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Session: Mycology, Fungal Infections and Antifungal Drugs

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Aspergillus flavus culture filtrate inhibits candidal biofilm development in-vitro

S. Bhattacharyya*, G. Banerjee, A. Jain, P. Gupta, M. Singh

CSMMU, Lucknow, Uttar Pradesh, India

Background: Invasive candidiasis, a major health concern in developing countries, has an attributable mortality of 15-40%. It is associated with biofilm formation over indwelling devices. Our study was aimed at finding low-cost alternatives to inhibit this biofilm development in-vitro.

Methods: One loopful of Aspergillus flavus clinical isolates were cultured in YPD(Yeast Extract-Peptone-Dextrose) broth for 1 week. Culture filtrate was prepared by filtering it through membrane filter of pore size 0.45 µm. Candia albicans and C. tropicalis clinical isolates were cultured in YPD broth at 37 °C for 48 hours. Dilutions of yeast cell suspensions were prepared in YPD broth as well as Aspergillus culture filtrate, and yeast cell density was adjusted to 106 cells/ml in both cases. Then 100 µl of each was poured in wells of a flat-bottomed microtitre plate. The plate was incubated at 37 °C and washed at intervals of 90 minutes, 24 hours and 48 hours with sterile Phosphate Buffered Saline (pH 7.2). Each time after washing, wells were reloaded with respective cell suspensions. Then the optical density(O.D.) was measured in a spectrophotometer at 450 nm wavelength after staining the wells with 1% Safranine in 95% ethanol. The plate was also observed under Inverted microscope to see biofilm development. Tests were done in triplicate.

Results: Aspergillus filtrate significantly reduced the biofilm formation in *C. albicans* and *C. tropicalis*, as observed microscopically and spectrophotometrically. Mean O.D.value of *Candida* spp. in YPD broth was 0.795 0.6 and that of filtrate containing *Candida* spp. suspension was 0.576 ± 0.1 (p value: <0.05).

Conclusion: Culture filtrate of *Aspergillus flavus* disrupts Candidal biofilm development in-vitro.

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Genetic relatedness of *Candida albicans* clinical isolates from Candida bloodstream infections in a Malaysian population

S.S. Chhabra 1,*, P.P. Chong 2, C.S.Y. Lim 1

¹ UCSI University, Cheras, Kuala Lumpur, Malaysia

Background: Candida is an opportunistic eukaryotic diploid fungus, which can cause bloodstream infections (BSIs) in immunocompromised or immunodeficient persons. With candidemia representing the most common BSI, its frequency is increasing

investigate the genetic relatedness of the most prevalent *Candida* species in Candida BSIs in a Malaysian population via Randomly Amplified Polymorphic DNA (RAPD)-PCR fingerprinting.

Methods: Forty-four Candida BSI blood cultures were originally obtained from University Malaya Medical Centre (UMMC). The blood cultures were then cultured and maintained on Sabouraud's Dextrose agar (SDA), after which genomic DNA was extractd from single colonies. Polymerase chain reaction (PCR) and gel electrophoresis was then carried out on the isolated DNA using *ITS1* and *ITS4* pan-fungal primers to amplify the fungal internal spacer conserved regions. CHROMagarTM Candida was used culture clinical isolates to confirm their identity. These two methods were combined to conclude the predominant causative species of Candida in these BSIs. Clinical isolates of the predominant Candida species were then genotyped via RAPD-PCR fingerprinting using *PST*, an arbitrary oligonucleotide primer. Computer-assisted clustering analysis and construction of the phylogenetic tree was carried out to analyze the genetic relatedness of these clinical isolates

Results: The consolidated results of the molecular and biochemical methods of *Candida* spp identification showed that *C. albicans* is the most prevalent species (31.81%), with fourteen clinical isolates out of forty-four. RAPD-PCR fingerprinting resulted in eight distinctive polymorphic bands. The average SAB value of the fourteen *C. albicans* clinical isolates was 0.733 ± 0.172 , hence describing the overall non-relatedness of these isolates. When analyzing the results individually, only five of the fourteen were observed to be similar, with SAB values of 1.00 each. Four isolates had SAB values of 0.80-0.99, which suggests that microevolution might have occurred and that these clinical isolates possibly belong to different strains. The remaining five isolates were unrelated (SAB of <0.80).

Conclusion: The observations from this study may provide useful insights into the *Candida albicans* population structure and epidemiology in Malaysian Candida BSIs, as well as its mode of propagation.

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A rare case of gastrointestinal mucomycosis following *Plasmodium knowlesi* infection

T. Chinniah*, K. Prabu, A. Jalihal

RIPAS Hospital, Bandar Seri Begawan, Brunei Darussalam

Background: A 59 years non-diabetic, known hepatitis B, HIV-negative male presented with tiredness, unwell, loss of appetite and coffee ground vomitus. He had tachycardia, central cyanosis with 64% SPO2 on 8 litre of oxygen. With laboratory investigations, revealing thromocytopenia, leucocytosis, impaired renal chemistry and blood gas analysis, diagnosis of sepsis with metabolic acidosis was made and was admitted to ICU, suspecting melioidosis starting on co-amoxyclav and ceftazidime continued for 12 days.

Results: Septic screen and blood cultures done reported negative, but following day, blood picture revealed *Plasmodium knowlesi* infection. Quinine IV and doxycycline were started and continued

² University Putra Malaysia, Selangor, Selangor, Malaysia