Genotyping of clinical and environmental *Aspergillus flavus* isolates using *AflaSTR* assay and antifungal susceptibility patterns

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Introduction: *Aspergillus flavus* is the second leading cause of fungal sinusitis, cutaneous infections, and endophthalmitis in tropical countries. Despite an increasing number of infections of *A. flavus* in Iran, the antifungal susceptibility patterns and molecular epidemiology of clinical and environmental strains has not been well studied. In the current study, we evaluated the in vitro antifungal susceptibilities of five antifungal agents and applying microsatellite typing in order to investigate the epidemiology of clinical and environmental *A. flavus* isolates from Iran.

Materials and methods: *A. flavus* strains were preliminarily identified to the species level based on microscopic and macroscopic characteristics; subsequently, their identity was confirmed by DNA sequencing of the partial β-tubulin gene. Antifungal susceptibilities of AMB, ITC, VRC, POS, and CAS against *A. flavus* isolates were determined in accordance with the CLSI M38-A2 document. The *A. flavus i* solates were genotyped by using microsatellite typing (*AflaSTR* assay). The genetic relatedness between the *A. flavus* isolates was investigated by comparing the profiles with BioNumerics v6.6 software. The discriminatory power of the microsatellite markers was calculated using Simpson's index of diversity (D).

Results: One hundred and ninety nine clinical (n = 171) and environmental (n= 28) *A. flavus* isolates were evaluated for their antifungal susceptibilities paterns. Caspofungin (MIC90=0.063 µg/mL), followed by posaconazole (MIC90=0.25 µg/mL), exhibited the lowest minimum inhibitory concentrations (MIC). All isolates had caspofungin MEC90 lower than the epidemiologic cutoff values, and 3.5% of the isolates had amphotericin B MIC higher than the epidemiologic cutoff values. Microsatellite typing of *A. flavus* isolates demonstrated 118 different genotypes. A possible outbreak at a pulmonary ward was discovered. The discriminatory power for the individual markers ranged from 0.4812 to 0.9457.

Conclusion: Our results suggest that Iranian *A. flavus* isolates are the most susceptible to posaconazole and caspofungin, followed by voriconazole, itraconazole, and amphotericin B. Microsatellite typing provided excellent discriminatory power in studying the molecular epidemiology of clinical and environmental *A. flavus* isolates in Iran.

Keywords: Antifungal susceptibility, *Aspergillus flavus*, Microsatellite strain typing, AflaSTR assay