to prevent mortality.

PO33

FP05

Competitive fitness and trade-offs associated with azole resistance in Candida albicans

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Poster Presentation

Objectives: Following the global emergence of Candida auris, this multi-drug-resistant yeast has become a concern of serious threat to public health. The evolution of such multi-drug-resistant pathogens depends on their relative fitness to their susceptible counterparts. Fitness costs are expressed in terms of reduced competitive ability of real-world absence of drugs, plays a key role in the drug resistance dynamics. The objectives of the study were to investigate the oxidative stress response (ORS) in C. albicans ATCC 90028 and C. auris ATCC 13801 with fluconazole and their flu-susceptible counterparts.

Methods: A total of 513 C. albicans clinical isolates (61 flu-susceptible and 250 flu-resistant) were screened for stress tolerance to fluconazole (2 μg/mL) by standard MIC test with several concentration range from 0.001 to 10 μg/mL. The percentage of fungal cell killing. Scanning electron microscopy (SEM) imaging were taken to check the cellular association under increasing fluD2 exposure.

Results: Of the 513 isolates, 505 (98.2%) were flu-susceptible and 8 (1.6%) were flu-resistant. The flu-susceptible isolates were further screened using a fluorescence-activated cell sorter to determine the percentage of fungal cell killing. Scanning electron microscopy (SEM) images were taken to check the cellular association under increasing fluD2 exposure.

PO34

FP06

Respiratory Aspergillus flavus clinical isolates and associated fitness cost

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Poster Presentation

Objectives: The black yeasts Aspergillus flavus is an opportunist pathogen that causes polysporon onychomycosis in both immunocompetent individuals and immunocompromised patients, resulting in localised cutaneous and subcutaneous infections to more severe systemic forms such as necrotising infections. Besides, A. flavus dermatitis was frequently found as a colonizer of pulmonary or renal tissue in patients, which appears to be associated with more advanced disease. Infections of A. dermatitidis are often chronic and recalcitrant. Previously, we have demonstrated that pyrimidine pneumonia (PA) and chronic sinusite with azoles against A. dermatitidis in vitro, which was confirmed in vivo in Galliera mellonella model.

Methods: Treatment of pyrimidine pneumonia resulted in significant growth restriction of A. dermatitidis, reduction of fungal burden, and significantly (p < 0.01) the effect of oxidative stress and G. H. However, the underlying mechanism and possible target of PA are still unknown. The aim of this study is to investigate the role of biofilm formation and cell cycle progression in the effect of PA against A. dermatitidis.

Conclusion: The glucose starvation caused a significant alteration in cell cycle progression and cell cycle progression. The PA treatment significantly reduced the growth rate and a lower virulence. A. dermatitidis and A. flavus are commonly associated with a fitness cost including a lower growth rate and a lower virulence.
Table 1. Primers used in this study.

| Primer name | Sequence (5’ → 3’) |
|-------------|-------------------|
| ExfrF       | ATCGATGACCTGTCGTGTC |
| ExfrR       | GATCTTCGGCAGTCCCGTCAGC |
| ExfrD       | GTGGTTCTCAATCGGCTAGCA |
| ExfrSr      | TCTGGACGGCAGTACCGGAC |
| ExfrF       | GTCCTACCTCTTCCTACGGCTTC |
| hphF        | CTCGGGAGTTGAGAAATGAGC |
| hphR        | CATACCACGCACCTCAGACG |

PS08
Molecular mechanisms associated with fluconazole resistance and genetic diversity in clinical Candida krusei isolates from North India

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

Objectives: Candida krusei accounts for 2.8% of invasive candidiasis worldwide. Fluconazole resistance and its underlying mechanisms in clinical isolates of C. krusei (n = 137) collected from eight hospitals in India were investigated. Also, genetic diversity of C. krusei strains among different hospitals was studied through short tandem repeat (STR) genotyping.

Material and Method: All the isolates were identified by MALDI-TOF MS. Antifungal susceptibility test was done by using broth microdilution method (CLSI M27). To evaluate the genetic relatedness among the strains, STR typing was done by using 9 STR markers. To understand the fluconazole-resistant mechanisms in C. krusei, known fluconazole resistance mechanisms such as alterations in target enzymes ERG11 and drug transporters ABC1 and ABC2 were investigated in 35 C. krusei isolates (14 fluconazole-susceptible [FLU-S], and 17 fluconazole-susceptible drug-dependent (FLU-SSD)). Furthermore, transcriptomics of one FLU-SSD (MIC-32 mg/l) and one FLU-S (MIC-4 mg/l) isolate was performed.

Results: Majority (77%) of C. krusei isolates were from Hospital-acquired infections. Notably, 70% of candidemia cases occurred in neonatal intensive care units (NICUs). Remarkably, 81% (n = 110) were detected as fluconazole-SSD (MIC-16-32 mg/l) and the remaining 19% were FLU-S (MIC ≥ 8 mg/l). Method genetic diversity with 51 diverse STR types was noticed among the 106 isolates. Importantly, two ongoing candidemia outbreaks were observed in two geographically separated hospitals both representing NICU isolates. In addition, a large cluster containing isolates from six different hospitals was observed. ERG11 mutation analysis revealed that it did not harbor any mutations contributing to the fluuczolation. Overexpression of the ABC1 gene in 11 FLU-SSD isolates out of 17 as compared to FLU-S isolates was noted. However, no alteration was observed in the expression of ERG11 and ABC2 in both groups.

Transcriptomics analysis revealed a significant number of differentially regulated genes were distributed in various geneontology terms including transport (16 genes), mRNA-activated protein kinases (MAPK) signaling (8 genes), NOS5, PTSEP, STE50, ERL1, OPY2, STE3, SKN7, and RLM1, reported biogenesis (3 genes, ERG24, ERG25, and ERG26) and transcription factors (7 genes). In addition to the up-regulation of exported pathway genes, overexpression of key transcriptional regulator of exported biogenesis genes UTR2 was observed in FLU-SSD isolates as compared with susceptible. Additionally, FLU-SSD isolates showed 2-fold increased expression of PDR12, plasma membrane ATP-binding cassette (ABC) transporter, NCL1 (locus of krusei), a major glycerol-synthesizing enzyme was found to be 5-fold downregulated in FLU-SSD isolate compared to susceptible. The loss of ICJ1 alters the expression of the FKS1, ERG11, and CDC2 genes in C. albicans. Taken together, the increased expression of PDR12 and altered MAPK signaling network may partially account for the fluuczolation in C. krusei FLU-SSD isolates.

Conclusion: Candida krusei isolates among different hospitals showed large genetic diversity (54 different genotypes). Also, the presence of C. krusei clonal strains in six different hospitals suggest possible introduction from a widespread environmental source and human-to-human transmission. In comparison to other Candida species, the resistance mechanism in C. krusei seems to be more complex. Therefore, an in-depth study of other resistance mechanism pathways in C. krusei is further warranted.

PS09
Investigation of in vitro antifungal susceptibility testing and genetic diversity of clinical isolates of Trichohyphon benhamiae and Trichohyphon eriotrephon in Iran

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Background: Trichohyphon benhamiae is a zoophilic dermatomycosis, known as one of the causative agents of dermatomycoses.

Objectives: The purpose of this study was to explore the genotypes of T. benhamiae strains isolated from geographically different areas of Iran and also to evaluate in vitro antifungal susceptibility profile of these strains against seven antifungal drugs.

Methods: A total of 22 strains of T. benhamiae and 2 strains of T. eriotrephon were isolated from patients with distinct types of dermatomycosis. DNA extraction and amplification of DNA regions of ITS1 and ITS4 primers were conducted on the isolates. The in vitro antifungal susceptibility of posaconazole (PS), voriconazole (VRC), itraconazole (ITC), ketocanazole (KET), econazole (ECON), griseofulvin (GRZ) and terbinafine (TRB) was evaluated according to CLSI M38-A2 protocol.

Results: The multiple alignments of the ITS-rDNA sequences of T. benhamiae indicated a mean similarity of 99.5%, with 0.5 inter-species nucleotide differences. The geometric mean (GM) values of minimum inhibitory concentrations (MICs) and minimum effective concentrations (MECs) across the all isolates were respectively: TRB: 0.025 mg/l, VRC: 0.032 mg/l, ITC: 0.035 mg/l, and GRZ: 0.078 mg/l with lower values and CAS: 0.11 mg/l, ETF: 0.66 mg/l, and GMZ: 0.78 mg/l with higher values.

Conclusion: Diverse ITS sequences types of T. benhamiae were shown in different geographical regions of Iran. The TRB, VRC, and ITC were the most effective drugs against T. benhamiae strains, respectively. Furthermore, in our study, two strains of T. eriotrephon as a zoophilic dermatomycosis species were described.