

# Oral Presentation Abstracts

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**O-01****Investigation of pulmonary fungal infections in immunocompromised patients referring to special lung clinic of Amir-Al-Momenin hospital, Arak University of Medical Sciences**

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**Introduction:** With increasing immunocompromised patients, fungal infections especially lung infection, have also increased. In this study, fungal contamination of respiratory system in immunocompromised patients referred to specialized lung clinic of Amir-Al-Momenin hospital in Arak city was evaluated.

**Materials and Methods:** This descriptive cross-sectional study was conducted from April 2017 to Jun 2018 on immunocompromised patients suspicious of pulmonary infections referring to Amir-Al-Momenin hospital in Arak city. Of these patients, 64 patients, including 35 women and 29 men, were selected. A bronchoalveolar lavage (BAL) sample was prepared by the physician from these patients and was immediately sent to medical mycology Laboratory of medicine school. Bronchoalveolar lavage specimens were investigated by gomori methenamine silver stains (GMS) staining and culture method. Data were analyzed and analyzed by SPSS software version 16.

**Results:** Of 64 patients, 9(14%) of patients were infected with pulmonary fungal infections. Among the patients infected with fungal infection, 9 patients (100%) were positive in the culture examine and 8(72%) by staining GMS. Among infected people, 7(77.8%) were female and 2(22.2%) were male. The most common isolated fungi were *Candida albicans*, *Aspergillus fumigatus* and *Mucor* spp. (2 cases). There was no significant relationship between fungal contamination with sex, age, occupation, marriage and type of disease.

**Conclusion:** The results of this study showed that immunocompromised patients are prone to fungal infections, especially candidiasis and aspergillosis. Therefore, the use of control methods to reduce the probability of such patients to fungal infections should be considered.

**Keywords:** pulmonary fungal infection, immunocompromised patients, Arak

**O-02****Oropharyngeal candidiasis in patient with head and neck cancer undergoing radiotherapy**

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**Introduction:** oropharyngeal candidiasis (OPC) caused by disturbing the microbial balance and minor changes in host defense system. The risk factors for development of OPC in patients with head and neck cancer

undergoing radiotherapy include local mucosal damage due to anticancer treatment and reduced salivation. Reduce saliva secretion or hyposalivation by itself increases the risk of *Candida* colonization and infection as much as using denture. OPC is relatively common in patients receiving radiation therapy for head and neck cancer which occurs in approximately 25% of patients. Oral candidiasis, as an inflammatory complication, occurs during the course of radiotherapy in one patient out of three patients at increased risk. In patients with locally or systemic impairment, OPC with a painful manifestation, affects the quality of life and can even spread to the esophagus or cause systemic infections.

**Materials and Methods:** In a cross-sectional study, 34 patients with head and neck cancer receiving radiotherapy (4000-7000 cGy) with suspicion for OPC were enrolled in Shahid Rajaie Babolsar Radiotherapy Center, Babolsar, Iran. The sample from oropharyngeal lesions were collected using a sterile swab and investigated by wet mount and culture. The yeast isolates were identified using conventional (colony color in CHROMagar *Candida* and germ tube formation), and then confirmed by PCR-RFLP method at the species level.

**Results:** In the present study, among 34 study patients under radiotherapy, 22(64.7%) patients had oral candidiasis and 7(20.5%) patients were diagnosed as a *Candida* colonization. There was a significant association between OPC and radiotherapy (P value= 0.019). The most common causative agent was *Candida albicans* 18(62%), followed by *Candida glabrata* 5(17%), *Candida tropicalis* 2(7%), *Candida parapsilosis* 2(7%), *Candida krusei* 1(3%), *Candida kefyr* 1(3%). The most clinical features were patches of pseudomembranous white slough with or without erythematous (atrophic form) lesions.

**Conclusion:** Radiotherapy increased the risk of OPC and *Candida* colonization with a trend towards the isolation of non-*albicans* *Candida* species. Accurate identification of causative agents can play an important role in choosing the right drug that leads to pain relief and improve the quality of life in irradiated patients.

**Keywords:** oropharyngeal candidiasis, head and neck cancer, *Candida* species, radiotherapy

### O-03

#### Evaluation of nasal lavage fluid for diagnosing invasive fungal infections in leukemic patients

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**Introduction:** One of the important problems in treating patients with acute leukemia after intensive chemotherapy is invasive fungal infections, especially invasive Sinopulmonary aspergillosis. Early diagnosis and timely treatment of these invasive fungal infections is one of the major challenges in managing these patients. In our study we would like to assess that we can use fluid of nasal lavage for detecting galactomannan or fungal elements for early detection of invasive fungal infections in leukemic patients.

**Materials and Methods:** During, 2017-2018 the leukemic patients who were admitted to hematology ward of Imam Khomeini Hospital of Tehran, and who had been suspected for invasive fungal infections after receiving chemotherapy were selected and following explanation of methods and objectives and obtaining informed consent from patients; each nostril was washed with 5ml saline using the 5ml syringe and collected in sterile receivers and sent to mycology laboratory for detecting galactomannan level and fungal smear and culture.

**Results:** Of the 32 patients with leukemia and probable or proven fungal infection with nasal discharge galactomannan cut off of 0.5, 12 patients (37.5%) had galactomannan higher than 0.5 and 20 patients had lower than 0.5, positive fungal culture were obtained by sinus endoscopy from 6 patients of 12 patients who had galactomannan higher than 0.5.

**Conclusion:** Besides the other methods, it may also be possible to use this safe diagnostic method for leukemic patients suspected of invasive Sinopulmonary aspergillosis as one of the indices of preemptive therapy.

**Keywords:** Galactomannan, invasive sinopulmonary aspergillosis, fever and neutropenia.

#### O-04

### Evaluation levels of IFN- $\gamma$ , IL-4, IL-6, TGF- $\beta$ and IL-17 in HIV-addicts patients suffering from fungal infections in Kerman

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**Introduction:** The most common sources of morbidity and mortality among HIV-positive individuals at the late stages of the disease are opportunistic infections resulted from agents such as fungi that rarely infect immunocompetent individuals. Reduced TCD4+ cells, both directly and indirectly affect immunological functions that cause of more opportunistic fungal infection. Prevalence of drug abuse is increasing among HIV-infected individuals rapidly. The present study aimed to investigate the effects of HIV infection and addiction on immune function in patients with fungal infections.

**Materials and Methods:** This study was a cross sectional study. A total of 144 men ranged between 18-50 years old referring to Kerman Behavioral Disorders Center (Kerman, Iran) were participate in this study. The measurement of the plasma level of cytokines was done by enzyme-linked immunosorbent assay (ELISA) method. Statistical analyses were done by SPSS (ver. 20; SPSS Inc.).

**Results:** The most fungal infection was detected in 72 subjects were Candidiasis. HIV has been significant effect on serum levels of IL-4, IL-6, IL-17, IFN- $\gamma$  and TGF- $\beta$ . Fungal infections have a significant effect on serum levels of IL-6, IL-17, IFN- $\gamma$  and TGF- $\beta$ . Drug dependence has a significant effect on serum levels of IL-17, IFN- $\gamma$  and TGF- $\beta$ .

**Conclusion:** The findings of this study showed each of the fungal infection, HIV infection, drug addiction, alone and in interaction with each other, have significant effects on the immune system that interfere with cytokine network and response of the immune system.

**Keywords:** fungal infection, addiction, HIV, cytokine

#### O-05

**A 7-year study of onychomycosis from Isfahan, Iran**Rasoul Mohammadi<sup>1</sup>, Mostafa Chadeganipour<sup>2</sup><sup>1</sup>Department of Medical Parasitology and Mycology, School of Medicine, Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran<sup>2</sup>Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

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**Introduction:** Onychomycosis is a fungal nail infection caused by yeasts, dermatophytes, and some non-dermatophyte molds. It is the most common nail disease in adults, which is responsible for almost 50% of all nail disorders. Its incidence is estimated at more than 10% among the healthy population and 40% in the elderly individuals, maybe associated with lack of maintain good foot care, impaired immune system, and decreased growth of the nail plate throughout the life. It is the purpose of this study to describe the prevalence of onychomycosis, and the range of fungal species isolated from nail infections in Isfahan, Iran.

**Materials and Methods:** The study was performed between June 2007 and June 2014 in Isfahan, Iran. A total of 9,785 suspected cases (3,295 male and 6,490 female) referred to the Shefa Mycology Reference Laboratory, Center for Medical Mycology in Isfahan, Iran. Nail clippings were taken from fingernails or/and toenails and collected in sterile Petri dishes for direct microscopic examination with 15% potassium hydroxide and culture on sabouraud dextrose agar with chloramphenicol and cycloheximide. Some additional tests containing culture on Trichophyton agars, hair penetration test, urea hydrolysis, growth on rice grains, germ-tube test, chlamydoconidia production test using corn meal agar supplemented by 1%

Tween 80, CHROMagar Candida, API 20C AUX, czapek dox agar were used to confirm the fungal identification.

**Results:** One thousand two hundred eighty-four patients of 9,785 (13.1%) had positive direct examination; 527 male (238 fingernail, 289 toenail) and 757 female (473 fingernail, 284 toenail). A total of 671 of 1,284 patients (52.2%) claimed for culture. Twenty-three samples (3.4%) did not grow due to unknown factors. The disease period was from a week to 3 years. Age range of patients was between 1 and 82 years (mean age, 45 years). Housewives were the commonest infected population. Four hundred and sixty-seven patients (36.4%) had distal onychomycosis, 438 patients (34.1%) had proximal, and 379 patients (29.5%) had lateral onychomycosis. Nine patients (0.7%) had both fingernail and toenail onychomycosis, and the etiologic agents of fingernail and toenail in six of nine patients (66.6%) were same. Three hundred and thirty-one *Candida* spp. (51.1%), 174 dermatophyte spp. (26.8%), and 143 non-dermatophyte spp. (22%) were isolated from nail infection in this investigation. *Candida albicans* was the most prevalent species isolated from patients in this study (34.9%) followed by *Trichophyton interdigitale* (11.7%), *Aspergillus flavus* (9.1%), *Epidermophyton floccosum* (8.5%), *C. parapsilosis* (6.9%), and *T. rubrum* (5.8%).

**Conclusion:** The profile of the fungal nail infection is changing in different areas, so repeated studies on the prevalence of onychomycosis and the etiologic agents of the infection seems to be essential. The present study provides novel and appropriate epidemiologic data of onychomycosis, which can lead to the better prevention and treatment of this fungal infection.

**Keywords:** identification, causative agents, onychomycosis

O-06

### Antifungal susceptibility pattern of tavaborole against molds and *Candida* isolates originated from onychomycosis

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**Introduction:** Fungal nail infections are usual and recurrent problems caused often by different species of *Candida* and filamentous fungi. It is estimated that the prevalence rate of onychomycosis in the different countries ranges from 0.5% to 30%. Despite common antimycotic agents such as itraconazole and terbinafine as well as the introduction of novel generations of topical formulations, including luliconazole and efinaconazole, achieving of complete cure remains challenging worldwide. Tavaborole (5%), a boronic acid quinolone compound is a topical drug for the treatment of onychomycosis, targeting the leucyl-tRNA synthetase enzyme and preventing protein synthesis. There was no comprehensive *in vitro* antifungal susceptibility information of tavaborole against a panel of clinical fungi isolated from nail disorders. Therefore, we evaluated *in vitro* activity of this novel drug against different fungi.

**Materials and Methods:** A total of 170 clinical nail isolates were included in current study. Fifty-two *Candida* isolates, and 118

molds, including *Aspergillus*, *Fusarium* and *Trichophyton* species were recovered from patients suffering from fingernail (n=70) and toenail (n =100) infections. *In vitro* antifungal susceptibility testing for yeasts and molds were performed based on Clinical and Laboratory Standards Institute (CLSI) documents M27-A3 and M38-A2, respectively using tavaborole and 4 comparators including itraconazole, voriconazole, terbinafine and fluconazole.

**Results:** Tavaborole exhibited high minimum inhibitory concentration (MIC) values against yeasts and molds, compared to those of the other antifungal agents. Tavaborole showed high MICs for the most *Candida* isolates (MIC<sub>50</sub> and MIC<sub>90</sub>, 16µg/ml), whereas the MIC<sub>90</sub> values of voriconazole, itraconazole, and terbinafine were lowest for this genus, at 0.25, 4, and 4 µg/ml, respectively. For *Aspergillus* strains, all antifungal agents except fluconazole showed better activity than tavaborole. MIC<sub>50</sub> values of fluconazole and tavaborole were 64 and 2 µg /ml, respectively, whereas all *Aspergillus* strains demonstrated MIC<sub>50</sub> values of 1 µg /ml for the remaining drugs. Overall, MIC<sub>50</sub> and MIC<sub>90</sub> values of tavaborole (8 and 16 µg/ml) had low activities comparable to those of itraconazole (0.0625µg/ml) and terbinafine (0.004 and 0.008µg/ml) used for treatment of onychomycosis due to dermatophytes.

**Conclusion:** We found that the *in vitro* antifungal activity of tavaborole against a panel of different agents of onychomycosis is inferior to those of terbinafine and azoles, except for fluconazole.

**Keywords:** tavaborole, antifungal susceptibility test, onychomycosis

### O-07

**Genome sequencing reveals novel fungal agent with high frequency and different antifungal susceptibility in otomycosis patient**

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**Introduction:** Otomycosis is a superficial infection of the ear caused by a spectrum of various fungal agents in particular species under *Aspergillus* and *Candida* genera. Black aspergilli (section Nigri), particularly *Aspergillus niger* is the most prevailing causative agents of otomycosis. However, using morphological criteria alone, discrimination of species within section Nigri -A number of different species whose morphological features resemble those of *A. niger* cannot be reliably achieved. Due to different susceptibility patterns among species under section Nigri to antifungal agents and appropriate treatment, species delimitation of this section is issue of great importance. The aim of this study was to determine the frequency of otomycosis in Tehran, Iran, with emphasis on molecular identification and determination the susceptibility pattern of a set of black aspergilli isolated from otomycosis patients.

**Materials and Methods:** From Apr 2016 to Jan 2017 a set of 412 subjects with a suspicion of external otitis were included. Clinical examination and specimen collection was performed by an otorhinolaryngologist. Subsequently, direct examination and culture was performed on specimens and isolated molds were identified morphologically. Yeast isolates were identified using CHROMagar candida medium and PCR-RFLP of ribosomal DNA whenever needed. Black *Aspergillus* isolates from otomycosis patients were identified by using the PCR-sequencing of the *βtubulin*

gene. Furthermore, the susceptibility of black aspergilli isolates to three antifungal drugs, including fluconazole (FLU), clotrimazole (CLT), and nystatin (NS), were examined according to CLSI M38-A2. Fungal agents number of patients *A. niger* + *C. glabrata* 7 *A. niger* + *A. flavus* 4 *A. flavus* + *C. glabrata* 2 *A. niger* + *C. parapsilosis* 1 *A. flavus* + *C. albicans* 1 *A. flavus* + *C. tropicalis* 1 Total 16 The different patterns of mixed fungal otitis due to *Aspergillus* and *Candida* species observed among 117 patients with otomycosis The lowest minimum inhibitory concentration (MIC) values were observed for NS with geometric means (GM) of 4.65 µg/ml and 4.83 µg/ml against *A. tubingensis* and *A. niger* isolates, respectively. CLT showed wide MIC ranges and a statistically significant inter-species difference was observed between *A. tubingensis* and *A. niger* isolates (p64 µg/ml).

**Results:** A total of 117/412(28.4%) included patients were diagnosed with otomycosis including 64(54.7%) males and 53(45.3%) females. Patients were within the age range of 10-75 years and the highest prevalence was found in the age group of 46-55 years (30.77%). Pruritus (89.74%) and auditory manipulation/trauma (83.76%) were the predominant symptom and predisposing factor, respectively. From 117 patients 126 isolates were recovered, black aspergilli (n=43, 34.1%) were the most common etiologic agents and *Candida glabrata* (n=25, 20%) was the predominant isolated yeast. Furthermore, 16 cases of mixed otomycosis were identified and coinfection due to *A. niger* and *C. glabrata* (seven cases) were the predominant pattern. While, with sequence-based methods the majority of black aspergilli isolates were identified as *A. tubingensis* (32/43, 74.42%) followed by *A. niger* (11/43, 25.58%). The lowest MIC values were observed for NS with geometric means (GM) of 4.65 µg/ml and 4.83 µg/ml against *A. tubingensis* and *A. niger* isolates,

respectively. CLT showed wide MIC ranges and a statistically significant inter-species difference was observed between *A. tubingenensis* and *A. niger* isolates (p64 µg/ml). The MIC values of three antifungal drugs against *Aspergillus* section Nigri isolated from otomycosis patients.

**Conclusion:** Species other than *A. niger* can be more frequent as observed in our study. In addition, considering the low and variable activity of tested antifungal drugs, empirical treatment can result in treatment failure. Accurate identification and antifungal susceptibility testing of isolates is, however, recommended.

**Keywords:** *Aspergillus niger*, *Aspergillus tubingenensis*, Iran, section nigri, otomycosis, antifungal agents

## O-08

### Study of skin and nail *Trichosporon* species as a normal flora in different healthy age groups

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**Introduction:** The skin as the body's largest organ, hosts heterogeneous inhabitants. Until now, there is a little information about the skin fungal flora and as we know, *Trichosporon* is occasionally found as normal flora of skin.

**Materials and Methods:** In this study, differences in *Trichosporon* community structure associated with 9 different skin sites of 238 healthy people during 10 months from July to March 2016, are described. These subjects were divided by age into 4 groups

including infants, children, adults and geriatrics. The collected samples in this study were examined by culture on sabouraud chloramphenicol agar and sabouraud cycloheximide chloramphenicol agar. Also for precise identification of isolates in the species level, *ITS1-5* and *8S-ITS2* DNA regions were sequenced.

**Results:** Our results showed that the frequency of *Trichosporon* species was not significantly different between age groups. The most *Trichosporon* isolations were related to the adult age group and the fewest in the infants. *T.asahii* was the predominant isolated species in all age groups. This study showed statistically significant effect of the subject's sex on *Trichosporon* population resident on human skin surface.

**Conclusion:** Our study showed that the lowest prevalence of *Trichosporon* isolation is related to the infant age group, this finding may be a result of this fact that the cases were at the age of 4-15 days and the chance of skin colonization with *Trichosporon* during this time is less than other age groups. Fetal skin is sterile, but *Trichosporon* colonization occurs through the hands of health care workers, parents and the infant's contact with objects and equipment related to neonatal care. The highest prevalence of *Trichosporon* isolation recorded from the skin of adult individuals. The main reason for this is possibly due to high level of daily activities in this age group and their greater contact with source of pollution compared with another age groups. In our study the frequency of *Trichosporon* isolation was significantly greater in females compared to males. Physiological and anatomical differences between male and female and cutaneous environments such as sweat, sebum and hormone production, partially account for the microbial differences seen between the genders. Isolation of *T.asahii* as the commonest species in all sites of the skin in our study was noteworthy. The prognosis

of infection caused by this yeast is very poor, its mortality being approximately 70%. This is higher than that of candidiasis, with a mortality of 40%.

**Keywords:** cutaneous *Trichosporon* composition, different age groups, DNA-sequencing, microbial epidemiology, Iran.

### O-09

#### Review of prevalence and etiologic agents of tinea capitis among nigerian children from 1978-2018

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**Introduction:** Dermatophytosis constitutes a group of cutaneous fungal infections of the epidermis, hair and nails. Of particular concern is the large number of people affected worldwide. The burden is usually worrisome especially among children. Tinea capitis affects predominantly, but not exclusively, children and manifests as hair loss, which may be associated with varying degree of inflammation and heavy psychological impact.

**Materials and Methods:** In this review we searched international electronic database (PubMed, Web of Science and Embase) and Google Scholar for publications between 1978 and 2018, on tinea capitis among children in Nigeria. The keywords used were 'tinea capitis', 'tinea favosa', 'scalp ringworm', 'endotrix', 'ectotrix', 'scalp dermatomycosis' with Boolean operator 'OR' used, while 'children', 'Nigeria' with 'AND' was used to narrow the search results. In addition, local medical and dermatology journals database was also searched. The available publications were thereafter

reviewed and findings qualitatively described.

**Results:** Total of 53 publications was available, out of which 19 passed the screening filter and were analyzed and reviewed. Our finding revealed that the prevalence of tinea capitis among Nigerian children ranges from 8.7% to 87%, with lowest prevalence from the southeast and highest from the northwestern region. Children between 4-12yrs were the most affected with M: F = 1.75:1. For the last four decades, *Microsporum audouinii* remains the leading cause (33.63%) of tinea capitis followed by *Trichophyton soudanense* up to the end of the 3<sup>rd</sup> decade, thereafter is being replaced gradually by *T. mentagrophyte*.

**Conclusion:** As an anthropophilic fungus is the leading cause of dermatophytosis in Nigeria, improving the status of personal hygiene among children should be considered in curtailing the disease. The gradual changes in the etiologic agents highlight the need for epidemiological surveys on dermatophytosis in Nigeria.

**Keywords:** tinea capitis, tinea favosa, endotrix, ectotrix, Nigeria.

### O-10

#### Molecular study of fungal agents causing urinary tract infection in hospitalized patients

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**Introduction:** Urinary tract infections are a common finding in hospitalized patients. *Candida* species are important agents of funguria. The aim of this study was molecular identification of *Candida* species based on sequence analysis of the internal

transcribed spacer (ITS) region of the rRNA gene.

**Materials and Methods:** This study was conducted on 114 patients referred to Rasoul-e-Akram hospital Medical and Educational Center of Tehran, Iran. The patients were entered to the study by a simple random sampling method. A questionnaire was completed for each patient about their age, gender and the medical conditions. Collected urine samples were identified by microscopic examination, all samples were cultured on CHROMagar *Candida* for identification of mixed infections of *Candida* spp. The clinical isolates were evaluated with specific PCR using *ITS1* and *ITS4* primers, and the *ITS* region of the rRNA gene, was amplified and sequenced. The sequences were submitted to the GenBank.

**Results:** In total twenty three *Candida albicans*, seventeen *Candida tropicalis*, five *Candida parapsilosis*, two *Candida glabrata*, and one *Candida africana* isolates were identified. **Conclusion:** *Candida* species have always been a major problem, especially in hospitalized patients. Accurate identification of *Candida* spp. isolates are critical to treatment management, since some strains showed varying degrees of resistance to antifungal drugs.

**Keywords:** funguria, candiduria, *Candida*, Urinary infection

## O-11

### Frequency and geographic distribution of *CARD9* mutations in patients with severe fungal infections

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**Introduction:** Susceptibility to fungal infections in otherwise healthy individuals with mendelian disorders is increasingly being recognized than before the widespread use of genome sequencing. Mutations in several proteins involved in the caspase recruitment domain containing protein 9 (*CARD9*) signaling protein has been demonstrated to cause primary immunodeficiencies in humans. The aim of this study was to evaluate the frequency, geographic distribution and nature of mutations in patients with *CARD9* deficiency.

**Materials and Methods:** The review process occurred in three steps, namely a literature search, data extraction and statistical analyses. A total of 21 relevant articles could be retrieved.

**Results:** Overall, 24 different genetic alterations in *CARD9* were described in the 60 patients. Three of those were identified most frequently: homozygous (HMZ) p.Q289X (c.865C > T), HMZ p.Q295X (c.883C > T) and HMZ p.D274fsX60 (c.819-820insG), which accounted for 25.8%, 17.7%, and 8.1% of the patients, respectively. The presence of the HMZ p.Q295X and HMZ p.Q289X mutations were associated with an elevated risk of candidiasis (OR: 1.6; 95% CI: 1.18–2.15;  $p = 0.004$ ) and dermatophytosis (OR: 1.85; 95% CI: 1.47–2.37;  $p < 0.001$ ), respectively. Also a strong association was evident between the presence of HMZ p.D274fsX60 and disseminated phaeohyphomycosis; 2.42 (95% CI 1.84–3.2,  $p < 0.001$ ). The main mutations in African patients were different from those in

Asians; HMZ p.Q289X and HMZ p.R101C (c.C301T), accounting for 75% and 10%, respectively, were the common mutations in Africa. The three most common mutations in Asia were HMZ p.Q295X, HMZ p.D274fsX60, and HMZ p.R70W (c.208C > T), which accounted for 34.5%, 17.2%, and 13.8% of the Asian cases, respectively. Notably, HMZ p.Q289X was the most common mutation observed in 75% of the Algerian patients (9 out of 12), while the HMZ p.Q295X mutation was reported in 8 out of 10 Iranian patients (80%). The spectrum of *CARD9* mutations in Asian patients was higher than in African. This finding is important as it provides a relationship between mutation and specific geographic occurrence in these patients.

**Conclusion:** Asia is the most populous continent in the world and may have a greater genetic burden resulting in more patients with severe fungal infections. The presence of a high diversity of mutations revealing 24 distinct variations among 60 patients emphasize that the unique genetic alteration in *CARD9* gene may be associated with certain geographical areas.

**Keywords:** severe fungal infections, *CARD9* deficiency, mutation.

## O-12

### Prevalance of severe asthma with fungal sensitization (SAFS) and asthma associated with fungal sensitization (AAFS) by focusing on the *Aspergillus* sensitization in Iran

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**Introduction:** A new and particular phenotype of severe asthma, has been described recently, is a severe asthma with fungal sensitization (SAFS) and another form of asthma is, asthma associated with fungal sensitization (AAFS); which may be seen in patients with mild to moderate asthma. These conditions may be progress to allergic bronchopulmonary aspergillosis (ABPA). The major complications of ABPA include bronchiectasis, chronic pulmonary aspergillosis, chronic cavitary pulmonary aspergillosis (CCPA) and fibrosis of lung. The exact diagnosis of fungal sensitization is increasingly important in patients with severe asthma due to therapeutic criteria for patients with ABPA or SAFS. This is the first comprehensive report of the prevalence of SAFS and AAFS in patients with allergic asthma from Iran.

**Materials and Methods:** Two hundred consecutive outpatients aged  $\geq 18$  years with diagnosis of moderate to severe allergic asthma, referred to Pediatric Respiratory Diseases Research Center of Doctor Masih Daneshvari hospital (Tehran, Iran) over a period of 25 months were screened for SAFS and AAFS by following criteria: 1. Bronchial asthma, 2. Positive type I skin prick test to *Aspergillus* allergens and/or raised *Aspergillus* specific IgE  $> 0.35$  kUA/L, 3. Negative (usually) *Aspergillus* specific IgG ( $< 26.9$  kUA/L), 4. Total IgE  $< 1000$  kIU/mL (usually less than 500 IU/mL), 5. Normal or central bronchiectasis less than 3 lobes, no centrilobular nodules/ mucoid impaction/ hyperdense mucus, 6. Eosinophil count generally  $< 500$  cells/ $\mu$ l.

**Results:** During this cross sectional study period, 200 outpatients, male 89 (44.5%) and female 111 (55.5%), with moderate (51.5%) to severe (48.5%) allergic bronchial asthma and no smoking were evaluated for SAFS and AAFS by focusing on the *Aspergillus* sensitization. The mean (range) of age was 45.8 (18-78) years and All patients underwent the *Aspergillus* skin prick test that 27 (13.5%) out of 200 patients were sensitive to *Aspergillus* antigens. Of these, 10 (37.0%) patients with overall prevalence 5.0% fulfilled all the diagnostic criteria for SAFS and AAFS. In the next step *Aspergillus* specific IgE values were measured by using immuno CAP assay for all subjects and 22 (11%) of asthmatic patients showed sensitivity to *Aspergillus* antigen and 10 (45.5%) of these, with overall prevalence 5.0% met all the diagnostic criteria for SAFS and AAFS. After all criteria were screened, the findings our study revealed a 7 (7.2%) prevalence of SAFS and 6 (5.8%) prevalence of AASF in outpatients with severe and moderate allergic asthma in Iran.

**Conclusion:** The detection of SAFS or AAFS should be done carefully, because there is probably a significant overlap with

ABPA, especially with ABPA-S. Therefore, in severe asthma or moderate asthma patients, who show signs of fungal sensitization, should consider the risk of SAFS and AAFS. Given the fact that, the therapeutic criteria for ABPA and SAFS is different, thus, we need more data to determine the exact state and prevalence of SAFS as a specific phenotype of severe asthma to managing it timely and accurately. **Keywords:** allergic asthma, SAFS, AAFS, *Aspergillus* sensitization, prevalence

### O-13

#### Identification of clinically common and uncommon yeasts species by molecular and MADLDI-TOF methods and Isolation *Candida haemulonii*, *Candida sorbosivorans* and *Cyberlindnera fabianii* from Iran

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**Introduction:** The incidence of fungal infections caused by the yeasts and yeast-like has

increased dramatically in immunocompromised patients, during the past several decades. However, a few of yeast and yeast-like species may colonize in skin and mucous membranes of healthy individuals. *Candida* species are the major cause of yeast

infections and *Candida albicans* is still the most frequently isolated yeast pathogen.

**Materials and Methods:** A total of 1600 clinical samples were taken from patients with suspected fungal infection and 230 (14.4 %) yeast and yeast like strains isolated and identified by PCR-RFLP, PCR amplification of *HWPI* gene, sequencing and MADLDI-TOF.

**Results:** In this study, 230(14.4 %) clinical samples from 1600 were positive in terms of yeast and yeast-like infection, which 190(82.6%) samples was related to females and 40(17.4%) samples obtained from males. The most of samples attributed to vagina (53%), followed by nail (20%), cutaneous (15.6%) and BAL (3.9%). Thirty seven (80.4%) yeasts species isolated from nail samples was belonged to females. In total, *Candida albicans* (n= 126) was the most frequently isolated species followed by *C. glabrata* (n= 24), *C. parapsilosis* complex (n =17), *C. tropicalis* (n= 13), *C. guilliermondii* (n= 12), *Issatchenkia orientalis* (n =11), *C. stellatoidea* (n= 6), *Kluyveromyces marxianus* and *Cyberlindnera fabianii* each (n= 4), *C. famata* (n= 3), *C. haemulonii* and *Cutaneotrichosporon jirovecii* and *C. africana* each (n= 2) and *C. intermedia*, *C. sorbosivorans*, *Pichia kudriavzevii* and *Trichosporon asahii* each (n=1).

**Conclusion:** In this study, the simultaneous use of PCR assays and MALDITOF MS turned out to be a powerful technique for the accurate identification of yeasts isolated from clinical samples. Interestingly, *C. haemulonii*, *C. sorbosivorans* *Cyberlindnera fabianii* were isolated from clinical samples, for the first time in Iran. *C. sorbosivorans* has near related with *C. magnoliae* in terms of phylogenetic relations, and *C. haemulonii* as a member of *C. auris* complex has emerged as an opportunistic fungal pathogen associated with candidiasis infections. With growing population at risk for fungal infections and the emergence of some less

virulent or non-pathogenic and uncommon yeasts which are not readily distinguishable with phenotypic assays, the accurate identification using molecular methods are warranted.

**Keywords:** uncommon yeasts, *Candida haemulonii*, *Candida sorbosivorans*, *Cyberlindnera fabianii*, *hwp1* gene, MADLDI-TOF

#### O-14

#### Simple and rapid detection of TR34/L98H mutations in the *Cyp51A* gene of triazole-resistant *Aspergillus fumigatus* by tetra-primer ARMS-PCR technique

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**Introduction:** Single nucleotide polymorphism (SNP) detection has been used extensively for genetic association studies. Alteration of the drug target (*Cyp51A*) is the principal mechanism of triazole resistance among *Aspergillus fumigatus* isolates. The most frequently characterized (hotspot) mutation in *Cyp51A* gene is at codons L98 accompanied by a tandem repeat of 34 base pairs in the 5'upstream region of *cyp51A* (TR34/L98H). Rapid identification of triazole-resistant point mutations is important for management of aspergillosis. Amplification-Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) is an inexpensive, reliable and rapid way to investigation and identification of point mutations. In this study we evaluated detection of TR34/L98H mutations in the *Cyp51A* gene of triazole-resistant *A. fumigatus* by tetra-primer ARMS-PCR technique.

**Materials and Methods:** Reference *A. fumigatus* strains (10042001/02 and 10042003/04) carrying wild-type and mutant (TR34/L98H) were used for the

establishment of ARMS-PCR assays. Optimization of ARMS-PCR was carried out in a step by step manner. In this technique, four primers in one reaction were done for amplification of indicative amplicons in wild-type and triazole-resistant *A. fumigatus* carrying TR34/L98H mutations. The assays were evaluated using 5 susceptible and 10 triazole resistant isolates.

**Results:** ARMS-PCR assay from reference triazole-resistant *A. fumigatus* isolate containing TR34/ L98H mutations at *cyp51A* yielded 942 bp & 212 bp DNA fragments. PCR amplification from reference *A. fumigatus* isolates containing wild-type sequence yielded 904 bp & 741 bp DNA fragments. The DNA sequencing data confirmed the results of ARMS-PCR assays for all the isolates analyzed in this study. None of the *A. fumigatus* isolates lacking TR34/L98H mutations yielded false-positive results by ARMS-PCR assays.

**Conclusion:** ARMS-PCR assays developed in this study is a fast, easy, low cost, and user friendly method that may also help in rapid identification of azole resistant *A. fumigatus* carrying TR34/L98H mutations for proper management of patients with invasive aspergillosis in developing countries.

**Keywords:** TR34/L98H mutations, *Aspergillus fumigatus*, ARMS-PCR

## O-15

### Design and synthesis of Moucoadhesive nanogel containing farnesol; investigation of the effect on *HWPI*, *SAP6* and *Rim101* genes expression of *Candida albicans* in vitro

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**Introduction:** Nowadays, the number of immunocompromised cases has been progressively

increased. On other hand, merging resistant *Candida* spp. to common antifungal drugs led to

developing the new therapeutic strategies including, mucoadhesive nano-gels against *Candida*

infaction. In addition to, the use of natural or bioactive compounds extracted from plants or

other microorganisms which have anti-fungal properties with minimum the side effects and increasing

the antifungal effect for the treatment of invasive fungal infections as well as Candidiasis

has been interested.

**Materials and Methods:** In the current study, nano-gels was designed and synthesized by using alginate and chitosan polymers. The physicochemical properties of the nano-gels were examined. Then the indicated concentration 300µM of farnesol was loaded into the gels. Farnesol release, toxicity and the inhibitory activity of nano-gels were investigated. Finally, the effect of nano-gel on genes expression of *HWPI*, *SAP6* and *Rim101* in *Candida albicans* ATCC10231 was assessed by Real-time PCR.

**Results:** Nano-gels containing farnesol showed spherical surface morphology and their

mean size was found about 42-70nm by SEM. Release of farnesol from chitosan and alginate nanogels determined as 58% and 44% respectively. Chitosan nano-gel showed the much more in inhibitory zone compared to farnesol (9 Vrs 7 mm). As well,

cytotoxicity assay indicated no significantly difference between control and treatment groups ( $p > 0.05$ ). Expression of *HWPI* and *SAP6* genes in *C. albicans* treated with chitosan and alginate nano-gels as well as farnesol were decreased, whereas, the expression of *Rim101* gene did not showed any difference in treatment and control groups.

**Conclusion:** Taken together, obtained finding denoted, Chitosan nano-gels contains farnesol

expressed proper antifungal activity with promising new candidate against *Candida albicans*

however, more studies in vitro and in vivo is needed in future.

**Keywords:** nanogel, farnesol, *Candida albicans*, *HWPI*, *SAP6*, *Rim101*

## O-16

### Microsatellite genotyping of *Candida glabrata* isolates from acute and multiple episodes of vulvovaginal candidiasis

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**Introduction:** *Candida glabrata* is an opportunistic fungal pathogen that has emerged as the second most common etiologic agent of vulvovaginal candidiasis (VVC). Population structure and genotype differentiation *C. glabrata* isolates that cause of vulvovaginal candidiasis is essential for epidemiological studies. Therefore, the aim of the current study investigate the genetic diversity and identify the most common genotype of *C. glabrata* isolates involved in vulvovaginal candidiasis with different episodes.

**Materiala and Methods:** At present study, we used microsatellite typing method that were amplified in a multiplex PCR with a set of three markers, RPM2, MTI and ERG3, on 53 independent *C. glabrata* strains, including 30 patients isolates with experienced a single episode of VVC in the last 1 year period and 23 multiple episodes isolates in women with a history of at least two episodes of *Candida* vulvovaginitis in the previous year, in Southwest of Iran.

**Results:** A total of 26 different alleles were observed, generating 22 distinct genotypes (with discriminatory power 0.93), among which 12 of them had a unique genotype and 10 clade of related genotypes were identified. Microsatellite typing demonstrated that the predominant genotypes of *C. glabrata* isolates, especially genotypes of GT18 (134, 242, 236), GT2 (127, 239, 200) and GT6 (128, 240, 235) were most common in patients with had a history one episode or acute vaginal candidiasis.

**Conclusion:** In conclusion, microsatellite typing methods are rapid and reliable, we recommend this approach for other similar epidemiological investigations for accurate identification of genetic diversity of *C. glabrata* isolates, as well as the identification of a specific *C. glabrata* population genetics structure in women with acute VVC may be an important marker for more studies on severity of pathogenicity of *C. glabrata* isolates and effective treatment of this infection.

**Keywords:** *Candida glabrata*, vulvovaginal candidiasis, microsatellite typing, Iran

## O-17

### Simultaneous targeting of HSP90 and lanosterol 14 $\alpha$ -demethylase could reverse azole resistance in some *Candida* species

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**Introduction:** Drug resistance in *Candida* species is an increasing threat which makes a big challenge for treatment of patients. Azole resistance, particularly fluconazole resistance, has increasingly been reported in recent years. Thus, looking for alternative treatment strategies or medications seem necessary.

**Materials and Methods:** In this study we investigated the in vitro interactions between fluconazole, itraconazole, caspofungin and anidulafungin with geldanamycin, an HSP90 inhibitor agent, against a set of resistant *Candida* species including *Candida auris*, *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. tropicalis* using checkerboard method. The interaction outcomes were interpreted based on fractional inhibitory concentration index (FICI).

**Results:** The synergistic interactions between geldanamycin and fluconazole or itraconazole was able to reverse azole resistance in *C. albicans* and *C. parapsilosis* by decreasing the minimum inhibitory concentrations up to 10 two-fold dilutions. Moreover, synergistic effect of these drugs was also observed for some *Candida glabrata* isolates. The remaining combinations resulted in indifference effect. No synergistic effect was recorded against *Candida auris* strains for any combinations.

**Conclusion:** HSP90 seems to be a potential target in *C. albicans* and *C. parapsilosis* for

reversal of azole resistance; however more should be done to prove it.

**Keywords:** Geldanamycin, *Candida auris*, combination therapy, azole resistance

## O-18

### *In vitro* and *in vivo* activities of novel azole compounds ATTAF-1 and ATTAF-2 against *Candida* species

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**Introduction:** Aryl-1, 2, 4-triazole-3-ylthio analogous of fluconazole [ATTAF-1 and TTAF-2] is triazole alcohol-derived analogues, being developed for the treatment of invasive *Candida* infection. ATTAF-1 and ATTAF-2 have demonstrated potent *in vitro* activity against a broad range of *Candida* species. Therefore, we aimed to determine the *in vitro* activity of ATTAF-1 and ATTAF-2 against fluconazole-susceptible and -resistant *Candida* isolates and combination of these compounds with fluconazole. Moreover, we have evaluated the *in vivo* efficacy of ATTAF-1 and ATTAF-2 against *C. albicans* isolate in an invasive candidiasis murine model.

**Materials and Methods:** The *in vitro* activities and interaction of two novel azole compounds (aryl-1, 2, 4-triazol- 3-ylthio

analogues of fluconazole [ATTAFs]) and fluconazole against 52 clinical *Candida* isolates from 5 different species were determined. Female 4-5 weeks old CD1 (ICR) mice were used. A clinical isolate of *C. albicans*, obtained from blood culture was used in this experiment. The day of infection, mice were challenged i.v. with  $1 \times 10^6$  CFU/animal of *C. albicans* in 0.2 ml into the lateral tail vein. Groups consisting of 15 immunocompetent mice were administered ATTAF-1, ATTAF-2, and fluconazole (3.5 and 35 mg/kg/day) intraperitoneally, once daily for 5 days. The efficacy of therapy was evaluated through survival time, the fungal tissue burden and histopathological studies. Data analysis was performed by using GraphPad Prism software.

**Results:** The novel azole compounds had the lowest geometric mean MICs, followed by fluconazole. Moreover, combinations of these compounds with fluconazole exhibited synergistic effects against fluconazole-susceptible (22 of 23 isolates), fluconazole-susceptible dose-dependent (10 of 13 isolates), and fluconazole-resistant (1 of 16 isolates) *Candida* isolates. Mortality was significantly delayed in mice that were administered ATTAF-1 and ATTAF-2 at a dose of 35 mg/kg compared with that in the fluconazole 35 mg/kg and control mice. Median survival time was 15, 15.5, and 15.5 days for mice threatened with ATTAF-1, ATTAF-2, and fluconazole (3.5 mg/kg), respectively and 20.5, 24.5, and 25.5 days for mice threatened with ATTAF-1, ATTAF-2, and fluconazole (35 mg/kg), respectively compared to 12 days for control mice ( $p < 0.05$ ). ATTAF-1 and ATTAF-2 compounds at a dose of 35 mg/kg significantly reduced the fungal burden in comparison with the fluconazole 35 mg/kg and control mice. Fungal burden was  $4.8 \pm 0.36$  log mean CFU/g of kidney for control mice and  $2.8 \pm 0.31$ ,  $2.8 \pm 0.31$ , and  $3.4 \pm 0.31$  for mice treated with ATTAF-1,

ATTAF-2, and fluconazole (35 mg/kg), respectively.

**Conclusion:** ATTAF-1, ATTAF-2 demonstrated potent *in vitro* activity against *Candida* species, which translated into *in vivo* efficacy against invasive candidiasis. Potential therapeutic dose of ATTAF-1 and ATTAF-2 (35mg/kg) were effective in treating invasive candidiasis caused by *C. albicans* in mice. Further studies with more isolates of fluconazole-susceptible and -resistant *Candida* species representing a wider range of MICs should be carried out to assess whether there is any relationship between MIC values and ATTAF-1 and ATTAF-2 efficacy.

**Keywords:** Triazole derivatives, *in vitro* susceptibility, animal model, invasive candidiasis, *Candida* species

### O-19

#### Down regulation of thioredoxin gene in caspofungin resistance clinical isolates of *Aspergillus flavus* using cDNA-AFLP

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**Introduction:** Drug resistance in *Aspergillus flavus* isolates impacts the management of aspergillosis, since echinocandin is the one of the best choices for prophylaxis and therapy. The treatment failure with caspofungin in aspergillosis has been recorded in clinical and laboratory isolates of *A. flavus*. However, to our knowledge, mechanisms underlying resistance are poorly understood. Mutation in S678P substitution in *Fks1p*, significantly contributed an echinocandin resistance in *Aspergillus fumigatus*.

**Materials and Methods:** To investigate differential gene expression between resistant and sensitive clinical isolates to echinocandin, a cDNA-AFLP approach was performed. The reliability of gene expression differences was confirmed by quantitative real-time RT-PCR (qRT-PCR).

**Results:** Fig 1. Pattern of TDFs which extracted from cDNA- AFLP PAGE. M; (50 bp) molecular weight marker. S: Sensitive. R: Resistant. Fig 2. Relative expression pattern of a *thioredoxin* gene by real time RT-PCR, which was measured in *A. flavus* CAS-resistance compared to sensitive. The expression of  $\beta$  *tubolin* was used to normalize the data. The values are the mean  $\pm$  SD:  $P < 0.001$ .

**Conclusion:** Several researchers showed the upper or under expression of genes related to regulation of the redox homeostasis and/or antioxidation systems contributes to resistance to common antifungals.

**Keywords:** caspofungin resistance, *Aspergillus flavus*, thioredoxin, cDNA-AFLP

## O-20

### Survey the effect of Nd: YAG laser irradiation and sodium hypochlorite 1% treatment on the residential *Candida albicans* and *Enterococcus faecalis* of dental canals

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**Introduction:** One major problem following the endodontic treatments is the raising of local high-resistance infections. *Candida*

*albicans* and *Enterococcus faecalis* are the main microorganisms which are remained after the mechanical and chemical debris in the dental canals, pulp, and pericardial tissues. Laser radiation is considered as one of the supplementary endodontic treatment methods. The goals of the current study were to evaluate the effect of Nd: YAG laser irradiation and sodium hypochlorite 1% treatments on the microbial burden of *C. albicans* and *E. faecalis*.

**Materials and Methods:** Root canals of 56 single-canal teeth were chemically-mechanically prepared, soon after removing the smear layer, the teeth were sterilized. Dental canals smeared with a suspension consisted from *C. albicans* and *E. faecalis* in the physiologic serum. Then, the teeth were randomly divided into four groups of 14 teeth. Group A was considered as the control group which was not subjected to any intervention. Group B is exposed to laser radiation 3 watts/30 seconds. In the third group, the canals were washed with sodium hypochlorite 1% for 15 minutes. In the fourth group, the canals were washed with 1% hypochlorite for 15 minutes and then exposed to laser radiation 3 watts/30 seconds. After laser irradiation, the teeth were cultured in the specific culture mediums under sterile conditions, and the cultures were examined for growth levels of *C. albicans* and *E. faecalis*.

**Results:** *Candida albicans* colony count showed that laser irradiation reduced the number of colonies from 24712 units to 16564 yeast cells. These changes are evident by using 1% sodium hypochlorite (27002 to 1344 yeast cells). Also, the use of both laser Nd: YAG and 1% sodium hypochlorite has been shown to severely reduce the number of colonies than the use of laser or sodium hypochlorite alone (27532 to 23 yeast cells).

**Conclusion:** According to the results of this study, application of both Nd: YAG laser and 1% sodium hypochlorite has a significant

effect on the microbial burden of *C. albicans* and *E. faecalis* which cooperatively reduce the of growth levels of them. Therefore using these methods seems to be effective in the eradication of secondary infections of the oral cavity and dental canals.

**Keywords:** Nd: YAG laser, 1% sodium hypochlorite, dental canals, *Candida albicans*, *Enterococcus faecalis*.

## O-21

### Development of a real-time PCR assay for detection of *Candida auris*

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**Introduction:** *Candida auris* is multidrug-resistant yeast causing invasive nosocomial infections. This emerging opportunistic pathogen has been rapidly spread across the world. Although standard microbiologic methods commonly misidentify *C.auris* as other yeast, Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) method has made precise identification of the yeast possible. In the lack of access to the MALDI-TOF in routine clinical laboratories, rapid and accurate methods are demanded for detecting and identifying of *C. auris*. Thus, we developed and validated a quantitative real-time PCR (qPCR) assay targeting the internal transcribed spacer 2 (*ITS2*) region of the ribosomal gene of *C. auris*.

**Materials and Methods:** The *ITS2* region of the nuclear ribosomal DNA of *C. auris* and other related yeasts were analyzed for finding an amplifiable specific target in *C. auris*. A123 base pair target was selected and primers and probe designed according to TaqMan chemistry. Serial dilutions of counted targets containing from 10<sup>5</sup> to 10<sup>0</sup> CFU of the yeast were used to establish a

standard curve for quantifying the yeast. The qPCR reaction was based on the simultaneous detection of a specific *ITS* target and also contained an internal control to compensate for variations in DNA extraction and the various compounds from human that inhibit PCR.

**Results:** The qPCR assay was able to identify and quantify *C. auris* with the detection limit of one *C. auris* CFU per reaction. The specificity was confirmed by the lack of amplification of other *candida* species, other yeast and molds, bacteria and human DNA. A qPCR using DNA extracted from a suspension contains one CFU of *C. auris* resulted in steady Ct-value (Ct) of 34. The assays resulted in a standard curve showed a highly significant linearity between the Ct-values and the dilution rates ( $R^2 = 0.99$ ; slope = -3.32).

**Conclusion:** The TaqMan qPCR assay could rapidly and accurately identify and quantify emerging opportunistic *C. auris* from a wider variety of specimen. The Assay time considering sample processing and DNA extraction would take less than 4 h with greater sensitivity and specificity in comparison with microbiological based identification and conventional PCR. This method shows great promise as a tool for rapid diagnosing exposures to *C. auris* in clinical laboratories.

**Keywords:** *Candida auris*, Real-time PCR

## O-22

### Simultaneous identification of medically important molds using PCR- Reverse line blot hybridization assay

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**Introduction:** Due to the high mortality rates of invasive fungal infections, particularly in immunocompromised individuals, rapid identification of the causative agent of infection is important since the appropriate treatment is usually related to the responsible species. We designed 10 species specific probes for evaluation the ability of a reverse line blot (RLB) assay to identify fungal species in culture as well as direct identification in clinical specimens.

**Materials and Methods:** We performed PCR/RLB assay on 10 reference strains including *Aspergillus* species (*A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*, and *A. clavatus*), *Mucor circinelloides*, *Rhizopus oryzae*, *Alternaria alternata*, *Cladosporium herbarum*, and *Fusarium solani*. Besides, twenty-two clinical specimens from patients were analyzed. The obtained results were then compared with the results of culture and sequence analysis.

**Results:** The fungal species-specific oligonucleotide probes were able to distinguish between all species represented in this study with the exception of cross-reactivity between the two probes; A.NIG and A.FUM, which were related to *A. niger* and *A. fumigatus* species. Results of the RLB assay were concordant with the culture and *ITS* sequencing results except in samples that mixed infection occurred.

**Conclusion:** Our result demonstrate that the RLB assay potentially is suitable for rapid and simultaneous identification of variety fungal pathogens directly from culture as well as from clinical specimens, hence could be performed with a high throughput for epidemiological and diagnostic purposes.

**Keywords:** Reverse Line Blot Hybridization (RLB) assay, Oligonucleotide probe, fungal species

## O-23

### Molecular identification of *Malassezia* species with direct DNA extraction from scalp of patients with dandruff and seborrheic dermatitis

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**Introduction:** *Malassezia* genus in dandruff and seborrheic dermatitis which together have affected more than 50% of humans, have increasing proliferation in scalp. Proliferation of yeasts leads to scalp-flaking and create physical and mental disorder in people. The conventional culture-based methods for the isolation and identification of the *Malassezia* species in relative disease are labour intensive and time-consuming. We aimed to conduct a molecular analysis with direct DNA extraction from scalp in such patients at least time without complexity.

**Materials and Methods:** In this cross sectional study, samples were taken from 65 patients with dandruff and seborrheic dermatitis. DNA extraction was performed directly from uncultured scalp by hexadecyltrimethylammonium bromide (CTAB method). Using species-specific primers derived from the 26s rDNA, PCR amplifications were performed. Identification of the species was carried out with enzyme CfoI in RFLP technique. CTAB method was applied as a more successful method for DNA extraction.

**Results:** *Malassezia restricta* was identified in %58.4 of the scalp specimens as the predominant species. In other 41.6% of cases, *Malassezia restricta* and *Malassezia globosa* were detected together.

**Conclusion:** Molecular-based method of this study is able to diagnosis *Malassezia* yeasts in scalp without time-consuming culture-based method. Also the study shows the invasive characteristics of *Malassezia restricta* and *Malassezia globosa* species.

**Keywords:** *Malassezia*, Dandruff, seborrheic dermatitis, CTAB, 26 S rDNA

## O-24

### Genotyping and antifungal susceptibility profile of clinical *Cryptococcus neoformans* species complex

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**Introduction:** *Cryptococcus neoformans* and *Cryptococcus gattii* are the major causative agents of human cryptococcosis. Cryptococcosis recognized as an acute, subacute or chronic infectious mycotic zoonosis of world-wide significance and have a wide range of appearances such as meningitis, meningoencephalitis, pneumonia, osteomyelitis, abscesses in various internal organs and ocular disorders. Meningitis is the most frequent manifestation of cryptococcosis. The aims of this comprehensive study were to determine the

molecular types of clinical *Cryptococcus neoformans* isolates by URA5-RFLP; to survey for the presence of the two mating types:  $\alpha$  and a; and to determine the antifungal susceptibility profile of the clinical isolates.

**Materials and methods:** 220 clinical samples from 212 patients with suspected cryptococcosis were collected from April 2016 to June 2018. The clinical specimens included: cerebrospinal fluid (CSF), broncho alveolar lavage (BAL), spinal cord abscess, aspirated vesicles, sputum, brain biopsy and paraffin block of brain abscess. PCR-RFLP of the *URA5* gene, mating type determination, in vitro susceptibility test to antifungal drugs alone and in combination (checkerboard technique) were performed.

**Results:** out of 220 samples, 14 samples were positive. The prevalence of cryptococcosis was 5.7% (12/220) and all patients affected to meningitis. The most prevalent molecular type was VNI (85.7%) followed by VNII (14.3%). The lowest MIC values ranged from 0.031 – 0.125 $\mu$ g/ml were observed for voriconazole. The checkerboard methods of antifungal combinations of amphotericin B with 5-fluorocytosine revealed synergistic interaction against 6 (43%) isolates, indifferent interactions against 8 (57%) isolates. The combination amphotericin B and fluconazole resulted synergistic interaction against 4 (28.5%) isolates, antagonism against 3(21.5%) isolates and indifferent against 7(50%) isolates. The combination of 5-FC with fluconazole yielded synergistic interaction against 3 (21.5%) isolates and indifferent 11(78.5%) isolates.

**Conclusion:** The majority of the isolates, 87.5% (n=12), were VNI (var. *grubii*, serotype A), which accords with the fact that this variety causes most human cryptococcal infections worldwide.

**Keywords:** *Cryptococcus*, Genotyping, Susceptibility test, Combination

### O-25

#### Evaluation of miR-146a expression level in macrophages exposed to *Candida glabrata*

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**Introduction:** MicroRNAs are small non-coding RNAs with 1924- nucleotides in length. Up- or down-regulation of many miRNAs has been shown by stimulation of Toll-like receptors (TLRs) in the innate immune system. Up-regulation of miR-146a has been reported by both TLR and heat-killed *Candida albicans*. In this study, we aimed to evaluate the expression of miR-146a in cultured monocyte-derived macrophages (MDMs) infected by *Candida glabrata* at 12, 24, and 48 hours.

**Materials and Methods:** miR-146a expression was evaluated by qRT-real time polymerase chain reaction (PCR) at three time points in *C. glabrata*-infected MDMs. The data was analyzed using repeated measures ANOVA.

**Results:** miR-146a expression was down-regulated in infected MDMs compared to the control group ( $P < 0.018$ ). The expression of miR-146a was at its highest level at 48 h, as compared to 12 and 24 h ( $P < 0.018$ ). The differences between the experimental group compared to the control group were statistically significant ( $P < 0.018$ ).

**Conclusion:** These results suggest that miR-146a can be involved in regulating macrophage function following TLR stimulation in *C. glabrata*-infected MDMs.

**Keywords:** *Candida glabrata*, macrophage, miR-146a

### O-26

#### Pathological study of *Candida albicans* PTCC5027 antitumor effects in rats

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**Introduction:** Nowadays, cancer is one of the biggest health problems in the world mostly due to the poor quality of life. Probiotics are viable and beneficial microorganisms that have beneficial effects on the health of the digestive system. The aim of this study was to evaluate the effect of immuno modulatory effect of standard strain of *Candida albicans* on intracellular colorectal cancer

**Materials and Methods:** Thirty two-week-old rats were prepared from Pasteur Institute and after grouping and induction of colorectal cancer for two consecutive weeks and once a week (15 mg/kg) by gavage using azoxymethane and dextran sodium sulfate followed by treating with *Lactobacillus plantarum* PTCC1058 and *Candida albicans* standard PTCC5027. Subsequently, the effect of treatments on the incidence of cancer was evaluated by histopathologic studies.

**Results:** In this study, cytological and morphological changes in *Candida albicans* group were statistically different from the control group and *Lactobacillus plantarum*, and the severity of changes in the *Candida albicans* group was lower ( $P < 0.05$ ).

**Conclusion:** The results of this study indicate that probiotic consumption reduces tumor cells and also reduces fibrous tissue and lymphocyte infiltration around the cancerous mass in the treated group with *Candida albicans* PTCC5027 compared to the positive control group and chemotherapy. Therefore, the use of probiotics can be an appropriate supplemental therapy for the improvement of colorectal cancer.

**Keywords:** *Candida albicans*, colorectal cancer, Rat

### O-27

#### Characterization of the differential effects of the *Aspergillus fumigatus* and *Aspergillus flavus* species on the platelets activity

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**Introduction:** *Aspergillus* is one of the common opportunistic fungal infections in immunocompromised patients. *Aspergillus* acts invasively into the vessels and causes the intravascular thrombosis. Several studies have been conducted on the interaction of *Aspergillus* and platelets. The findings confirmed that platelets secrete antifungal peptides and have a key role in inherent immune responses to aspergillosis. Further observations revealed that platelets are able to attack *Aspergillus* by blocking and

delaying hyphal elongation. Although the platelets do not have the ability to kill and digest *Aspergillus*, but they strongly stimulates the immune system by secretion of inflammatory factors to inhibit the growth of *Aspergillus* hyphae.

**Materials and Methods:** The collected platelets from blood of healthy volunteers were treated hyphae and conidia of standard strains of *Aspergillus fumigatus* and *Aspergillus flavus*. Flow cytometry was applied to evaluate the platelets activation. Platelets activation was checked by the well documented surface exposure of the integral  $\alpha$ -granule membrane protein P-selectin (CD62P) using FITC-conjugated monoclonal antibodies against CD62P.

**Results:** Our results revealed, *A. fumigatus* and *A. flavus* are able to activate platelets in comparison with negative control of platelets without any stimulation ( $p < 0.05$ ). *Conidia* and in particular hyphae markedly induced an increase in surface expression of CD62P as assessed by flow cytometry. In fact, the hyphae-induced expression of CD62P was similar to the effect of the thrombin receptor agonist SFLLRN, and the combination of these two stimuli showed additive effect on CD62P expression.

**Conclusion:** Data from flow cytometry showed that *A. flavus* and *A. fumigatus* activated the platelets 15.62% and 12.97% respectively. The activity upon the stimulation by hyphae was far more significant than conidia. Considering the evidence from previous studies, these increases can be interpreted as a consequence of platelet activity. However, *A. fumigatus* is often linked with fungal pneumonia. Cellular and animal model studies confirmed that *A. flavus* is more virulent and more resistant to antifungal drugs compared to the other *Aspergillus* species. It has been shown that *A. flavus* is also more resistant than *A. fumigatus* to hyphal damage which was induced by polymorphonuclear leukocytes. With this

evidence, we can conclude that although the two fungi have many similarities, they act in a different manner on human cells.

**Keywords:** human platelet, flow cytometry, *Aspergillus fumigatus*, *Aspergillus flavus*

## O-28

### Molecular analysis of genes involved in zinc metabolism in *Aspergillus fumigatus*

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**Introduction:** Zinc ion is a necessary micronutrient that fungal cells need them to grow and have pathogenesis. In the metabolism of zinc ion in fungal *Aspergillus fumigatus*; *pacC*, *zafA* genes are affecting as a regulator and in case of deficiency of zinc ion, they control the growing of fungus. This investment has done for performance appraisal of the effects of zinc ion on *Aspergillus fumigatus*. Different isolates were examined in a culture medium and analyzed by molecular analysis

**Materials and Methods:** 52 isolated *Aspergillus fumigatus* in aspergillus minimal media cultivated with various concentration of zinc ion including 0 µm, 50 µm, 500 µm, 2000 µm, 10000 µm and 20000 µm cultured and the rate of growing defined by colony diameter. The *zafA* and *pacC* genes were molecular analyzed. To obtain different patterns of *zafA* gene Hae III and EcoRV enzymes were used and to obtain different patterns of *pacC* gene EcoRI and BGL1 enzymes were used. The molecular results were compared with the growth of isolates.

**Results:** In the absence of Zn ion, the growth of the fungus was incomplete, and by adding 2000 µm of Zn the growth of the fungus was complete. Also, with increased zinc concentration, the growth of the fungus was less or stopped. In molecular analysis 1096 bp fragment was obtained for *zafA* gene, and RFLP showed 4 different genetic patterns.

2300 bp fragment was obtained for *pacC* gene, and RFLP showed 3 different genetic patterns.

**Conclusion:** Also in the investments showed that Zinc is an important factor in growth of *Aspergillus fumigatus* in specific concentration. It has increasing effect on growth and in higher concentration it has toxic and reduced effect. The greatest reduction was observed in environmental isolates. The *zafA* and *pacC* genes have shown several different patterns with RFLP.  
**Keywords:** Zn, *zafA*, *pacC*, *Aspergillus fumigatus*