Poster Presentation Abstracts

(Presenters' last names alphabetically sorted)

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

P-01

Clinical manifestation and diagnosis of invasive fungal sinusitis in 24 patients with hematological malignancy

Masoud Mardani[1], Yazdanali Faghani[2], Mahdi Tabarraee[3], Sara Abolghasemi[1]

[1] *Infectious Diseases and Tropical Medical Research Center, Shahid Beheshti University of Medical Sciences,Tehran, Iran*

[2] *Infectious Disease Specialist, School of Medicine, Islamic Azad University of Tehran, Iran*

[3] *Hematology and Oncology Center, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Email: drmasoudmardani@yahoo.com

Introduction: Invasive fungal sinusitis (IFS) is a potentially lethal infection, especially in immunocompromised patients. The current study aimed to evaluate clinical manifestations, outcomes, and factors that may affect IFS in immonucompromised patients' survival.

Materials and Methods: This cross-sectional, descriptive study was performed on patients admitted to Taleghani Hospital of Shahid Beheshti University of Medical Sciences, Tehran, Iran, during one year from October 2012. The clinical data of 24 patients with IFS were reviewed. All the patients had hematologic malignancies and had received broad-spectrum chemotherapy. Demographic data, clinical characteristics, presenting symptoms and signs, underlying diseases, and outcomes of the patients were studied.

Results: The age range of the patients was 15-60 years. IFS was identified as proven in 25%, probable in 66.7%, and possible in 8.3% of the cases. Serum galactomannan antigen was positive in 41.6% of the cases. In general, 15 out of 24 IFS patients had received antifungal chemoprophylaxis before diagnosis, while 54% of the patients received fluconazole and 8.3% itraconazole. *Aspergillus flavus* (33%), *A. fumigatus* (20.8%), *A. niger* (16.7%), and *Mucor* (16.7%) were responsible for incidence of IFS; moreover, 54% of IFS incidences occurred in summer. We found that 91.6% of IFS cases occurred during hospital construction, which was a risk factor in 91.6% of cases.

Conclusion: Our study revealed that *Aspergillus flavus* was the most common fungal isolated pathogen in sinusitis. In addition, *Aspergillus fumigatus* was the second isolated pathogen in the IFS patients. Hospital construction was an important environmental risk factor for acquisition of infection in hematological malignant patients. Additionally, the common causes of mortality in patients with IFS were primary disease and refractory to chemotherapy (37.5%).

Keywords: Sinusitis, Hematologic neoplasm, Mycoses, *Aspergillus*, *Mucor*

P-02

Impact of stored grains contamination with *Aspergillus* species on farmer's lung disease

Seyed Reza Aghili[1, 5], Ali Reza Khosravi[2], Tahereh Shokohi[3], Bahar Salmanian[4]

[1] *Department of Medical Parasitology and Mycology, Invasive Fungi Research Centre (IFRC), School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[3] *Department of Medical Parasitology and Mycology, Invasive Fungi Research Centre (IFRC), School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[4] *Department of Sciences, Seddighe-e Tahereh branch, Farhangian University, Sari, Iran*

[5] *Department of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran,*

Email: aghili70@yahoo.com

Introduction: Farmer's lung disease (FLD), also known as extrinsic allergic alveolitis, is an immunologically mediated inflammatory disease of the lung involving the terminal airways. Repeated inhalational exposure to biologic dusts including various *Aspergillus* species is one of the causes of this disease. Exposure to large quantities of contaminated wet agricultural products such as hay is the most common source of disease and grain farmers usually are not at risk for the development of the disease. However, many types of mold, particularly *Aspergillus* species, grow on insufficiently dried stored crops in granary. While working with moldy grains, the farmers inhale mold spores released as dust; inhalation of a large amount of this dust can be health risk for FLD.

Materials and Methods: For the purpose of data collection, 100 samples of paddy rice and 100 samples of polished rice collected from 100 granaries in Mazandaran, Iran, were studied for fungal contamination. For each sample, 24 grains were plated (12 per plate) on potato dextrose agar medium containing chloramphenicol (50 mg/l). All plates were incubated at 27°C for 7-10 days. Following incubation, the developing fungal colonies were counted and each morphologically unique fungal colony was sub-cultured and purified using standard techniques. The cultures were identified at genus or species level on the basis of macroscopic and microscopic features with the help of literature. With the aim of determining levels of fungal contamination of farmer's stored rice in granaries, we discuss the potential risk of FLD in farmers and their households.

Results: The mortality rate of farmer's lung is reportedly 0-20%. Acute farmer's lung manifests as fever, chills, nonproductive cough, chest tightness, dyspnea, headache, and malaise. If the inhalational exposure is high, the patients may develop acute respiratory failure. The *Aspergillus* antigens, by humoral and cell-mediated immune responses, are responsible for the hypersensitivity reaction in FLD. *Aspergillus* colonization and infection must also be considered as potential triggers of airway symptoms in symptomatic farmers. Following inhalation of moldy grain, levels of interleukin-1 and interleukin-8 and tumor necrosis factor-alpha, as inflammatory mediators, were increased. In this study, the frequency rates of sample contamination with *Aspergillus* species were 21.7% and 43.9% in stored paddy rice and polished rice, respectively. *A. flavus* (42.0%), *A. niger* (32.4%), and *A. fumigatus* (11.9%) were the most frequent *Aspergillus* species identified in 64/100 samples of paddy rice. However, *A. flavus* (57.9%), *A. fumigates* (17.5%), *A. niger* (9.0%), and *A. flavipes* (7.1%) were the most isolated species in 61/100 samples of polished rice. Other species of *Aspergillus* isolated from stored grain included *A. oryzae*, *A. glaucus*, *A. nidulans*, *A. versicolor*, and *A. candidus*.

Conclusion: Multiple factors are responsible for FLD. The evidence showed that in addition to genetic causes, inhalation and repeated exposure to *Aspergillus* species, particularly *A. fumigatus*, is one of the important causes of FLD in work environments as it activates adaptive response and leads to subsequent inflammation-driven lung damage. Prevention of fungal contamination of grain in the pre-harvest and post-harvest stages reduces humidity of granary. Therefore, using masks by farmers may reduce exposure with the amount of antigen.

Keywords: *Aspergillus*, Farmer's lung disease, Fungal contamination, Inhalation, Antigen, Inflammation

P-03

Onychomycosis caused by *Aspergillus* species

Seyed Jamal Hashemi[1], Mohsen Gerami shoar[1], Roshanak Daie ghazvini[1], Ensieh Zibafar[1], Leila Hosseinpour[1], Zeinab Borjian[1], Kazem Ahmadikia[1], Zahra Zareh[1], Omid Raeesi[1], Fereshteh Zarei[1]

[1] *Department of Medical Parasitology and Mycology, School of Public Health, National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran*

Email: kazem_ahmadikia@yahoo.com

Introduction: Onychomycosis, a common cause of nail dystrophy, is ordinarily caused by yeasts; however, dermatophytes and non-dermatophytes molds (NDM) are being increasingly isolated from such cases. According to the previous studies, the isolation rates of opportunistic NDMs in nails range from 2-25%. Epidemiological studies have indicated that *Aspergillus* species are emerging fungal agents

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

of toenail infections. It is important to identify the causative agent of onychomycosis to ensure that the appropriate treatment is employed for each case. Therefore, the aim of this study was to determine the role and pattern of *Aspergillus* species as the most common causative agent of onychomycosis due to NDM in Iran.

Materials and Methods: With the aim of determining the frequency of onychomycosis caused by *Aspergillus* species, a retrospective study was carried out during December 2014-September 2016 at the Medical Mycology Laboratory of Tehran University of Medical Sciences in Tehran, Iran. The results of direct and culture examination of 722 patients suspected of onychomycosis are presented in this study.

Results: According to the results, a total of 259 cases were diagnosed as onychomycosis out of which 85 cases (32.8%) were caused by NDM. *Aspergillus* species were the most frequently isolated etiological agent of onychomycosis due to NDMs (40.0%, N=34). Distributions of *Aspergillus* species were as follows: *A. flavus* (55.8%, N=19), *A. niger* (N=9), *A. fumigatus* (N=2), *A. terreus* (N=1), *Aspergillus* species (N=1), *A. candidus* (N= 1), and *A. versicolor* (N=1). Furthermore, the frequency of *Aspergillus* species in females was 74.5% (26 cases). The mean patients age was 49 years. The most affected age group ranged between 50 and 73 years. The prevalence of toenail and fingernails onychomycosis were 84% and 16%, respectively. Distal and lateral subungual onychomycosis (DLSO) was the most clinical form of the infection. Among predisposing factors of onychomycosis, trauma, diabetes mellitus, and vascular disease were highlighted.

Conclusion: This study indicated that the frequency of onychomycosis due to *Aspergillus* species was 13.1%. Moreover, *A. flavus* was found to be the most prevalent species. Consequently, the identification of the etiological agents of onychomycosis is necessary for appropriate therapy.

Keywords: Onychomycosis, non-dermatophytes molds (NDM), *Aspergillus* species

P-04

Disseminated Aspergillosis as the Herald Manifestation of Chronic Granulomatous Disease in an Adult Patient

Ilad Alavi Darazam[1], Davood Mansouri[1], Hossein Akhavan Zanjani[1], Davood Sanaee[1]

[1] *Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Email: ilad13@yahoo.com

Introduction: Chronic granulomatous disease (CGD) is an inherited defect in intracellular killing of ingested microorganisms, which are characterized as recurrent life-threatening bacterial and fungal infections, such as invasive aspergillosis, in early childhood.

Case Presentation: In this report, a 20-year-old girl, who was admitted due to low back pain, subcutaneous masses in the left thigh and left temporal part of scalp, as well as perihilar mass in the right lung, hypodense splenic lesions and heterogeneous infiltrative soft-tissue density in pelvic fosse, was assessed. Subcutaneous masses, bone marrow aspiration and spleen aspiration revealed septate, acute-angle hyphae in Gomori methenamine using silver staining and culture. The serum galactomannan was strongly positive, and a diagnosis of CGD was confirmed through nitroblue tetrazolium test (NBT= 0), demonstrating no dihydrorhodamine oxidation and abnormality. Results of this study were indicative of the delayed presentation of CGD until twenty years of age without previous infections and secondly, the high burden of *Aspergillus* infection involving multiple organs, particularly the isolation of this germ from the spleen and bone marrow. While *Aspergillus* infection in phagocytic disorders, especially CGD, is often presented as a as local slowly progressive infection, evaluation of our case revealed that multiple organs (skin, lung, pelvic cavity, spleen, vertebrae and bone marrow) were affected. Another intriguing finding in this case report was the dramatic response to voriconazole, echinocandin and gamma interferon.

Conclusion: According to the results of this study, CGD must be considered in all patients with recurrent catalase-positive microorganism and fungal infections regardless of age, despite the fact that this condition usually manifests in children.

Keywords: Disseminated Aspergillosis, CGD

P-05

Analysis of *Aspergillus* spp. spore load in indoor air samples of hospitals in Ardabil, Iran

Saeideh Amani Ghayum[1], Sadegh Hazrati[2], Farnaz Valedeiny Asl[3], Solmaz Basiri Khiavi[4]

[1] *Department of Medical Mycology and Parasitology, Ardabil University of Medical Sciences, Ardabil, Iran*

[2] *Department of Occupational and Environmental Health Engineering, School of Public Health, Ardabil University of Medical Sciences, Ardabil, Iran*

[3] *Department of Vice Chancellor in Treatment Affairs, Ardabil University of Medical Sciences, Ardabil, Iran*

Email: saidehamani@yahoo.com

Introduction: Prevalence of aspergillosis is on the rise in hospitalized immunocompromised patients, often presenting as an invasive pulmonary disease. With the continuous increase in the number of severely immunocompromised patients, hospitals are faced with the growing rates of invasive aspergillosis and other opportunistic fungal infections. Considering their challenging treatment and fatal outcomes, preventive measures are of paramount importance in the control of invasive filamentous fungal infections. Until recently, inhalation of airborne *Aspergillus conidia* was believed to be the primary route of acquiring aspergillosis. Although efforts for the filtration of hospital air has led to the reduction of airborne conidia and frequency of invasive fungal infections, the association between the concentration of *Aspergillus conidia* in hospital air and risk of invasive fungal infections remains unclear.

Materials and Methods: Concentrations of airborne fungi were monitored during six months in different special care units of four hospitals in Ardabil, Iran. All airborne fungal samples were isolated from the air samples of four different hospital wards using a V-4 vacuum pump. To increase isolation accuracy, sample collection was performed using an active method. In addition, Czapek-Dox Agar containing chloramphenicol (40 mgl^{-1}) incubated at the temperature of 30˚C was used as the culture medium. For sample discrimination, duplicate-slide culture assay was performed on PDA and CMA media. Moreover, microscopic examination was carried out using lactophenol cotton blue stain. Colony counts, CFU and other indices were analyzed statistically.

Results: Out of 200 samples collected from different patient care environments, an average of 18 CFU/m3 was recovered at the temperature of 28°C and 35% humidity. In total, 57 species were identified as potential opportunistic fungi, including *Aspergillus niger* (55%), *A. flavus* (26.6%), *A. fumigatus* (8.3%), *A. terreus* (3.5%), *A. clavatus* (1.6%), and three isolates of *Aspergillus* spp. (5%). In the thoracic surgery and emergency wards, the most average spore load contamination values in the air were within the range of 24-35 CFU/m3. However, degree of fungal air contamination and species composition had no significant difference inside special care units with hospital corridors.

Conclusion: This study aimed to evaluate the concentrations of different airborne taxa of *Aspergillus* in four hospitals, where immunocompromised patients are at a high-risk of developing fatal aspergillosis. Our findings were indicative of an association between human activities and the number of viable fungal particles in indoor air, so that the highest level of fungal contamination was observed in the emergency ward. Furthermore, no significant barriers were established against air exchange between supposedly protective areas in the thoracic surgery unit and the surrounding rooms. Therefore, it is recommended that fungal exposure be closely monitored in

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

immunocompromised patients using a personal air-sampling device in intensive care units. However, no fungal sampler is currently available for this purpose.

Keywords: *Aspergillus*, Aspergillosis, Airborne conidia, Immunocompromised patients

P-06

Antifungal susceptibility profile of clinical and environmental *Aspergillus flavus* strains from Iran

Saham Ansari[1], Mojtaba Taghizadeh-Armaki[2,3], Mohammad T. Hedayati[2,4], Paul E. Verweij, Seyedmojtaba Seyedmousavi[5,6,7]

[1]*Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2]*Department of Medical Mycology and Parasitology School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[3]*Department of Medical Mycology and Parasitology, School of Medicine, Babol University of Medical Sciences, Babol, Iran*

[4]*Invasive Fungi Research Center, Mazandaran University of Medical Sciences, Sari, Iran*

[5]*Department of Medical Microbiology, Radboudumc, Nijmegen, The Netherlands*

[6]*Department of Medical Microbiology and Infectious Diseases, ErasmusMC, Rotterdam, The Netherlands*

[7]*Current Address: Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, United States of America.*

Email: samansari1988@yahoo.com

Introduction: *Aspergillus flavus* has been frequently reported the leading cause of invasive aspergillosis in certain tropical and sub-tropical countries. The molecular epidemiology, and antifungal susceptibility profile of clinical and environmental *A. flavus* strain in Iran is poorly documented.

Material and methods: Two hundred *A. flavus* strains originating from clinical and environmental sources were phylogenetically identified at the species level using sequences of beta-tubulin gene. *In vitro* antifungal susceptibility testing was performed against seven antifungals by using the Clinical and Laboratory Standards Institute (CLSI-M38-A2) broth-microdilution method. Final concentrations of the following antifungal agents ranged from 0.016 to 16 mg/L: Amphotericin B (AMB), Isavuconazole (ISA), Itraconazole (ITC), Voriconazole (VRC), Posaconazole (POS), Caspofungin (CAS) and Anidulafungin (AFG).

Results: The geometric means of the MICs of the antifungals for all isolates were as reported below in increasing order; AFG 0.05, POS 0.11, ITC 0.20, CAS 0.35, VRC 0.57, ISA 0.98 and AMB 3.02 mg/L. The MIC ranges of antifungal agents were the following: AFG (0.016-0.125 mg/L), POS (0.016-4 mg/L), ITC (0.032-4.0 mg/L), CAS (0.016-0.5 mg/L), VRC (0.062-1.0 mg/L), ISA (0.032-1.0 mg/L) and AMB (0.1-4.0 mg/L), respectively.

Conclusions: Anidulafungin and posaconazole showed the greatest *in vitro* activity among systemic azoles and echinocandins, respectively. Antifungal susceptibility of *A. flavus* was not linked with the clinical or environmental source of isolation.

Keywords: Antifungal susceptibility, *Aspergillus flavus*, Iran.

P-07

Invasive *Aspergillus* sinusitis successfully treated with voriconazole

Narges Aslani[1], Ghasem Janbabaie[2], Mahasti Babaeian[2], Saman Rezaei[3], Melika La'al Karghar[4], Sahar Ghazanfari[4], Nina Zahedi[1], Hamid Badali[5]

[1]*Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

[2]*Hematology & Oncology Research Center, Mazandaran University of Medical Sciences, Sari, Iran*

[3] *Department of Otorhinolaryngology (ENT), Mazandaran University of Medical Sciences, Sari, Iran*

[4] *Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*

[5]*Invasive Fungi Research Center, School of Medicine, Mazandaran University of Medical Sciences; Sari, Iran*

Email: narges.aslani987@gmail.com

Invasive *Aspergillus* sinusitis is a rare disease largely attributable to *Aspergillus* and *Mucor* species, widely recognized as a cause of morbidity and mortality in immunocompromised patients, with a high risk of neutropenia. Earliest indications of this disease in immunocompromised patients include fever, headache, nasal obstruction and facial swelling. However, this disease has a poor prognosis. This study aimed to describe a case of invasive *Aspergillus* sinusitis successfully treated with voriconazole. A 22-year-old male patient presented with a history of acute lymphoblastic leukemia for nine months. A broviac catheter was inserted into the right subclavian vein, and the patient underwent chemotherapy with daunorubicin, cyclophosphamide and vincristine. After seven chemotherapy sessions, neutropenia (600/mm3) occurred in the patient. On day three of chemotherapy (session seven), while neutropenic, the patient developed a fever of 38.7˚C with eye, throat and chills. All blood cultures were negative, and he had no history of tuberculosis, diabetes mellitus, asthma, corticosteroid use and long-term antibiotic therapy. Fiberoptic endoscopic sinus surgery was performed, and direct microscopic examination (10% KOH) revealed abundant septate fungal hyphae (dichotomous hyphae). A biopsy specimen was inoculated on Sabouraud dextrose agar (supplemented with chloramphenicol and brain-heart infusion agar) and incubated at temperatures of 25ºC and 37ºC for 48 h. Growth of yellowish colonies was observed, and the fungi were morphologically classified as *Aspergillus flavus*. Patient was started on intravenous ciprofloxacin, clindamycin, vancomycin, and liposomal amphotericin B (3 mg/kg/day for 5 weeks). Seven days after amphotericin B consumption, the patient developed fever (39.3°C) and chills. MICs of itraconazole, voriconazole and posaconazole were adjusted in accordance with the Clinical and Laboratory Standards Institute (M38-A2 document). Itraconazole had a low MIC value (0.25 µg/ml), followed by posaconazole (0.5 µg/ml), and voriconazole (0.5 µg/ml). Patient had a good response to voriconazole treatment (200 mg/day). However, despite antifungal therapy, patient conditions continued to deteriorate, and he died due to leukemia.

Keywords: Leukemia, *A. flavus*, Voriconazole

P-08

Highly Itraconazole Susceptible *Aspergillus fumigatus* and resistant *Fusarium* species Isolated from Avian Farms in Iran

Narges Aslani[1], Hamid Badali[2]

[1]*Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

[2]*Invasive Fungi Research Center (IFRC)/Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

Email: narges.aslani987@gmail.com

Introduction: *Aspergillus fumigatus* as a facultative pathogen, is major cause of aspergillosis approximately 95% of cases that frequently inhaled through the air route and remains a major respiratory pathogen in animals in particular birds and immunocompromised host with various clinical manifestations. Fusariosis is a rare but often fatal fungal infection in avian farms. Use of azole therapy, frequent exposure to azole fungicides in the environment, widespread uses of azole prophylaxis in avian farms are thought to be potential risk factors for the development of azole-resistant species in the environment over time and these factors can lead to increase fungal infection and failure of management. To assess the potential risk of azole-resistance emergence in avian farms where azole compounds are used for the control of avian mycoses, we conducted a drug susceptibility study including *A. fumigatus* and *Fusarium* isolates from avian farms.

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

Methods: Environmental samples (nest material and compost heaps) from five nesting sites were collected and examined in terms of the growth of triazole-resistant *A. fumigatus* isolates. Cultures were prepared on a sabouraud dextrose agar plate, supplemented with 4 and 1mg/L of itraconazole and voriconazole, respectively, at 45°C for 72 h in the dark. All colonies growing on the plates, mimicking *A. fumigatus* complexes, were sub-cultured. Strain identities were reconfirmed by DNA sequencing of the partial b-tubulin gene. Afterwards, *in vitro* antifungal susceptibility tests against triazole agents were performed based on the Clinical and Laboratory Standards Institute (CLSI) M38- A2 document.

Results: The MIC of itraconazole against *A. fumigatus* (15 strains) was less than breakpoint and epidemiological cut-off value, but *Fusarium* species (19 strains) had high MIC value (>16 µg/ml) which were resistant.

Conclusion: Although the number of azole-resistant isolates was limited, strict supervision of persistent environment azole-resistant isolates screening of azole resistance are vital to the development of approaches for the management of azole resistance in avian and human pathogenic fungi.

Keywords: *Fusarium* species, *Aspergillus fumigatus*, Avian Farms, Itraconazole

P-09

Streptomyces rochei as a sources of novel antifungal active compounds against azole-resistant *Aspergillus fumigatus*

Sanaz Hadizadeh[1], Hamid Forootanfar[2], Gholam Hosain ShahidiBonjar[3], Hamid Badali [4], Karamy Robati A[1], Sahar amir rostami[5], Seyyed Amin AyatollahiMousavi[1]

[1]*Dept. of Medical Mycology & Parasitology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman*

[2]*Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman*

[3]*Dept. of Plant Pathology & Biotechnology, College of Agriculture, Bahonar University of Kerman*

[4]*Department of Medical Mycology and Parasitology/Invasive Fungi Research Center (IFRC), Mazandaran University of Medical Sciences, Sari, Iran.*

[5]*Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.*

Email: aminayatollahi@kmu.ac.ir

Introduction: Azole resistance in *Aspergillus fumigatus* is a major challenge and is emerging into a global health problem. Azole resistance may develop due to long terms of azole therapy or exposure of the fungus to the azole fungicides. Therefore, we aimed to evaluate the *in vitro* activity of *Streptomyces rochei as a* sources of novel antifungal active compounds against azole-resistant *Aspergillus fumigatus*

Material and Methods: In the present study, we report the isolation and characterization of actinomycetes compounds with a focus on the antifungal activity (minimum inhibitory concentration) against azole resistant *A. fumigatus*.

Results: *Streptomyces rochei* were isolated and confirmed by DNA sequencing (KP137826.1) and demonstrated antifungal activities against azole resistant *A. fumigates* with high efficacy.

Conclusion: The observations from this study showed that these strains represent a particular interest for enlarged investigation, including isolation and characterization of pure active compounds and study of their biosynthesis.

Keywords: *Streptomyces rochei*, azole resistant, *Aspergillus fumigates*.

P-10

Aspergillus species in indoor environment of a burn hospital in Sari, Iran

Zeinab Bandalizadeh[1], Mahin Tavakoli[1], Afsane Vaezi[1], Mohamad T. Hedayati[1], Tahereh Shokohi[1], Hamid Badali[1]

[1]*Invasive Fungi Research Center, Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

Email: z.bandali93@gmail.com

Introduction: Nosocomial infections are mainly associated with high morbidity and mortality rates in healthcare systems. The presence of airborne fungi in hospital environments is of great concern due to their potential role as a source of hospital-acquired infections (HAI). Therefore, this study aimed to determine and compare the concentration of *Aspergillus* species in indoor environment of a burn hospital in Sari, Iran.

Materials and Methods: A cross-sectional analysis of *Aspergillus* was performed in a burn hospital in Sari, using the quick take 30 pump-air sampler and carpet sterile fragments for sampling of air and surfaces in different wards of the hospital (e.g., operating room, intensive care unit, as well as surgery and burn wards, respectively). Collected samples were cultured on petri dishes containing Sabouraud dextrose agar with chloramphenicol. Plates were incubated at 27-30˚C for seven days. Grown *Aspergillus* spp. were identified in the level of species using morphological methods including macroscopic and microscopic characteristics.

Results: In this study, a total of 42 samples were collected and cultured. Mean level of recovered fungi was determined at 25.7 CFU/m3. Assessment of samples obtained from the air, surfaces and healthcare setting revealed that *Aspergillus* spp. were the most common fungi. Moreover, a total of 38 *Aspergillus* were isolated. Among the different *Aspergillus* species, *A. fumigatus* (20.5%) was the most common, followed by *A. flavus* (17.9%), *A. niger* (7.7%) and *A. clavatus* (1.3%), respectively.

Conclusion: According to the results of this study, *A. fumigatus* was spread as an important agent of nosocomial infections in hospital environments. It is recommended that contributing factors for the airborne fungal level in hospital environments be properly managed to minimize the risk of HAIs.

Keywords: indoor environment, burn hospital, *Aspergillus* spp.

P-11

Introduction of an *Aspergillus* polymerase chain reaction assay to clinical mycology service in Iran

Kambiz Diba[1], Shahram Khademvatan[1], Froozan Zarza[2]

[1] *Cellular and Molecular Research Center, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Imam Educational Hospital, Urmia University of Medical Sciences, Urmia, Iran*

Email: kambiz37diba@gmail.com

Introduction: *Aspergillus* species are abundant and widely distributed in soil, water, air, seed, and food. These species are associated with allergic bronchopulmonary disease, mycotic keratitis, otomycosis, nasal sinusitis and invasive infection. In this study, we developed a polymerase chain reaction-single-strand conformational polymorphism method (PCR-SSCP) to identify the most common *Aspergillus* species.

Materials & Methods: The current study utilized *Aspergillus* clinical isolates of an educational hospital in Urmia, Iran, as well as some *Aspergillus* standard species obtained from Japan Collection of Microorganisms. All *Aspergillus* isolates were identified, using the morphological (colonies and microscopic) features. For the molecular identification, the ITS2 region of rDNA gene (approximate length size: 330 bp) was amplified in PCR. The PCR product was incubated at 95ºC for 5 min, and then moved quickly into ice bath for immediate quenching. A vertical electrophoresis with 6-12% gradient poly-acrylamide gel was used full time cooling at 4ºC.

Results: The tested Aspergillus species including *A. nidulans*, *A. fischeri*, *A. fumigatus*, and *A. niger* discriminated. Consequently, SSCP assay enabled us to identify above *Aspergillus* species within 8-12 h after overnight incubation.

Conclusion: As the results of the study indicated, SSCP was concluded to be a simple and rapid method for identification of some medically important *Aspergillus* species. However, we

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

recommend this method as a compliment test to be used with other molecular methods such as PCR-restriction fragment length polymorphism in order to identify more *Aspergillus* species.
Keywords: Rapid identification, *Aspergillus*, Clinical case

P-12

Aspergillus identification in clinical and hospital sources, Urmia, Iran

Kambiz Diba[1], Davood Jabari[2], Elnaz Hosseianlipoor[2]
[1] *Cellular and Molecular Research Center, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*
[2] *Department of Mycology and Parasitology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*
Email: kambiz37diba@gmail.com

Introduction: In spite of a low percent (1%) of fungal hospital acquired infections, *Aspergillus* species are the main agents of fulminate fungal infections. This study aimed to detect the sources of nosocomial infections through the identification of *Aspergillus* species isolated from hospital, clinical and environmental samples.
Materials and Methods: Samples in this study included *Aspergillus* isolates with confirmed hospital-acquired infections and environmental specimens collected from air, swabs of the walls, curtains, beds, blankets, trolleys, air conditioners and medical devices. A morphological diagnosis was performed on Sabouraud glucose agar and Czapek dox agar for the identification of fungal agents, which was confirmed by the PCR-RFLP method. In addition, random amplified polymorphic DNA (RAPD) technique was performed based on the polymerase chain reaction (PCR) for a molecular typing of environmental isolates.
Results: In this study, the final results revealed the presence of *Candida* spp., *Aspergillus* spp. and other fungi in 110 fungal isolates. Among the clinically isolated *Aspergillus*, the most frequent species were *A. flavus* (47%), *A. fumigatus* (29.4%) and *A. niger* (23.6%), respectively. Environmental specimens contained *Aspergillus* isolates as follows: *A. niger* (43.7%), *A. flavus* (41.8%) and *A. fumigatus* (14.7%). Comparison of clinical and environmental isolates demonstrated RAPD settings with similar patterns for the two clusters.
Conclusion: According to the results of this study, while molecular typing of *Aspergillus* species by PCR-RFLP and RAPD might be a simple method to find hospital *Aspergillus* sources, the final results are mostly inaccurate.
Keywords: *Aspergillus* species, hospital sources, RAPD-PCR, PCR-RFLP

P-13

Survey of fungal contamination in hospital wet cooling systems in Arak city, Iran

Mojtaba Didehdar, Mahdi Mosayebi, Zahra Eslamirad, Reza Hajihossein
Department of Medical Mycology and Parasitology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
Email: didehdar_m@yahoo.com

Introduction: Fungal infections are common complication during hospitalization, especially in immunocompromised patients. Wet cooling systems in hospitals are considered as a major source of nosocomial infections. This study aimed to evaluate the wet cooling systems of hospitals in terms of fungal contamination in Arak city, Iran.
Materials and Methods: This descriptive, cross-sectional study was conducted during May-September 2016. Samples were randomly collected from the water and straws of 84 wet cooling systems in four hospitals in Arak, Iran. Samples were cultured in Sabouraud dextrose agar containing chloramphenicol. Identification of fungi was performed using the slide culture method.
Results: Out of 84 wet cooling systems, fungal contamination was detected in 32 cases (38.1%). The highest rate of fungal contamination was observed in oncology wards and coronary care units. Moreover, the most frequent fungi isolates in the selected hospitals were *Aspergillus* spp. and *Candida* spp., respectively.
Conclusion: According to the results of this study, significant fungal contamination was present in the hospital wards using wet cooling systems, particularly *Aspergillus* contamination. Therefore, it is recommended that non-aqueous or closed-cycle air conditioning systems be used in hospitals, especially in the care environment of susceptible patients.
Keywords: Fungi, Wet cooling system, Hospital, Arak

P-14

Use of CSP typing for genotyping azole-resistant and susceptible *Aspergillus fumigatus* isolates in Iran

Mahsa Falahati[1], Hamid Badali[1], Hamed Fakhim[1], Saham Ansari[1], Mahdi Abastabar[1]
[1] *Invasive Fungi Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
Email: mahsafalahati776@gmail.com

Introduction: *Aspergillus fumigatus* is globally known as a leading cause of various clinical diseases, accounting for an alarmingly high mortality rate in immunocompromised hosts. Recognition of pathogen dispersion and relatedness is essential for determining the epidemiology of nosocomial infections and designing effective pathogen control techniques. This study aimed to investigate the diversity and putative origins of clinical and environmental susceptibility and resistance of *A. fumigatus* isolates in Iran.
Materials & Methods: In total, 79 *A. fumigatus* isolates were evaluated, including 15 azole-resistant and 64 azole-susceptible isolates, which were genotyped using partially cell surface protein (*CSP*) gene.
Results: Seven distinct repeat types (r01, r02, r03, r04, r05, r06, and r07) and 11 different *CSP* variants (t01, t02, t03, t04A, t06A, t06B, t08, t10, t18A, t18B, and t22) were observed among the isolates. Interestingly, t06B, t18A and t18B were exclusively found in azole-resistant *A. fumigatus* isolates (TR34/L98H or non-TR34/L98H). Simpson's diversity index (D) was calculated at 0.78.
According to the results, isolates with significant resistance were genetically less diverse compared to azole-susceptible isolates. However, azole-resistant *A. fumigatus* without TR34/L98H were more diverse than azole resistant with TR34/L98H. In addition, the relatively close genetic relationships and limited CSP type diversity of the TR34/L98H isolates, compared to those of the azole-susceptible wild-type isolates, indicates that the independent and repeated emergence of the TR34/L98H mechanism to be unlikely.
Conclusion: It seems that resistant *A. fumigatus*, which has been emerging across the world, could spread easily through producing a large number of asexual airborne conidia. Furthermore, it has been suggested that *CSP* types might have a common ancestor that developed locally, migrating to different regions of the world.
Keywords: CSP typing, *Aspergillus fumigatus*, Azole-resistant and susceptible, Iran

P-15

Evaluation of antifungal activity of graphene oxide conjugated with indolicidin against *Aspergillus* spp. and *Candida albicans*

Ali Farzanegan[1], Maryam Rudbary[1], Mehraban Falahati[1]
[1] *Department of Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
Email: alifarzanegan1349@yahoo.com

Introduction: Infections caused by opportunistic and saprophyte fungi are the most common diseases in immunocompromised patients such as organ transplant recipients, HIV-infected patients, and those under long-term, broad-spectrum antibiotic therapy. Due to increasing drug resistant, the use of natural components with proper antimicrobial effect is gaining growing

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

interest. This study was performed to determine antifungal activity of graphene oxide conjugated with indolicidin as compared to amphotericin B and fluconazole on standard strains of *C. albicans* and *Aspergillus* spp.

Materials and Methods: Graphene oxide (GO) was synthesized using Homer method and carboxyl groups were increased on it by $C_2H_3BrO_2$ (Bromoacetic acid) and sodium hydroxide; thereafter, it was activated by dimethylaminopropyl chloride hydrochloride (EDC) and N-hydroxysuccinimide (NHS). Then the cationic antimicrobial peptide (indolicidin) was added to graphene for nanocomposite synthesis, which was confirmed by FTIR , NMR , TGA, and SEM analysis. For minimum inhibitory concentration (MIC) assessment, *C. albicans* (ATCC 10231) was cultured on Sabouraud dextrose agar (SDA) and incubated at 35°C for 48 hours. Moreover, *A. fumigatus* (ATCC204305), *A. flavus* (CBS 625166), and *A. niger* (ATCC 1105) were cultured on Czapek Dox Agar and incubated at 30°C for one week. Fungal suspensions were prepared at concentration of 1×10^3 (CFU/ml). MIC and minimum fungicide concentration (MFC) were determined by microdilution broth method with ranges 200-0.39 µg/m for nanocomposite, 128-0.25 µg/ml for fluconazole, 100-0.19 µg/ml for IN, 200-0.39 µg/ml for GO, and 32-0.06 µg/ml for amphotericin B. Each test was carried out in triplicate. In addition, negative and positive controls were considered.

Results: Our results indicated that nanocomposite had strong inhibitory effect against *C. albicans* (MIC: 3.12 µg/ml) compared to indolicidin and GO alone. Furthermore, at concentration of 25 µg/ml it had candidacidal activity, whereas this nanocomposite did not show any inhibitory effect on *Aspergillus* spp.

Conclusion: Designing a new drug delivery system via nanotechnology, which is able to inhibit fungal growth, can be one of the drug delivery system objectives for improving treatment with minimum side effects. According to our results, this nanocomposite with suitable MIC against *C. albicans* can introduce an appropriate component for inhibition of candida growth. The integrity of *Aspergillus* cell wall may explain the resistance of *Aspergillus* to nanocomposite penetration. However, further in-vitro and in-vivo studies should be performed on the safety and detailed mechanisms of this nanocomposite.

Keywords: Graphene oxide, Indolicidin, MIC, Nanocomposite, *Candida*, *Aspergillus*

P-16

Isolation of *Aspergillus* species from Educational hospitals in Ahvaz, Iran

Mahnaz Fatahinia[1], Neda Kiasat[1], Ali Zarei Mahmoudabadi[1,2]

[1] *Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[2] *Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Email: Fatahinia@yahoo.com

Introduction: Nosocomial aspergillosis is a common complication among immune-compromised individuals and high-risk patients. Aspergillosis has become a major invasive fungal infection in hospitals in recent decades. *Aspergillus*, a saprophytic fungus with wide distribution, is associated with systemic infections in predisposed patients with a mortality rate of about 85%. *Aspergillus* speices produce microscopic conidia, which can easily spread through the air. These conidia can enter alveolar through the airway and may cause different types of aspergillosis. This study aimed to evaluate diversity and abundance of *Aspergillus* in air samples of educational hospitals in Ahvaz, Iran.

Materials and Methods: The air was sampled from seven wards of five hospitals using Quick Take 30 Sample Pump (SKC, USA). As a result, a total of 175 air samples were collected, and then analyzed. Species of *Aspergillus* were identified, using macroscopic and microscopic features. Finally, the number of colonies forming units per cubic meter of air (CFU/m³) was calculated:

$$CFU/m3 = \frac{colonies\ number}{Debbie(28.3) \times Time(\min)}$$

Results: According to the results of the study, 420 colonies of *Aspergillus* species were detected. The highest and least concentration of conidia in the air was related to surgery (1366 CFU/m^3) and burn wards (412 CFU/m^3), respectively. The most abundant *Aspergillus* species were as follows*: A. niger* (17.6%), *A. terreus* (15%), *A. fumigatus* (11.4%), and *A. flavus* (8.3%).

Conclusion: The World Health Organization (WHO) has suggested limit of 50 CFU/m^3 for fungi in the hospital air. Regarding this, this study demonstrated that the contamination level is too high. Considering the presence of *Aspergillus* species in the indoor hospital as well as the resistance of *A. terreus* to amphotericin B *in vivo* and *in vitro*, monitoring is necessary to prevent possible hospital infections.

Keywords: *Aspergillus*, Hospital, Air, Nosocomial

P-17

Study of hemolysin gene "*aspHS*" and its phenotype in *Aspergillus fumigatus* isolates

Farzaneh Ganj[1], Majid Zarrin[1]

[1] *Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Email: mktn1285@yahoo.com

Introduction: *Aspergillus fumigatus* is the most pathogenic of all aspergilli fungi, with more than 90 percent of mortality rates of diseases. The major cause of this virulence is pathogenic factors dependent on specific gene sequences. Such a factor is the release of haemolysin which is encoded by *aspHS* gene. Haemolysin helps fungi to kill blood cells and has cytotoxic effects on endothelial cells and macrophages. Diagnosis merely based on morphological properties provides difficulties and is prone to uncertainties. Molecular biological methods with higher speed and precision are however able to differentiate morphologically identical genus, and help with detection of genotypes and polymorphs. *In vitro* studies indicate that the path of haemolysin release is different in diverse*A. fumigatus* isolates. The present study provides a phenotypic examination of haemolysin enzyme in these isolates through real-time PCR-RRLP to detect differences in *aspHS* gene sequence.

Materials and Methods: Fifty three *A. fumigatus* isolates including 4 standard isolate, 10 clinical, and 39 *environmental* isolates were approved morphologically for study. Due to higher resolution in haemolytic activity in *A. fumigatus*, 10 *A. niger* isolates were selected as control group. To measure haemolytic activity, isolates were incubated in blood agar medium at 37 °C for 48h. F-Asphs and R-Asphs primers in PCR was able to obtatin a 180bp band and Afhem2 and Afhem1 primers recovery a 450bp band. Restriction enzymes *NcoI* and *TaqI* also were able to identification of different genotypes. AnoR and AnoF primers, redesigned according to *A. niger* haemolysin sequences, provided was able to identification of wider sequences in *A. fumigatus* haemolysin.

Results: A phenotypic comparison of haemolysin activity in *A. fumigatus* and *A.niger* indicated that all isolates were able to create haemolytic cover with varying degrees (6 to 7.6) around blood agar colonies. PCR products from sequence were strikingly similar (99 percent) to haemolysin in data base. After digestion of PCR products by restriction enzymes *Nco*I and *Taq*I, a similarity of 97 percent also was shown, with only 3 percent polymorphism. Using haemolysin sequences in *A. niger* and its primers, and AnoF and AnoR primers, a band were detected for *A. fumigatus* 1200bp long. An outcome of priming of this sequence was only similar to that of 450bp band.

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

Conclusion: The present study indicated that despite little polymorphism in haemolysin gene, it is still a proper sequence for genetic marking of *A. fumigatus.*

Key words*: Aspergillus fumigatus*, RCR-RFLP, aspHS

P-18

In vitro susceptibility testing of *Aspergillus* species against five antifungal agents

Parisa Badiee[1], Fatemeh Ghasemi[2], Hadis Jafarian[1],Seyed Ahmad Ali Dastgheib[1]

[1] *Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*

[2]*Shiraz Islamic Azad University, Shiraz, Iran*

Email: fatemeh.ghasemi30@gmail.com

Introduction: The incidence of serious infections caused by opportunistic fungi has increased dramatically in immunocompromise patients. *Aspergillus* species are the most common etiologic agent. The aim of this study was to determine the *in vitro* activities of five antifungal agents, namely amphotericin B, voriconazole, itraconazole, posaconazole, and caspofungin, against *Aspergillus* species isolated from patients.

Materials & Methods: For the purpose of data collection, a total of 68 *Aspergillus* species were collected from the patients. All samples were plated on Sabouraud 4% dextrose agar and identified by routine and restriction fragment length polymorphism methods. Susceptibility tests were performed for five antifungal agents, using Clinical and Laboratory Standards Institute M38-A2 microdilution reference method. Data were analyzed by SPSS version 18.

Results: The collected species included *A. flavus* (33), *A. fumigatus* (29), *A. niger* (4), and *A.* species (2). The MIC 50 and MIC 90 values for amphotericin B, voriconazole, itraconazole, posaconazole, and caspofungin were found to be 2.0 and 8.0 µg/ml; 0.25 and 0.75 µg/ml; 0.03 and 1.5 µg/ml; 0.03 and 0.13 µg/m, and 10.03 and 0.50 µg/ml, respectively.

Conclusion: Regarding the high MIC 90 value for amphotericin B and itraconazole, the effective antifungal agents for treatment of *Aspergillus* infections in our region were voriconazole, posaconazole, and caspofungin.

Keywords: *Aspergillus fumigatus*, *Aspergillus flavus*, Voriconazole, Amphotericin B

P-19

Iatrogenic inter-abdominal and injection site abscess formation due to *Aspergillus fumigatus* in two patients: A case report

Nahid Gholineghad[1], Tahereh Shokohi[2], Seyed Reza Aghili[2], Hosssein Karami [3], hassan Asghari lamoki [4], Zahra Seifi[1], Zeynab Bandalizadeh[1], Somayeh Alizadeh[5]

[1] *Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Invasive Fungi Research Center (IFRC), Mazandaran University of Medical Sciences, Sari, Iran*

[3] *Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran*

[4] *Specialist in Infectious Diseases & Tropical Medicine, Zare Hospital, Sari, Iran*

[5] *Boali Sina Hospital, Sari, Iran*

Email: shokohi.tahereh@gmail.com

Introduction: Aspergillosis is an opportunistic infection with a very high mortality rate, which especially occurs in diabetic, neutropenic, and immunosuppressive patients. The cutaneous aspergillosis is a rarely encountered form of aspergillosis that may occur as either primary or secondary infection. In primary cutaneous aspergillosis, the lesion occurs as a result of direct inoculation of *Aspergillus* spores at the site of injury following intravenous catheter, trauma, occlusive dressings and tapes, burns, or surgery. Among *Aspergillus* species, the most common causative agent of opportunistic infections in humans is *A. fumigatus*. Herein, we present two rare cases of iatrogenic *Aspergillus* abscess formation following neglected left foreign body and indwelling angiocatheter.

Case Presentation

Case 1: A 4-year-old girl with a history of acute lymphoblastic leukemia was diagnosed with high fever and abscess in injection site of catheter into her right hand. The specimen was obtained from aspiration of the abscess and stained with Calcofluor White for direct microscopy. Dichotomous branching septate hyphae were observed in direct examination. Culture of the purulent exudates was performed on Sabouraud dextrose agar plates and fungal colonies were grown after five days at 30°C. *Aspergillus fumigatus* was identified using morphological characterization and confirmed by molecular method. Treatment with voriconazole was administered and the patient's signs improved.

Case 2: A 45-year-old woman with a history of type II diabetes was diagnosed with chronic wound with purulent exude localized on suture line after gynecological surgery. Radiographic imaging showed foreign object and exploratory laparotomy revealed unintentional retained surgical sponge in the patient's abdomen during a surgical procedure a week previously. With sampling from the abscess in site of suture and then staining with Calcofluor White, dichotomous branching septate hyphae were observed in direct examination. The purulent exudates were cultured on Sabouraud dextrose agar plates. After growth of fungal colonies, *A. fumigatus* was identified using morphological characterization and molecular method. The patient's signs improved after treatment with voriconazole.

Conclusions: Cutaneous aspergillosis may manifest with non-specific cutaneous lesion and abscess presented by swelling and redness and exudes as an iatrogenic complication following a common care and surgical procedure. These cases are being presented to increase awareness of clinicians and pathologists regarding the fact that cutaneous aspergillosis could present as a chronic abscess that should be diagnosed accurately.

Keywords: *Aspergillus fumigatus*, abscess formation

P-20

Fungal rhinosinusitis in immunocompromised patients

Parisa Badiee, Hadis Jafarian

Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Email: hdsjafarian@yahoo.com

Introduction: Fungal rhinosinusitis can cause fatal infections in immunocompromised patients. In this study, we investigated the etiology of such infections among immunocompromised patients admitted to Nemazee Hospital, Shiraz, Iran.

Materials and Methods: In general, 67 patients with suspected rhinosinusitis, with underlying diseases such as diabetes, organ transplantation, and hematological disorders were entered into this study. Tissue samples from sinuses were cultured on Sabouraud dextrose agar. The isolated fungi were identified by routine mycology lab methods. Pathology and computed tomography scan results were collected from patient records.

Results: A total of 36 patients had documented infections by histopathology smear, and fungi were isolated from 18 patients (18/39, 46.2%). The isolated agents were 10 *Mucoraceae*, 13 *Aspergillus flavus*, 1 *Aspergillus fumigates*, and 4 *Candida* spp.

Conclusion: *Aspergillus* spp. is one of the most frequently isolated fungi from infected patients. Accurate and early diagnosis is very important for successful treatment, especially in immunocompromised patients.

Keywords: *Aspergillus flavus*, *Aspergillus fumigates*, Rhinosinusitis

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

P-21

Relationship between aflatoxin exposure and low birth weight

Aghili Seyed Reza [1], Javad Javidnia[2], Bahar Salmanian[3]
[1] *Assistant Professor, Faculty Member of Department of Medical Parasitology and Mycology /Invasive Fungi Research Centre (IFRC), School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[2] *PhD student of Medical Mycology, Student Research committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[3] *Faculty Member of Department of Science, Farhangian University, Department of Sciences, Sari, Iran*
Email: aghili70@yahoo.com

Introduction: Low birth weight (LBW) is a term used to describe babies who are born weighing less than 2,500 grams. LBW is a risk for children's health and a problem of public health in under developing countries. Prevalence of LBW based on Iranian hospital data in 2013, was stimated7% and the evidence suggests that it is on the rise during two decades. Poor-quality diets and high rates of infection in pregnancy result LBW, but the relative contributions to this subject are unknown.This study tried to show information available on the potential impact of exposure to mycotoxins, including aflatoxin in the incidence of LBW.

Materials & Methods: In this retrospective study, we did an extensive literature review of published studies about mycotoxins particularly aflatoxin and low birth weight and infant growth.

Results: Aflatoxins are toxic metabolites produced by certain fungi (*Aspergillus flavus* and *Aspergillus parasiticus*) in/on foods and feeds. Aflatoxins are a cause of hepatotoxicity, cancer and in high doses, have caused deaths from aflatoxicosis. More recently, have been reported that there were significant negative effects of aflatoxin on child growth, as well as immune modulation in prenatal and after birth. These observations are consistent with impaired fetal development, immune deficiency and gut dysfunction in animal models. Many studies suggest that aflatoxin exposure contributes to stunting, independent of and with other risk factors (About 27% impact) and high exposure aflatoxin during pregnancy lead to fetal growth restriction and LBW. A study in The Gambia found a significant association between in utero aflatoxin exposure and growth faltering in infants.

Conclusion: Our study concludes that surveillance information on exposure to mycotoxins such as aflatoxins are generally lacking outside in the development countries such as Iran. Available data from measurements of contaminated crops and through the use of disposal biomarkers in exposed society indicate that mycotoxin exposures can be high throughout development countries, as well as in Iran and other parts of Asia. However, the validity of the findings from studies on aflatoxin and LBW is uncertain because they have small sample sizes for adverse birth outcomes, and thus may not be sufficiently powered to detect important outcomes. So, to confirm this issue, need further investigation.

Key words: Mycotoxin, *Aspergillus*, Aflatoxin, Food contamination, Low birth weight

P-22

Epidemiology and microbiology of fungal diseases: A survey in South of Iran

Azadeh Karami Robati, Seyyed Amin Ayatollahi Mousavi, Sanaz Hadizadeh
Department of Mycology and Parasitology, Afzalipoor Faculty of Medical Sciences, Shahid Bahonar University of Kerman, Kerman, Iran
E-mail: karamy8926@yahoo.com

Introduction: Invasive fungal infections are a growing public health problem and cause high morbidity and mortality rates in the nosocomial setting.

Materials and Methods: In a two-year retrospective study of over 248 transplantation patients, the incidence of community-acquired fungal infections in three hospitals of Kerman, Iran was investigated. Isolated molds were identified based on colonial morphology and slide cultures. Yeast colonies were detected by growth on Corn Meal Agar (CMA) and PCR- RFLP. Community-acquired infection isolates from 127 (70.5%) patients were obtained.

Results: *Candida* spp. were the most commonly isolated invasive yeasts, and *Aspergillus* spp. were the most commonly (15%) isolated invasive moulds. *C. albicans* was the most common (51.8%) microorganism, followed by *C. glabrata*, *C. parapsilosis*, *C. kafier*. *A. fumigates* remains the most frequently isolated mold, followed by *A. niger*.

Keywords: Community-acquired fungal infections, *Aspergillus* spp., *Candida* spp.

P-23

Morphological identification of *Aspergillus* species in Zahedan

Nasser Keikha[1], Ali Jalali [2], Mohadese Shahraki [2] , Nasimeh Marghzari[1], Sanaz Aghaei gharebolagh[3], Bahman Fouladi[4]
[1] *Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*
[2] *Islamic Azad University of Zahedan, Zahedan, Iran*
[3] *Department of Medical Mycology and Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[4] *Department of Medical Mycology and Parasitology, Zabol University of Medical Sciences, Zabol, Iran*
Email: nasserkeikha@yahoo.com

Introduction: *Aspergillus* species are saprophytic fungi. Since these species are ubiquitous in the environment, people may inhale hundreds of *Aspergillus* conidia per day. *Aspergillus* conidia can cause a variety of clinical manifestations depending on the immune status of the host. The present study aimed to identify *Aspergillus* species isolated from environmental samples, using morphological (macroscopic and microscopic) characteristics.

Materials & Methods: This descriptive study employed two differential media, namely Czapek Dox agar (CZA) and Sabouraud glucose agar (SGA) with chloramphenicol for air sampling. Air samples were collected by placing the media plates in the exposure of air flow for 15 min at a height of 120 cm from the ground in five areas of Zahedan, Iran. After seven days of incubation, the plates (in triplicates) were observed for macroscopic characteristics such as colony diameter, exudates, and colony reverse and microscopic characteristics including conidiophores, vesicle, metulae, phialides, and conidia. For microscopic characteristics slides were stained with lectophenol cotton blue.

Results: According to the results of the study, a total of 330 fungal isolates were obtained. The most common *Aspergillus* species were demonstrated to be *A. fumigatus* (47.5%), *A. niger* (40.5%), and *A. flavus* (18%), respectively.

Conclusion: Our findings demonstrated that morphological methods are useful to identify the species of *Aspergillus*; however, molecular techniques are improved and become highly available.

Keywords: *Aspergillus*, Morphological identification, Zahedan

P-24

Molecular characterization of *Aspergillus niger* and *Aspergillus flavus* isolated from Tehran air using RAPD-PCR

Firoozeh Kermani[1], Masoomeh Shams-Ghahfarokhi[2], Seyed Reza Aghili[1], Mehdi Razzaghi-Abyaneh[3], Samira Dodangeh[1]
[1] *Department of Medical Mycology and Parasitology, Mazandaran University of Medical Sciences, Sari, Iran*
[2] *Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
[3] *Department of Mycology, Pasteur Institute of Iran, Tehran, Iran*
Email: kermani.f94@gmail.com

Introduction: *Aspergillus* species have recently caused increasing numbers of life-threatening acute invasive infections, particularly respiratory diseases in immunocompromised

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

patients. *Aspergillus* spores exist in the indoor and outdoor air; therefore, they can easily reach the respiratory systems. The antifungal drug resistance is currently the subject of detailed investigations and reviews. Regarding this, the use of molecular characterization for identification of *Aspergillus* species in the inhaled air is very important and usually random amplification of polymorphic DNA polymerase chain reaction (RAPD-PCR) technique is used for detecting the genetic variability.

Materials and Methods: For the purpose of data collection, 18 *Aspergillus* isolates were collected including two species of *A. niger* and *A. flavus*. Out of the collected samples, 11 isolates were *A. niger* obtained from the air of districts 1, 4, 8, 11, and 18 and seven isolates were *A. flavus* obtained from the air of districts 2, 3, 19, and 22 of Tehran Municipality. The two species were subjected to RAPD-PCR using 7 primers for determining the molecular characterization and fingerprint patterns.

Results: The dendrogram obtained from the data by 7 random decamer primers showed that 11 isolates of *A. niger* had 41 % similarity to each other and they were resided in one group with three branches. However, 7 isolates of *A. flavus* had 31% similarity. In this species, 6 isolates were in one group with two branches and only one isolate were resided in one branch similar to *A. niger* cluster. In addition, *A. niger* and *A. flavus* were 15 % similar.

Conclusion: Our results showed that the examined *Aspergillus* species from air of different extents of distribution in Tehran had a significant genetic variation. Consequently, the genetic variation frequently happens among isolates of different areas. RAPD was concluded to be a useful technique for phylogenetic evaluation, which can be used as a complementary technique for differentiation of *Aspergillus* species when a little variation in morphological characters exists.

Keywords: *Aspergillus*, Outdoor air

P-25

Outdoor and indoor profile and seasonal variation of airborne Aspergilli spores in Shiraz (southern Iran)

Hossein Khodadadi[1], Ladan Karimi[2], Mohammad Reza Samaei[3], Fariba Abbasi[3]

[1] *Department of Medical Mycology and Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[2] *Dr. Beheshti Hospital, Social Security Organization, Shiraz, Iran*

[3] *Department of Environmental Health Engineering, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran*

Email: h_khodadadi@sums.ac.ir

Introduction: *Aspergillus* species are important causes of respiratory disorders. However, data is limited regarding the diversity, load, seasonal distribution and environmental sources of airborne *Aspergillus* spores. Although recent studies suggest that *Aspergillus flavus* is a dominant clinical isolate in Iran, *A. fumigatus* is responsible for the majority of pulmonary aspergillosis cases in other regions of the world. This study aimed to describe the indoor and outdoor air levels and profiles of *Aspergillus* spores and determine their associations in Shiraz, Iran.

Materials and Methods: Via According to the CDC bioaerosol sampling method (NIOSH 0800), 128 air samples were collected with a portable Andersen Air Impactor from different points of a compost facility, two hospitals and urban parts of Shiraz. Every point was sampled three times in summer and winter 2015. samples were cultured on Czapeck and Sabouraud chloramphenicol agar media. *Aspergillus* species were identified conventionally and viable *Aspergillus* CFU/m3 were counted. Differences between the selected environments were investigated statistically.

Results: In total, 125 *Aspergillus* species were isolated in this study, the foremost of which were *A. flavus* (38%), *A. niger* (34%), *A. fumigatus* (26%), and *Aspergillus* spp. (2%), respectively. *A. flavus* was determined as the predominant species in all regions during summer, while *A. niger* was the predominant species in the compost facility during winter. Level of *A. fumigatus* spores was mostly lower than the recommended quantity, while the concentrations of *A. flavus* and *A. niger* were extremely high in most outdoor sites. Seasonal variations of outdoor *Aspergillus* concentrations were affected by temperature, wind speed and humidity. Moreover, levels of *Aspergillus* spores were lower than the recommended amount in all indoor regions.

Conclusion: Profiles and seasonal distribution patterns of *Aspergillus* spores depend on the changes in atmospheric parameters (e.g., temperature, wind speed and humidity). In this study, profile patterns of airborne fungal spores were different due to the hot and dry climate of the southern region of Iran compared to areas with a moderate climate. Domination of environmental *A. flavus* spores in the majority of the studied regions could explain the higher isolation of *A. flavus* from Iranian patients compared to *A. fumigatus*. High concentration of airborne fungal spores in landfill sites is alarming. Therefore, fungal infections originated from outdoor environments must be prevented in workers and high-risk populations.

Keywords: *Aspergillus*, Spore levels, Outdoor, Seasonal variations

P-26

Coexistence of aspergilloma and pulmonary hydatid cyst

Sadegh Khodavaisy[1], Shirin Sadat Hashemi Fesharaki[2,3], Hamid Badali[3], Mohammadali Boroumand[2], Maryam Sotoudeh Anvari[2], Mohsen Gramishoar[1], Zahra Abtahian[4], Setareh Agha Kuchak Afshari[1], Elahe Sasani[1]

[1] *Department of Medical Parasitology and Mycology, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Department of Pathology and Laboratory Medicine, Tehran Heart Center, Tehran University of Medical Science, Tehran, Iran*

[3] *Department of Medical Mycology and Parasitology, Invasive Fungi Research Center (IFRC), School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[4] *Shahid beheshti university of medical sciences, Tehran, Iran*

Email: sadegh_7392008@yahoo.com

Introduction: Hydatid cyst is a zoonotic disease caused by *Echinococcus granulosus* and *Echinococcus multiloculari.* The liver and lungs are the most common sites of infection although other organs are involved, as well. Pulmonary echinococcal hydatid cysts have been reported coexistent with aspergilloma.

Case Presentation: In the current study, we report the case of successful treatment of the most disseminated coinfection of the hydatid cyst and aspergilloma due to *Echinococcus granolosus* and *Aspergillus flavus* (as identified based on molecular tools), respectively, in a 34-year-old female. *In vitro* antifungal susceptibility tests revealed that the minimum inhibitory concentrations for the antifungals used in this case in increasing order were posaconazole (0.031 µg/ml), itraconazole (0.125 µg/ml), voriconazole (0.25 µg/ml), and amphotericin B (1 µg/ml). The minimum effective concentration for caspofungin was 0.008 µg/ml.

Conclusion: This unique coexistence of active pulmonary echinococcosis and aspergillosis is reported because of its rarity and clinical importance for its management.

Keywords: Aspergilloma, Hydatid cyst, *Aspergillus flavus*

P-27

In vitro antifungal activities of amphotericin B, caspofungin, and fluconazole against *Aspergillus terreus*

Neda Kiasat[1], Mahdis Zargaran[1], Simin Taghipour[1], Ali Zarei Mahmoudabadi[1,2]

[1] *Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[2] *Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Email: nedakiasat1402@gmail.com

Introduction: *Aspergillus terreus* is the fourth leading cause of aspergillosis and one of the agents of morbidity and mortality

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

among immunocompromised and high-risk patients. The prevalence of *Aspergillus terreus*, a cause of opportunistic infections (from superficial to serious invasive infections), is on a growing trend. Although invasive aspergillosis is often treated empirically with amphotericin B, most *A. terreus* isolates are drug resistant both *in vivo* and *in vitro*. The current study aimed to evaluate antifungals susceptibility profile of different environmental strains of *Aspergillus terreus* against amphotericin B, caspofungin, and fluconazole.

Materials and Methods: Forty *A. terreus* strains were isolated from environmental sources (air and soil) and identified, using macroscopic and microscopic features. Three antifungal drugs including amphotericin B, caspofungin, and fluconazole were applied for susceptibility test according to CLSI Broth Microdilution Method (M38-A2).

Results: The results of the study demonstrated that all the tested isolates had caspofungin MEC90 (4 µg/ml) higher than the epidemiological cutoff value, whereas only 20% (8) of the isolates exhibited amphotericin B MICs of ≤ 4 µg/ml. On the other hand, fluconazole showed a MIC90 of ≥ 128 µg/ml with a range of ≤ 1 to ≥ 128 µg/ml.

Conclusion: This study demonstrated that caspofungin had a better *in vitro* activity against all tested isolates of *Aspergillus terreus*, with MICs lower than two other antifungal drugs.

Keywords: amphotericin B, caspofungin, fluconazole, *Aspergillus terreus*

P-28

Role of drain fly (Diptera: Psychodinae) as a mechanical vector of *Aspergillus* spp. in hospitals

Mohsen Karami[1], Ramazan Rajabnia[2], Saeid Mahdavi Omran[1], Ali Heidarpour[3], Aynaz Khademian[2], Jaber Alipour[1], Maryam Sadat Shafiee[1]

[1] *Infectious Diseases & Tropical Medicine Research Center, Faculty of Medicine, Department of Medical Mycology and Parasitology, Babol University of Medical Sciences, Babol, Iran*

[2] *Infectious Diseases & Tropical Medicine Research Center, Department of Microbiology, Babol University of Medical Sciences, Babol, Iran*

[3] *Health Center, Babol University of Medical Sciences, Babol, Iran*

Email: s.mahdavi@mubabol.ac.ir

Introduction: Some arthropods, such as drain flies (moth flies), could act as mechanical vectors for pathogenic microorganisms, including fungi. Drain flies are found in many human and animal dwellings. This study aimed to isolate and identify the *Aspergillus* spp. carried by drain flies.

Materials and Methods: This study was conducted on 54 adult flies collected from three hospitals and one human dwelling in Babol, located in the north of Iran, during June-September 2016. *Aspergilli* were isolated from the external and internal body surfaces of the flies using standard methods.

Results: In total, 100 specimens were cultured from 50 adult drain flies. *Aspergilli* were isolated from the external and internal body surfaces of the flies, and five species of *Aspergillus* were identified, as follows: *Aspergillus flavus* (15%), *A. fumigatus* (14%), *A. terreus* (3%), *A. niger* (2%), *Aspergillus* spp. (2%) and mixed isolates (14%). Moreover, infection and colonization rates of external and internal surfaces were determined at 44% and 54%, respectively.

Conclusion: According to the results of this study, drain flies could act as potential vectors of nosocomial infectious agents. Therefore, presence of drain flies in human environments must be taken into account, particularly in healthcare and medical centres. Drain flies could pick microbial and fungal agents from breeding places.

Keywords: *Aspergillus*, Drain flies, Diptera, Psychodinae, Babol, Iran

P-29

A retrospective study on invasive aspergillosis in patients referred to the Medical Mycology Laboratory, School of Public Health, Tehran, Iran

Mohsen Gerami Shoar, Seyed Jamal Hashemi, Roshanak Daie Ghazvini, Ensieh Zibafar, Leila Hosseinpour, Zeinab Borjian, Shahram Mahmoudi, Ladan Nazemi, Niloofar Rashidi, Mohamad Yarahmadi

Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences

Email: sh.mahmoudi93@gmail.com

Introduction: Invasive aspergillosis is a life-threatening infection caused by the common *Aspergillus* species in immunocompromised patients. This study was conducted on patients referred to the Medical Mycology Laboratory, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran to provide data on the incidence of Aspergillosis, which could be beneficial for both medical mycologists and clinicians.

Materials and Methods: This study was conducted on all patients suspected of having invasive fungal infections during a course of 30 months (April 2014-September 2016). Samples of *Aspergillus* species were aseptically obtained in different hospitals and transferred to the Medical Mycology Laboratory. Direct examination was performed on all the samples using 10% potassium hydroxide and through culture on Sabouraud dextrose agar with chloramphenicol and brain-heart infusion medium. Moreover, causative agents were recognized using additional slide cultures.

Results: Among the total 1232 evaluated specimens, direct examination for fungal elements was negative for 1001 (81.25%) of the cases, and 53 (22.9%) species of the 231 positive cases were identified as *Aspergillus*. The majority of direct examination and culture positive cases of *Aspergillus* spp. were among the specimens collected from the respiratory system (36 of 53). Moreover, the most common causative agents were *Aspergillus flavus* (60.38%), *A. niger* (26.41%), *A. fumigatus* (11.32%) and *A. nidulans* (1.89%), respectively.

Conclusion: According to the results of this study, *A. flavus* was identified as the most common pathogenic species. While *Aspergillus* fungal infection could occur in many human tissues, our results were indicative of rare infections caused by *Aspergillus* spp.

Keywords: *Aspergillus*, Fungal infection, Retrospective studies

P-30

Incidence of *Aspergillus* onychomycosis in Noor Pathobiology Laboratory of Tehran, Iran

Mohsen Gerami Shoar, Ensieh Zibafar, Shahram Mahmoudi, Omid Raiesi

Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Email: sh.mahmoudi93@gmail.com

Introduction: In addition to dermatophytes and yeasts, non-dermatophyte moulds constitute the third group of fungal agents to cause onychomycosis. As ubiquitous organisms, *Aspergillus* species are considered to play a key role in fungal nail involvement.

Materials and Methods: This retrospective study was conducted within a two-year period (September 2014-September 2016) on all the patients clinically suspected of onychomycosis. Mycological investigations, including direct examination and culture on appropriate media, were performed. Etiological agents were identified based on the macroscopic and microscopic characteristics of the colonies.

Results: In total, 334 patients were enrolled in mycological investigations, and onychomycosis was confirmed in 54 cases (16.16%). *Aspergillus* species were determined as the most important etiological agents in this regard, accounting for 23 cases of onychomycosis (42.59%), followed by *Candida* species (n=17, 31.48%), dermatophytes (n=11, 20.37%), and non-

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

Aspergillus non-dermatophyte moulds (n=3, 5.5 %). *Aspergillus flavus* was the most dominant *Aspergillus* species isolated from 15 patients, followed by *Aspergillus niger* (n=3) and *Aspergillus fumigatus* (n=1). Moreover, four isolates of *Aspergillus* were not identified to the species level.

Conclusion: According to the results of this study, saprophytic onychomycosis was more prevalent compared to dermatophyte onychomycosis. Furthermore, *Aspergillus* species were the leading agents to cause onychomycosis, among which *Aspergillus flavus* was the most frequent species.

Keywords: *Aspergillus*, Onychomycosis, Noor Pathobiology Laboratory

P-31

Management of aflatoxin producing fungi in peanut (*Arachis hypogaea* L.) varieties in Mereb Leke through soil solarization and planting time

Kahsay Tadesse Mawcha[1], Amare Ayalew[2], Dereje Assefa[3]
[1] *Aksum University Shire Campus Shire-Ethiopia*
[2] *Haramaya University college of Agriculture Haramaya-Ethiopia*
[3] *Mekelle University college of Agriculture Mekelle-Ethiopia*
Email: tadesekahsay@gmail.com

Introduction: Groundnut (Peanut) is economically important oil and food crop. However, owing to lack of appropriate management practices, aflatoxigenic fungi and subsequent aflatoxin contamination can be an extremely serious health problem. Soil solarisation could increase the soil temperature and could minimize fungal inoculum in the soil. Use of different planting times could help overcome conditions of drought stress and elevated soil temperature, which are known to favour preharvest aflatoxin contamination.

Materials and Methods: Laboratory and field experiments were conducted in northern Ethiopia. These experiments were performed at two locations to determine the effect of soil solarization on *Aspergillus* species inoculums in the soil and to evaluate the effect of soil solarization along with time of planting on *A. flavus* seed invasion. As a result, 80 soil samples were taken in three rounds and analysed for aflatoxigenic species. Seed samples (80) were plated on Czapek-Dox agar medium. Individual and total CFU $g^{-1}$of soil were determined before and after solarization and at harvest time.

Results: The results of the current study revealed that soil solarization reduced fungal inoculums. The identified *Aspergillus* species included *A. flavus*, *A. parasiticus*, *A. niger*, and *A. terreus*. In addition, after soil solarization, *A. flavus* and *A. parasiticus* reduced in the solarized plots by 53.8% and 45% CFU g^{-1} in Ramma and 36.4% and 44% CFU g^{-1} at 5 and 10 cm soil depths in Mayweyni, respectively. Moreover, *A. niger* was the most dominant species (0.6 x 10^3 and 1.6 x10^3 CFU g^{-1}soil) at 5 cm soil depths in Mayweyni and in Ramma, respectively. At harvest time, *Fusarium* species, *A. flavus* and *A. terreus* were detected from soils. Three *Aspergillus* species namely, *A. flavus*, *A. niger*, and *A. parasiticus* were isolated from seed samples and early planting of the varieties showed the lowest level of seed invasion by *A. flavus* (22.8%).

Conclusion: Preharvest seed invasion of groundnuts could be minimized by using soil solarisation and appropriate agronomic practices, *i.e*., planting times and planting suitable varieties.

Keywords: Peanut, Aflatoxin, Solarization, *Aspergillus*

P-32

Comparative and molecular study of fungal species contaminating livestock and poultry feed by aflatoxin in Gonabad, Iran

Mohammad Hassan Minooeian Haghighi [1], Ali Marvi Shahri[2], Farzad Katiraei [3]
[1] *Department of Medical Microbiology, Faculty of Medicine, Gonabad University of Medical Sciences, Gonabad, Iran.*
[2] *Faculty of Sciences, Damghan Islamic Azad University, Damghan, Iran*
[3] *Faculty of Veterinary Medicine, University of Tabriz, Tabriz,Iran.*
Email: drminooeian@gmu.ac.ir

Introduction: Humans keep domestic animals in their homes due to the need for livestock and poultry products. In addition, molds, such as *Aspergillus*, infect animal and human food products with dangerous toxic substances, including aflatoxin that can cause primary and secondary mycotoxicosis in animals and humans. In the current study, we aimed to compare fungal species producing aflatoxin in livestock and poultry feed in Gonabad, Iran.

Materials and Methods: In this experimental study, we randomly collected feed samples from two livestock feed factories and two poultry feed factories during summer and autumn three times according to sanitary conditions. Four forms of samples, consisting of solid disinfected samples, solid non-disinfected samples, suspension disinfected samples, and suspension non-disinfected samples, were cultured in Sabouraud dextrose agar with chloramphenicol for five days at 28°C. Microscopic and molecular diagnostic tests, as well as polymerase chain reaction (PCR) technique were employed to determine fungal species in the culture based on β-tubulin gene sequencing.

Results: Microscopic diagnostic tests of culture for fungi in 24 samples of two livestock and two poultry factories detected a mean of 27.25% and 31.70% *Aspergillus* contamination, respectively; the rest were other types of fungi. Molecular PCR test detected 4 *Aspergillus flavus*, 2 *A. fumigatus*, 2 *A. versicolor*, 1 *A. parasiticus*, 1 *A. ochraceus*, 1 *A. tereus*, and 2 *A. niger* contamination cases. The results demonstrated a significant difference between products of the two poultry feed factories (P=0.017) and between products of the livestock feed factories and poultry feed factories (P=0.008) regarding *Aspergillus* infection. There was also a significant difference between the mixture of all livestock and poultry feed factories in summer and autumn (P=0.008). Independent t-test and Mann-Whitney tests were run using SPSS.

Conclusion: Compared to livestock feed, poultry feed is more commonly contaminated to *Aspergillus*, a producer of mycotoxins like aflatoxin. One of the poultry feed factories showed the highest rate of contamination and one of the livestock feed factories had the lowest rate of contamination to *Aspergillus*. The rate of contamination in autumn was higher than in summer. The use of molecular diagnostic test based on β-tubulin gene sequencing is more reliable than the morphological method. Therefore, this method can help provide educational and preventive programs to prevent contamination of livestock and poultry feed, which can in turn, infect human feed products with dangerous toxic substances such as aflatoxin.

Keywords: Livestock feed, Poultry feed, *Aspergillus*, Aflatoxin, β-tubulin gene

P-33

Solid lipid nanoparticles as an effective carrier of voriconazole to overcome the resistant isolates of *Aspergillus fumigatus*

Maryam Moazeni[1, 2], Hamid Reza Kelidari[3], Roghayeh Babaei[4], Sara Gholami[1, 2], Mojtaba Nabili[4], Atefeh Abdollahi Gohar[4]
[1]*Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[2]*Invasive Fungi Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
[3]*Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
[4]*Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*
Email: moazeni.maryam@gmail.com

Introduction: Recently, Emergence of triazole-resistant *Aspergillus fumigatus* infections has become a major healthcare concern due to the long-term use of azole antifungals. According to recent studies, prevalence of azole-resistant *A. fumigatus* in Iran has increased remarkably from 3.3% to 6.6% in comparison with earlier epidemiological research. These findings may indicate a novel phase in the management of invasive

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

aspergillosis. This study aimed to prepare voriconazole-loaded solid lipid nanoparticles (VRC-SLNs) and investigate the efficacy of the optimal formulation on *A. fumigatus* species.

Materials and Methods: In this study, VRC-SLNs were prepared through probe ultrasonication. Properties of the obtained SLNs were characterized by photon correlation spectroscopy for average particle size and zeta potential at the temperature of 25◦C and a fixed angle of 90◦. Moreover, morphology of the obtained nanoparticles was determined via transmission-electron microscopy. Minimum inhibitory concentrations (MIC) for the new formulations against *Aspergillus* strains were investigated in accordance with the Clinical and Laboratory Standards Institute document M38-A2 as a guideline on 62 clinical and environmental *A. fumigatus* isolates.

Results: VRC-SLNs presented a spherical shape with a mean diameter and zeta potential of 114.3±21.3 nm and -15.6±2.1 mV, respectively. Drug release from VRC-SLNs exhibited burst-release behaviors in the initial stage, followed by sustained release over 24 h. In addition, the new formulation of voriconazole significantly decreased the MICs for all *Aspergillus* isolates (VRC-susceptible or VRC-resistant) ($P<0.05$). MIC_{50} drug concentration was obtained as 0.015 µg/ml for both VRC-susceptible and VRC-resistant strains of *A. fumigatus*, while it was determined at 0.25 µg/ml for VRC-susceptible isolates.

Conclusion: In this study, we evaluated a novel drug delivery system, which could be incorporated into a strategy to improve the antifungal activity of voriconazole against *A. fumigatus* strains with different susceptibilities to conventional formulations of voriconazole.

Keywords: *Aspergillus*, Solid lipid nanoparticles, Voriconazole, Resistant, TEM

P-34

Determination of antifungal susceptibility patterns among the environmental isolates of *Aspergillus fumigatus* in Iran

Faezeh Mohammadi[1], Seyed Jamal Hashemi[1], Parvin Dehghan[2], SeyedMojtaba Seyedmousavi[3]

[1] *Department of Medical Mycology and Parasitology, School of Hygiene and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Department of Medical Mycology and Parasitology, Isfahan University of Medical Sciences, Isfahan, Iran*

[3] *Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Health (NIH), Bethesda, MD, USA*

Email: faezehmohamadi119@yahoo.com

Introduction: In recent years, triazole-resistant environmental isolates of *Aspergillus fumigatus* have emerged in Europe and Asia. Azole resistance has been reported in patients treated with long-term azole therapy or exposure of the fungus spores to the azole fungicides used in agriculture. To date, a wide range of mutations in *A. fumigatus* have been described conferring azole-resistance, which commonly involves modifications in the *cyp51A* gene. Herein, we investigated antifungal susceptibility pattern of environmental isolates of *A. fumigatus.*

Materials and Methods: A total of 170 environmental samples were collected from indoor surfaces of three hospitals in Iran. We used β-tubulin gene to confirm all *A. fumigatus* isolates that were identified by conventional methods. Furthermore, the antifungal susceptibility of itraconazole, voriconazole, and posaconazole was investigated using broth microdilution test, according to European Committee on Antimicrobial Susceptibility testing reference method.

Results: From a total of 158 environmental fungi obtained from hospitals, 58 isolates were identified as *A. fumigatus* by amplification of expected size of β-tubulin gene. In this study, *in-vitro* antifungal susceptibility testing demonstrated that minimum inhibitory concentration values of triazole antifungals were not high in all the 58 environmental isolates of *A. fumigatus*.

Conclusion: Our findings demonstrated that environmental isolates of *A. fumigatus* were not azole-resistant. Medical triazole compounds have structural similarity with triazole fungicide compounds in agriculture; therefore, resistance development through exposure to triazole fungicide compounds in the environment is important, but it seems that drug resistance in environmental isolates has not caused serious health problems in Iran.

Keywords: Azole resistance, *cyp51A* gene, Triazole

P-35

Evaluation of anti-aspergillosis properties and identification of chemical constituents of *Thymus kotscuyanus*

Abdelnasser Mohammadi Gholami

Faculty of Science, Department of Biology, Lorestan University, Khorramabad, Iran

Email: nnnasser@yahoo.com

Introduction: *Aspergillus* genus is filamentous fungi consisting of various groups and species. Species identification of these fungi is important from pathogenic, toxigenic, and industrial points of view. Due to the development of drug resistance of microorganisms and increasing interest in the use of herbal medicine, in this study, the chemical composition and anti-aspergillosis effects of *Thymus Kotschyanus* against *Aspergillus fumigatus* (PTCC 5009), *A. flavus* (PTCC 5004), and *A. niger* (5013) were studied.

Materials and Methods: Herein, *Thymus kotscuyanus* compounds were identified using gas chromatography–mass spectrometry, and its antifungal activity against *Aspergillus* species was determined by means of microdilution broth method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).

Results: The chemical analysis of *Thymus kotscuyanus* resulted in identification of 45 compounds, of which carvacrol (15.35%), Thymol (12.2%), and gamatrpynn (4.6%) formed the major oil mixtures. MICs and MBCs of fungi were determined to be within the range of 0.125-0.25 µg/ml and 0.25-0.05 µg/ml, respectively.

Conclusion: Our results indicated that the antifungal activity of *Thymus kotscuyanus* is high. Therefore, regarding the drug resistance and side effects of chemical antifungal drugs, further studies should be performed on *Thymus kotscuyanus* as a natural antifungal agent.

Keywords: *Thymus kotschyanus*, Essential oil, Microdilution broth method, Anti- aspergillosis

P-36

Evaluation of antifungal activity of silver nanoparticles synthesized by the method of green on *Aspergillus niger*

Abdelnasser Mohammadi Gholami

Assistant Professor, Faculty of Science, Department of Biology, Lorestan University, Khorramabad, Iran

Email: nnnasser@yahoo.com

Introduction: Currently, fungal infections caused by opportunistic fungi have been common, particularly in people with special conditions, including weak immune system, pregnancy and diseases similar to HIV. Drug resistance, especially in recent decades, has led to a search for different approaches and methods to discover new compounds against bacteria and fungi. This study aimed to evaluate the effects of silver nanoparticles synthesized by the green method against *Aspergillus niger* isolates obtained from environmental samples.

Materials and Methods: In this study, green synthesis of silver nanoparticles was carried out using natural honey as a reducing agent reaction. Six isolates of *Aspergillus* were separated from food and air in different environments. The isolates were subcultured on sabouraud dextrose agar and Czapek dox agar, followed by the purification of colonies. All isolates were identified to species level using slide culture and sequencing of gene ITS1 across species. Antifungal activity of silver nanoparticles was detected according to CLSI method (M38-A2)

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

using broth microdilution. Plates were incubated at 35°C for 72 h, and *A. niger* (PTCC 5013) and amphotericin B was used as control.

Results: The stability and size distribution of synthesized silver nanoparticles were described using scanning electron microscope, as well as UV-visible spectroscopy, SEM, energy dispersive X-ray (EDX) and Fourier transform infrared spectroscopies (FTIR). MIC and MBC of silver nanoparticles and amphotericin B on all *Aspergillus* isolates were estimated at (4-8 µg/ml, 8-16 µg/ml) and (0.5-1 µg/ml, 1-2 µg/ml) in microdilution method, respectively**.**

Conclusion: According to the results of this study, significant antifungal activities were observed in silver nanoparticles, compared to the standard dose of amphotericin B. Therefore, it is recommended that further studies be carried out to evaluate clinical applications of this compound and replacing it with antifungal medications.

Keywords: Silver nanoparticles, Broth microdilution method, *Aspergillus niger*

P-37

Morphological and molecular identification of clinical *Aspergillus* species based on beta-tubulin gene sequencing

Rasoul Mohammadi[1,2], Mahnaz Kheirkhah[1]

[1]*Department of Medical Parasitology and Mycology, School of Medicine*
[2]*Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

Email: dr.rasoul_mohammadi@yahoo.com

Introduction: *Aspergillus* species are opportunistic pathogens among immunocompromised patients. In terms of pathogenesis and mycotoxin production, they are in great value. The aim of the present study is evaluating of beta-tubulin gene for identification of clinical *Aspergillus* species by PCR-sequencing method compared to morphological features of clinical isolates.

Methods: In this study, 465 patients referred to the Shefa laboratory of Isfahan were evaluated. Morphological and molecular identification of clinical samples was performed using culture on sabouraud agar, malt extract agar, czapek dox agar, direct microscopy, and PCR-sequencing of beta tubulin gene, respectively. Sequences were analyzed in comparison with gene bank data.

Results: Thirty nine out of 465 suspected cases (8.4%) had aspergillosis. The most prevalent species were *Aspergillus flavus* (56%), *A. oryzae* (20%), and *A. fumigatus* (10%), *Aspergillus tubingensis* (5%), *Aspergillus niger* (2.5%), *Aspergillus terreus* (2.5%), and *Aspergillus awamori* (2.5%), respectively. Fifty nine percent of patients were females and 49% were males. Clinical samples were obtained from nail (59%), sputum (13%), BAL (13%), skin (8%), ear (5%), and paranasal sinuses (2%).

Conclusion: In comparison with phenotypic tests, sequencing of beta-tubulin gene for identification of *Aspergillus* species is at great value. Replacement of molecular techniques with conventional tests is recommended for precise identification of microorganism for better management of infection.

Keywords: *Aspergillus*, Beta tubulin, Sequencing

P-38

Aspergillus vaccine challenges in immunocompromised patients

Hamid Morovati

Department of Medical Mycology and Parasitology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Email: morovatihamid1989@gmail.com

Introduction: Some of the vaccine candidates have already gone through clinical trials phase I in humans, showing good progress toward the development of an efficient method of fungal immunization. However, some other candidates are experiencing setbacks due to several issues. The fact that fungal pathogens mostly affect immunocompromised subjects greatly limits the generation of fungal vaccines. Thus, it is important that fungal vaccines could also elicit protection in immunocompromised subjects without any risk of aggravation of the underlying disease or development of the fungal disease due to vaccination.

Heat killed saccharomyces (HKS)-Immunity against *Aspergillus* and other fungi has been observed after vaccination with heat killed saccharomyces, which are able to induce Th1, Th2, Th17 cytokine profiles (antibodies?).

Antibodies-Recombinant antibodies that induce Th1 immunity includeAsp16f, Asp3f, Pep1p, Gellp, Crfl, which also act as monoclonal antibodies against beta-1,3-glucan and HSP-90 (Mycograb).

Antigens and cell wall components -Antigens and cell wall components could induce cell-mediated immunity and antibodies, as well as β-1,3-glucan and cell wall glucanase growth inhibitors (p41or crfl).

Recently, a pan-fungal vaccine was demonstrated using β-glucans of *S.cerevisiae* to generate protection against several pathogenic fungi (e.g., *A. fumigatus*). Interestingly, there was no need for an adjuvant to generate protection.

Conclusion: The world population is changing and fungal infections inimmunocompromised subjects is continuously rising, which will eventually lead to the preference of recombinant vaccines. On the other hand, development of a pan-fungal vaccine, especially in dendritic cell-based vaccines area, which protects human beings from this disease, might be one of the most promising strategies so far. Given the consideration of development of new antifungal agents as a priority in academia and industry, we must invest in the field of fungal vaccines, even if the revenue is less than those for bacterial and viral vaccines.

Keywords: *Aspergillus*, vaccine, immunocompromised patients

P-39

Overview of the epidemiology of antifungal susceptibility of *Aspergillus* section *Fumigati*

Vida Mortezaee[1,2], Newsha Hedayati[2], Mahshid Vakili[1], Mahin Tavakoly[1], Maedeh Maleki[1], Mahdi Abastabar[1], Mojtaba Taghizadeh Armaki[1], Mohammad T. Hedayati[1]

[1]*Invasive Fungi Research Center (IFRC), Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[2]*Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

Email: hedayatimt@gmail.com

Introduction: *Aspergillus* section *Fumigati* includes the fungal species that are the common causes of threatening infections in humans and animals. These important organisms could degrade organic matter, such as foodstuffs. Due to their similar appearance, morphological differences of the teleomorphic and anamorphic species of *Aspergillus* section *Fumigati* are extremely limited. Therefore, molecular techniques are routinely used for their differentiation and identification. Currently, section *Fumigati* constitutes more than 50 species of anamorph (*Aspergillus*) and teleomorph (*Neosartorya*) genera.

Materials and Methods: This review was conducted in various databases, including Google Scholar, Scopus, Wiley Online, Springer, Elsevier , PubMed, and ScienceDirect, using key words such as *A. fumigatus, A. fumigati* section, antifungals, *A. lentulus*, azole resistance, and *Neosartorya fischeri*. Articles accepted or published during 1997-2016 were reviewed in this study.

Results: In this section, we determined the resistant species to the main antifungal drugs used for aspergillosis treatment (e.g., itraconazole, miconazole, posaconazole, ravuconazole, and voriconazole). Resistant species to the mentioned drugs were remarkable in *A. lentulus*, *N. udagawae* and *N. pseudofischeri*. Moreover, some of these species such as *A. lentulus* showed more resistance to available antifungal agents, while *N. udagawae* and *N. pseudofischeri* showed variable sensitivity to triazoles.

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

Therefore, accurate identification of these fungal species is of paramount importance for the optimization of aspergillosis treatment.

Conclusion: According to the results of this review, prevalence of antifungal resistance in *Aspergillus* species of different sections, especially section *Fumigati*, must be determined, in order to improve patient outcomes and reduce the rate of unsuccessful aspergillosis treatment. Considering the increasing number of high-risk patients for fungal infections due to immune system defects, long-term use of corticosteroids, chemotherapy, immunosuppressive diseases, *Aspergillus* infections and resistance in the agents causing aspergillosis are considered as an important research field.

Keywords: *Aspergillus fumigatus*, *Aspergillus* section *Fumigati*, Antifungal susceptibility, Resistant *Aspergillus*

P-40

Two different *Aspergillus* species in the sputum and BAL of a CF patient with allergic bronchopulmonary aspergillosis: A case report

Maryam Hasanzad[1], Vida Mortezaee[1,2], Mohammad Taghi Hedayati[2], Somayeh Sharifynia[3], Maedeh Maleki[2], Ali Akbar Velayati[3]

[1] Pediatric Respiratory Diseases Research Center (PRDRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2]Invasive Fungi Research Center (IFRC), Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

[3]Clinical Tuberculosis and Epidemiology Research Center (CTERC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

Email: vidamortezaee@gmail.com

Introduction: Airway inflammation and chronic lung infection are common findings in cystic fibrosis (CF). Allergic bronchopulmonary aspergillosis (ABPA) is the manifestation of a hypersensitivity reaction to *Aspergillus* spp., especially *Aspergillus fumigatus*. ABPA could be caused by other species of *Aspergillus* as well. In CF patients, ABPA is accompanied by a significant reduction of lung function and pulmonary insufficiency.

Materials and Methods: In this paper, we report a 12-year-old male patient as a confirmed case of CF with ABPA diagnosis (based on the Rosenberg-Patterson criteria), by elevated *Aspergillus*-specific IgE and IgG using the ImmunoCap method and increased total IgE using the ELISA method. CT-scan and radiological examination of the patient revealed lung collapse and central bronchiectasis. In addition, laboratory tests were indicative of a significant increase in peripheral eosinophil count. Bronchoscopy was performed for controlling the lung collapse, bronchial washing, and pulmonary discharge sampling. Moreover, three sputum samples and two bronchoalveolar lavage (BAL) samples of the patient were cultured. Afterwards, we carried out antifungal susceptibility testing for the *Aspergillus* species isolated from the clinical samples of the CF patient and molecular tests for the diagnosis of *Aspergillus species* was carried out.

Results: In the sputum and BAL cultures, two *Aspegillus* species of two different sections were isolated by PCR test and sequencing method. Additionally, *A. flavus* was detected in three cultured samples of sputum, while *A. flavus* and *A. terreus* were isolated from two cultured BAL samples. According to antifungal susceptibility testing, *A. terreus* was resistant to amphotericin B, and both species were sensitive to voriconazole. Therefore, the patient was prescribed with voriconazole, which resulted in a favorable treatment outcome.

Conclusion: According to the results, satisfactory treatment outcomes could be attained through detecting various *Aspergillus* species and their sampling regions and types, as well as performing antifungal susceptibility testing for different isolates of *Aspergillus*.

Keywords: Cystic fibrosis, Allergic bronchopulmonary aspergillosis, *Aspergillus flavus*, *Aspergillus terreus*, Bronchiectasis

P-41

Growing incidence of non-dermatophyte onychomycosis in Tehran, Iran

Marjan Motamedi[1,2], Hossein Mirhendi [1,3], Zeinab Ghasem [1], Mohammad Reza Shidfar[1], Leila Hosseinpour[1], Kambiz Zomorodian[2], Hossein Khodadadi [2]

[1] Department of Medical Parasitology and Mycology, School of Public Health, National Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Medical Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[3] Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

E-mail: mirhendi@tums.ac.ir

Introduction: Non-dermatophyte onychomycosis (NDO) is caused by a wide range of mold fungi other than dermatophytes and has been reported at various rates in different countries worldwide. Studies on the incidence of NDO in the community are essential for understanding its epidemiology and control, as well as for the appropriate treatment of these infections. In this study, the incidence of NDO in Tehran, Iran, was compared to the incidence of onychomycoses due to dermatophytes and yeasts.

Materials and Methods: During 2014-2015, samples from a total of 1,069 patients with suspected fungal nail diseases, who were referred to three medical mycology laboratories in Tehran, Iran, were collected and subjected to direct examination (all samples) and culture (788 samples). Differentiation of the causative agents of onychomycosis was based on microscopic observation of characteristic fungal elements in the nail samples and growth of a significant number of identical colonies on the culture plate.

Results: Based on only direct microscopy, onychomycosis was diagnosed in 424 (39.6%) cases, among which 35.8% were caused by dermatophytes, 32.7% by yeasts, and 29.3% by non-dermatophyte molds (NDMs), while 2.2% were mixed infections. Direct exam was significantly more sensitive than culture for the diagnosis. The most commonly isolated NDMs were *Aspergillus* spp. (69.3%, n=52), followed by *Fusarium* spp. (n=7). The other isolated species were *Paecilomyces* spp., *Scopulariopsis* spp., *Acremonium* spp., *Cladosporium* spp., and *Chrysosporium* spp., with only one case of each.

Conclusion: An increasing frequency of NDO compared to onychomycosis due to other causative agents has been noticeable over the past few years in Iran. This epidemiological data may be useful for the development of preventive and educational strategies.

Keywords: Onychomycosis, Non-dermatophyte molds, Epidemiology

P-42

In vitro antifungal susceptibility testing of terbinafine and terbinafine nanomedicine on the growth of clinical isolates of *Aspergillus* species

Nazila Motedayen[1], Nakisa Sohrabi[1], Hooman Rounaghi[1]

1. Department of Pathobiology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Email: mo250n @yahoo.com

Introduction: *Aspergillus* species are filamentous fungi that cause several infections especially in immunocompromised patients. The most common species causing different diseases are *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. Since the attempts of many researchers to treat fungal infections with common antifungal agents have ended in failure, they have tried to find new antifungal drugs with fewer harmful side effects. The

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

use of nanotechnology in medicine is an attractive and underdeveloped approach, which has been employed only in a small number of mycological studies. In the present study, we aimed to evaluate the antifungal activity of terbinafine and terbinafine nanomedicine against clinical isolates of *Aspergillus* species.

Materials and Methods: For the purpose of data collection, 15 clinical isolates of *Aspergillus* species, including 6 *A. niger*, *4 A. fumigatus*, *2 A. terreus*, and *3 A. flavus* were collected from the patients for susceptibility testing. Macroscopic features in *Aspergillus* species identification were the colony diameter and colony texture and the microscopic characteristics was slide culture method. Microdilution broth method was performed according to CLSI M38-A2 guidelines. Plates were incubated at 35°C for 48 h. The nanoterbinafine based on myristic acid chitosan was used in this study

Results: According to the results of the present study, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of nanoterbinafine and terbinafine against all species of *Aspergillus* were as follows: *A. terreus* (6.25 µg/ml, 12.5 µg/ml and 12.5 µg/ml, 25 µg/ml); *A. niger* (12.5 µg/ml, 25 µg/ml and 25 µg/ml, 50 µg/ml); *A. flavus* (0.7 µg/ml, 1.5 µg/ml and 3.1 µg/ml, 6.25 µg/ml); and *A. fumigates* (1.5 µg/ml, 3.1 µg/ml and 6.25 µg/ml, 12.5 µg/ml). The current study demonstrated that antifungal activity of nanoterbinfine of all the *Aspergillus* species was higher than terbinafine.

Conclusion: This study demonstrated that MIC90s were different for every species of *Aspergillus* due to virulence variation in these species. Furthermore, nanoterbinafine and terbinafine were found to be more effective in *A. flavus* and *A. fumigatus* than *A. tereus* and *A. niger*.

Keywords: *Aspergillus*, Terbinafine, Nanoterbinafine

P-43

Microsatellite Typing of large collection *Aspergillus fumigatus* Strains in Iran

Mojtaba Nabili[1,6], Tahereh Shokohi[2], Hamid Badali[2], Maryam Moazeni[2], Sadegh Khodavaisy[3], Ferry Hagen[4], Jacques F. Meis[4,5]

[1] *Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Department of Medical Mycology and Parasitology/Invasive Fungi Research Center, School of Medicine Mazandaran University of Medical Sciences Sari, Iran*

[3] *Department of Medical Mycology and Parasitology, Tehran University of Medical Science, Tehran, Iran*

[4] *Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands*

[5] *Department of Medical Microbiology, Radboud, Nijmegen, The Netherlands*

[6] *Social Security Organization, Mazandaran, Iran*

Email: m.nabili2010@gmail.com

Introduction: Because of the growing incidence of *Aspergillus* infection, typing methods of *Aspergillus* species are increasingly being used, i.e., phenotypic and genotypic analysis to study the spread and population dynamics in clinical and environmental settings, at levels ranging from a single host to large-scale ecosystems. So, we carried out a genetic study based on the analysis of nine microsatellite loci in a large sample of isolates from different regions of Iran to explore the genetic diversity between azole resistant and susceptible environmental and clinical *A. fumigatus* strains and to compare the data with isolates from other continent.

Marerials and Methods: Sixty-six clinical and environmental strains were collected from ten provinces of Iran. The collection consisted of 43 clinical isolates from a variety of specimens, in addition 23 environmental isolates collected from soil and Hospital air samples. All strains were initially identified as *A. fumigatus* based on macroscopic and microscopic characters, the ability to grow at above 45°C, and finally reconfirmed by DNA sequencing of the partial b-tubulin gene. All *A. fumigatus* strains were subjected to microsatellite typing using three separate multiplex PCRs with a panel of nine short tandem repeats (STR) to evaluate the genetic relatedness between the isolates. The genetic relationship between the *A. fumigatus* isolates was established by comparing the profiles with BioNumerics v6.6 software.

Results: The Simpson's index of diversity for all nine markers combined was calculated to be less than 0.9. The STR typing of 66 *A. fumigatus* isolates revealed 38 distinct genotypes distributed among environmental and clinical isolates. We identified 12 clones including 40 different isolates representing 60% of all isolates tested, which each clone included 2–7 isolates.

Conclusion: In concordance with previous studies, STR typing provided to be a valuable tool for studying the molecular epidemiology and genotypic diversity of clinical and environmental *A. fumigatus* isolates with excellent discriminatory power.

Keywords: Microsatellite, *Aspergillus fumigatus*, *Iran*

P-44

Reduction of AFM1 in milk; natural or chemical: A review

Fatemeh Naeimipour[1], Saman Ayobi[2]

[1] *Department of Mycology, Fac of Vet. Med, Science and Research Branch of Islamic Azad University, Tehran, Iran*

[2] *Mycobacteriology Research Centre, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Email: angelicaparisa@gmail.com

Introduction: Quality and safety of food is of paramount importance. Contaminated animal feed leads to the transfer of diseases to humans through the consumption of animal products, such as milk and meat. . Among Microorganisms, fungal toxins, especially aflatoxin *B-1* is at the top of consideration. AFM1 is a metabolite produced by the conversion and hydroxylation of AFB1. Both these toxins could cause acute and chronic mycotoxicosis mainly through the ingestion of contaminated milk. Therefore, control and mitigation of these toxins is considered crucial.

Conclusion: Despite cost-effective efforts, prevention of aflatoxin contamination in food is a highly expensive and challenging process. Considering the high resistance of these toxins, especially AFM1 in milk and dairy products, could not be eliminated completely even by the most efficient agricultural monitoring methods before and after harvest. Several studies have investigated the approaches used for milk detoxification, expressing the benefits of reducing AFM1 in this regard. By determining the strengths and limitations of available procedures (e.g., preventative strategies, use of probiotics and antibodies, chemisorptions, and additives), we could select the most effective approach or a combination of methods to properly eliminate or reduce AFM1 in milk and its byproducts.

Keywords: Aflatoxin B1, Aflatoxin M1, Milk, Reduction methods

P-45

Evaluation the morphological changes and exploring the expression of *ERG3 and ERG11* genes in *Aspergillus fumigatus* under the influence of diclofenac sodium

Sanaz Nargesi, Sasan Rezaie

Department of Medical Mycology and Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Email: nargesi.sanaz@gmail.com

Introduction: *Aspergillus fumigatus* has become the most prevalent airborne fungal pathogen in developed countries, causing allergic diseases, fungus balls, and fatal invasive aspergillosis. Ergosterol is involved in numerous biological functions such as cellular cycle. The protein encoded by *ERG3*

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

gene participates in the transcriptional regulation of genes in controlling biological rhythm. The *ERG11* gene encodes lanosterol demethylase, the target of the azole antifungals. Diclofenac sodium is a non-steroidal anti-inflammatory drug. Antimicrobial effects of this drug have been proven in several studies. On the other hand, the effects of a variety of antibiotics have been examined on inhibition of *ERG3 and ERG11* genes. Therefore, we aimed to study the effects of diclofenac sodium on *ERG3* and *ERG11* genes expression.

Materials and Methods: According to the Clinical Laboratory Standards Institute protocol for molds, a standard strain of *Aspergillus fumigatus* (ATCC14489) was cultured on potato dextrose agar medium. Fungal suspension was prepared at concentration of 5×10^4 CFU/ml and minimum inhibitory concentration of diclofenac sodium was determined to range between 50 µg/ml and 900 µg/ml. For RNA extraction, *A. fumigatus* was inoculated into SDB treated with 500, 700, and 900 µg/ml diclofenac sodium. cDNA was generated using a reverse transcriptase and Real-time PCR performed for measuring the exact level of mRNA-*ERG3* and *ERG11*.

Results: It was observed that with increasing concentrations of diclofenac sodium, mycelium production was reduced. Diclofenac sodium concentrations of higher than 500 µg/ml had a significant inhibitory effect on the growth of *Aspergillus fumigatus*. No change was noted in *ERG3 and ERG11* genes expression.

Conclusion: Our finding suggested that diclofenac sodium, with dose-dependent effects, could reduce the growth of *Aspergillus fumigatus*. Although further studies are required, diclofenac sodium can be considered as one of the most effective pharmacological agents for the treatment of aspergillosis.

Keywords: *Aspergillus fumigatus*, *ERG3* gene, *ERG11* gene, Diclofenac sodium

P-46

Cockroaches as a vector of *Aspergillus* species in the environment

Hassan Nasirian

Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Email: hanasirian@yahoo.com

Introduction: The emergence of nosocomial fungal infections highlights the necessity of their prevention. Vectors such as cockroaches have been reported as carriers of fungal infections. Herein, we conducted this systematic review and meta-analysis on the contamination of cockroaches to fungi.

Materials and Methods: Relevant scientific papers on the contamination of cockroaches to fungi were collected during January 2015-July 2016. A total of 209 papers were retrieved; after a preliminary review, 25 articles were selected to become part of the detailed synthesis review and meta-analysis.

Results: The global mean contamination rates of cockroaches and the American cockroach species to the fungi has increased, while this rate slightly decreased in German cockroaches. There is no significant difference between the hospital and household environments in terms of mean contamination rates of cockroaches to fungi ($P>0.05$); however, there is a significant difference between urban and rural environments regarding the mean contamination rates of cockroaches to fungi ($P<0.05$). A total of 38 fungal species were isolated from the cockroaches including 38 species from the American cockroaches, 23 species from the German cockroach, and 13 species from the brown-banded cockroaches (*Supella longipalpa*). In addition to the fungal species that were isolated from the German and brown-banded cockroaches, 15 fungal species were isolated from the American cockroaches. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, and *A. versicolor* were the most frequent *Aspergillus* fungi isolated from the cockroaches. Statistical analyses indicated that the external and internal surfaces of cockroaches in contamination to fungi are more dangerous than the entire cockroaches' surfaces.

Conclusion: The internal surfaces of cockroaches in contamination to fungi are also more dangerous than the external cockroaches' surfaces. German and the brown-banded cockroaches in contamination to fungi are more dangerous than the American cockroaches in the hospital environments.

Keywords: *Aspergillus*, *Blattella germanica*, Fungi, Cockroaches, Nosocomial infection

P-47

Evaluation of antifungal susceptibility of isolated *aspergillus* species in the ICU of hospitals

Ayatollah Nasrollahi Omran

Department of Medical Mycology, Faculty of Medicine, Tonekabon Branch, Islamic Azad University, Tonekabon ,Iran

Email: ayat51@yahoo.co.in

Introduction: Invasive aspergillosis is a life-threatening disease which takes hold in patients' with defects in immune system and results in nosocomial fungal infections. In addition, this infectious disease is associated with high mortality rates. This study aimed to evaluate the resistance to antifungal drugs in patients infected by *Aspergillus* spp. isolated from the ICU**.**

Materials and Methods: In total, 160 plates containing Sabouraud dextrose agar medium were collected from the ICU of hospitals. Afterwards, the samples were incubated, followed by the approximate assessment of macroscopic features and microscopic characteristics of suspected fungal colonies. Following that, DNA was extracted and all the species were identified using DNA sequencing. Moreover, drug susceptibility testing for the existing agents was performed in 96-well microplates using the microdilution technique and CLSI M38-A2 guidance on isolates.

Results: In this study, 40 plates containing 11 colonies were suspected of *A. fumigatus* contamination after confirming the sequencing of *A. flavus* (n=5), *A. sydowii* (n=3), *A. fumigatus* (n=1) and *A. oryzae* (n=2). According to susceptibility testing, *A. sydowii* and *A. fumigatus* showed resistance to itraconazole and amphotericin and sensitivity to voriconazole. In addition, *A. sydowii* was resistant to caspofungin while *A. fumigatus* was sensitive to the drug. Sensitivity to amphotericin, itraconazole, voriconazole and caspofungin was observed in *A. flavus* , while this strain showed resistance against miconazole and ketoconazole.

Conclusion: According to the results of this study, diagnosis delay, inappropriate treatment of various diseases and underlying cause of neutropenia were responsible for the high mortality rate of patients with aspergillosis, especially in the patients admitted to the ICU.

Keywords: antifungal susceptibility, ICU, *Aspergillus* spp.

P-48

Antifungal effects of lanoconazole and luliconazole against azole-resistant and susceptible *Aspergillus fumigatus* strains

Mahdi Abastabar[1], Nooshin Rahimi[2], Mohammad Taghi Hedayati[1], Tahereh Shokohi[1], Seyed Reza Aghili[1], Jacques F. Meis[3, 4], Ali Rezaei-Matehkolaei[5], Hamid Badali[1], Koichi Makimura[6], Narges Aslani[2], Iman Haghani[1, 7], Susan Hosseinpoor[2]

[1] *Invasive Fungi Research Center (IFRC), Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

[3] *Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Ziekenhuis Hospital, Nijmegen, The Netherlands*

[4] *Department of Medical Microbiology, Radboud University, Nijmegen Medical Center, Nijmegen, The Netherlands*

[5] *Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

[6] *Laboratory of Space and Environmental Medicine, Graduate School of Medicine, Teikyo University, Japan/Asia International Institute of Infectious Diseases Control, Teikyo University, Japan; Teikyo University Institute of Medical Mycology, Tokyo, Japan,*
[7] *Department of Medical Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
Email: Mabastabar@gmail.com

Introduction: The majority of *A. fumigatus* isolates are susceptible to the antifungal drugs used to treat invasive infections (IA). Since a gradual increase has been observed in the number of isolates of azole-resistant *A. fumigatus* (ARAF), it is essential to evaluate the efficacy of new antifungal agents against azole-resistant *A. fumigatus*. Limited data are available on the in vitro activity of lanoconazole and luliconazole against *Aspergillus* species. This study aimed to assess the in vitro activity of these two new imidazoles and five comparators, including itraconazole, voriconazole, posaconazole, caspofungin and amphotericin B against a large collection of azole-resistant and susceptible *A. fumigatus* strains with various point mutations from clinical and environmental sources.

Materials and Methods: A total of 168 *A. fumigatus* strains were collected from culture collection of Invasive Fungi Research Center (IFRC). Samples included azole-resistant (n=27) and susceptible (n=141) strains originated from nail, sputum, bronchoalveolar lavage, sinus discharge and skin biopsy. Environmental samples were collected from soil and air. Most of the azole-resistant *A. fumigatus* strains (n=10) harbored a leucine-to-histidine substitution at codon 98, along with a 34-bp tandem repeat in the cyp51A promoter region. Moreover, TR46/Y121F/T289 (n=2) and other point mutations (n=8), such as G54, M220, G138C and G432C, were included. Minimum inhibitory concentrations (MICs) were determined based on the clinical and laboratory standard institute M38-A2.

Results: In this study, potent activities of novel imidazoles (luliconazole and lanoconazole) against all *A. fumigatus* isolates were demonstrated. MICs of lanoconazole and luliconazole against all *A. fumigatus* isolates were within the range of <0.001-0.5 and <0.001-0.016 µg/ml, compared to 0.064->16 µg/ml for itraconazole, 0.064->16 µg/ml for voriconazole, and 0.008-8 µg/ml for posaconazole. MICs of luliconazole and lanoconazole for the resistant isolates with various point mutations in the cyp51A gene were approximately similar to those of susceptible isolates. Meanwhile, strains with TR46/Y121F/T289 mutations revealed less susceptibility with a 4-log2-dilution step to other resistant strains harboring TR34/L98H, G54, M220, G138C and G432C.

Conclusion: According to the results of this study, the in vitro antifungal activity of luliconazole and lanoconazole were apparently superior against susceptible and resistant *A. fumigatus* isolates, compared to those of polyenes, other azoles and echinocandins.

Keywords: Lanoconazole, Luliconazole, *Aspergillus fumigatus*

P-49

Aspergillus clavatus with resistance to common antifungal drugs causing onychomycosis

Aynaz Ghojoghi[1], Mehraban Falahati[1], Mahdi Abastabar[2], Mohammad Taghi Hedayati[2], Tahereh Shokohi[2], Zeinab Ghasemi[3], Abdol Sattar Pagheh[4], Ali Rezaei-Matehkolaei[5], Shirin Farahyar[1], Maryam Roudbary[1], Mojtaba Taghizadeh Armaki[2], Akbar Hoseinnejad[2], Tahereh Hosseini[4], Javad Javidnia[4]
[1] *Department of Medical Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
[2] *Invasive Fungi Research Center, Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[3] *Department of Medical Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[4] *Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*
[5] *Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
Email: a.r.matehkolaie@gmail.com

Introduction: Onychomycosis is a common fungal infection of nails caused by yeasts species, dermatophytes and non-dermatophyte molds (e.g., *Aspergillus* species). A case of onychomycosis caused by *Aspergillus clavatus* with resistance to common antifungal drugs was observed for the first time.

Case Report: Our case was a 32-year-old woman identified with psoriasis of the nail. Our housewife patient was presented with no history of trauma, diabetes and other predisposing factors. Hematological and biochemical tests (e.g., complete blood count, hepatic enzymes, glucose, and cholesterol levels) in the patient revealed normal results. In vitro antifungal susceptibility of the isolate was performed based on the clinical and laboratory standards institute guidelines (M38-A2) for susceptibility testing of filamentous fungi. In this study, the presence of *A. clavatus* in the nail sample was confirmed using microscopic and culture analysis, followed by the sequence analysis of a beta-tubulin gene. After antifungal susceptibility test, it was indicated that the isolate was resistant to the majority of common antifungal agents (e.g., itraconazole, posaconazole, voriconazole, amphotericin b, terbinafine, miconazole, caspofungin, butenafine and econazole). However, the patient was successfully treated with daily administration of 200 mg itraconazole.

Conclusion: According to the results of this study, resistance of *A. clavatus* to a wide spectrum of antifungal drugs has not previously been reported in onychomycosis. Moreover, it was demonstrated that lanoconazole, an unused agent in Iran, had the most effective impact on fungi, compared to the other antifungal drugs.

Keywords: *Aspergillus clavatus*, Resistant to common antifungal agents

P-50

Detection of cytotoxicity of gliotoxin extracted from *Aspergillus flavus*

Parisa Badiee[1], Sara Roeen[2]
[1] *Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[2] *Department of Microbiology, Shiraz Branch, Islamic Azad University, Shiraz, Iran*
Email: sara.roeen@yahoo.com

Introduction: *Aspergillus flavus* can lead to tissue damages such as necrotizing pulmonary aspergillosis that has a high mortality rate. Gliotoxin is one of the virulence factors in *Aspergillus* spp. In this study, cytotoxic effects of gliotoxin in environmental and clinical *Aspergillus flavus* isolates were evaluated.

Materials and Methods: In general, 10 *Aspergillus falavus* strains isolated from clinical samples and 10 environmental isolates from soil were studied. The isolates were identified by culture and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using MwoI restriction enzyme. The cytotoxity of gliotoxin on MRC-5 lung cell lines was evaluated by XTT test.

Results: All the 20 isolates were confirmed as *Aspergillus flavus* by PCR-RFLP. Cytotoxic effect of gliotoxin on MRC-5 cell lines was established in clinical and environmental isolates, but there was not any significant difference between these two groups of samples (P>0.05).

Discussion: Few studies have been conducted on virulence factors of this species. Cytotoxicity of gliotoxin on lung cell lines in clinical and environmental isolates was identical.

Keywords: *Aspergillus flavus*, Gliotoxin, PCR-RFLP, XTT

P-51

Overexpression of *HSP90* gene and amphotericin B resistance in the clinical isolates of *Aspergillus* in bronchoalveolar lavage

Maryam Roudbary[1], Mehraban Falahati[1], Nayereh Ghods[1], Sara Mardani[2]

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

[1] *Department of Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
[2] *Department of Mycology, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran*
Email: m_roudbary@yahoo.com

Introduction: Aspergillosis is an opportunistic infection caused by *Aspergillus* spp. in patients with underlying diseases. This study aimed to investigate the role of *HSP90* gene in the polyene resistance of *Aspergillus* spp. using polymerase chain reaction (PCR) and minimal inhibitory concentration (MIC).
Materials and Methods: In total, 400 bronchoalveolar lavage (BAL) specimens were collected from the patients prone to aspergillosis referring to the bronchoscopy unit of Masih Daneshvary Hospital of Tehran during 2015-2016. After homogenizing the samples with 0.5% pancreatin, direct microscopic examination was performed, and samples were cultured on Sabouraud dextrose agar. *Aspergillus* species were identified using morphological methods, and amphotericin B susceptibility testing was conducted based on the CLSI approach (document M38-A). Moreover, presence of *HSP90* gene in the isolates was detected via PCR using a specific primer.
Results: Out of 400 BAL samples, 16 *Aspergillus* species were detected. Distribution of the isolates was as follows: *A. flavus* (n=6; 37.5%), *A. fumigatus* (n=7; 43.75%), and *A. terreus* (n=3; 18.75%). MIC value of amphotericin B for 14 *Aspergillus* species was estimated at >8 µg/ml, while it was <0.5 µg/ml for two *Aspergillus* species. In addition, results of MIC revealed that 87.5% of the isolates were resistant to amphotericin B, in which the *HSP90* gene could be successfully amplified.
Conclusion: According to the results, amphotericin B resistance in *Aspergillus* spp. is on a rising trend and could be detected via useful methods such as PCR and MIC.
Keywords: Amphotericin B, *Aspergillus*, MIC, *HSP90* gene

P-52

Study of *Med A* gene expression related to the virulence of *Aspergillus* spp. isolated from bronchoalveolar lavage

Maryam Roudbary[1], Sedigheh Rafiei Tabatabaei[2], Shahla Roudbar Mohammadi[3], Roya Vahedi Shahandashti[4], Mohsen Shirdost[2]
[1]*Department of Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*
[2]*Pediatric Infections Research Centre, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[3]*Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran*
[4]*Department of Microbiology, Faculty of Sciences, Alzahra University, Tehran, Iran*
Email: m_roudbary@yahoo.com

Introduction: Invasive aspergillosis (IA) is probably the most devastating of *Aspergillus*-related diseases. Identification of *Aspergillus* species in immunocompromised patients is imperative for effective antifungal therapy. Several genes are responsible for the pathogenesis of IA. *Med A* gene is an important virulence factor in the adhesion of *Aspergillus* to lung tissue. This study aimed to identify and investigate the expression level of *Med A* gene in *Aspergillus* spp. isolated from bronchoalveolar lavage (BAL) specimens in patients suspected of IA.
Materials and Methods: BAL specimens were collected from high-risk hospitalized patients and cultured on Sabouraud dextrose agar. Samples were identified through morphological characterization. For quantitative real time reverse transcriptase PCR (qRT-PCR), total extracted RNA and cDNA were synthesized using specific primers.
Results: In the direct examination and culture of BAL specimens, 22 *Aspergillus* spp. isolates were identified. Moreover, results of gene expression analysis were indicative of *Med A* gene mRNA overexpression (4-8 fold) in 22 out of 30 isolates (73.3%), while the expression of *Med A* gene was observed to decrease in eight isolates (26.6%).
Conclusion: According to the results, overexpression of *Med A* in a significant number of *Aspergillus* spp. isolates could explain the key role of this gene in the pathogenesis of IA. However, presence of other virulence genes cannot be disregarded. Identification of *Aspergillus* species could contribute to the effective management and care of patients with IA in order to prevent mortality and morbidity in high-risk cases.
Keywords: *Med A* gene, Real-time PCR, Invasive aspergillosis, Immunocompromised patients

P-53

The first reports on fungal contamination in the swimming pools of Hamadan, Iran

Zainab Sadeghi Dehkordi
Department Of Pathobiology, Faculty of Paraveterinary Sciences, Bu-Ali Sina University, Hamadan, Iran
Email: dehkordisz@gmail.com

Introduction: Cutaneous fungal infections might be transmitted to human hosts through using public facilities, such as swimming pools and saunas. These infections could be prevented through assessing the causes of opportunistic and pathogenic fungi and eliminating fungal contamination in these environments. This study aimed to determine the level of fungal contamination in indoor swimming pools of Hamadan, Iran in 2016.
Materials and Methods: In this study, we evaluated three indoor swimming pools in terms of fungal contamination in Hamadan over a nine-month period in 2016. To measure the levels of contamination with dermatophytes and saprophytic fungi, water samples were collected in test tubes with a sterile screw. Samples were obtained from different environmental surfaces in each pool (showers, dressing room surfaces, sauna seats, baths, platforms, and pool surroundings) using the sterile carpet collection method. Collected samples were inoculated and cultured separately on the specific media (Mycosel Agar and Sabouraud dextrose agar via standard methods.
Results: Out of 720 cultivated samples, 513 cases were positive for contamination with one or more fungi, including saprophytes (n=288, 56%), *Aspergillus* (41%), *Penicillium* (15%), and yeast (n=216, 42.1%). Additionally, the highest rate of fungal contamination with saprophytes was observed in the showers (59.3%), saunas (25%), and pool walls of indoor swimming pools (6.25%), respectively.
Conclusion: According to the results of this study, although saprophytes were the most frequent agents to cause fungal contamination, low rate of fungal infections in dealing with the host was associated with a high risk of developing asthma and allergic diseases.
Keywords: *Aspergillus*, swimming pools

P-54

Identification of causative agents of aspergillosis in formalin-fixed paraffin-embedded tissue specimens by real-time quantitative PCR assay

Elham Salehi [1], Mohammad T. Hedayati[2, 3], Jan Zoll[4], Haleh Rafati[5], Maryam Ghasemi[6], Atosa Doroudinia[7], Mahdi Abastabar[2,3], Ali Tolooe[8], Eveline Snelders[4], Henrich A. van der Lee[4], Antonius J. M. M. Rijs[4], Paul E. Verweij[4], Seyedmojtaba Seyedmousavi[3, 4, 9], Willem J.G. Melchers[4]
[1] *Student Research Committee, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[2] *Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[3]*Invasive Fungi Research Center (IFRC) Mazandaran University of Medical Sciences, Sari, Iran*
[4]*Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands*
[5] *Department of Biochemistry, Erasmus University Medical Center, Rotterdam, The Netherlands*
[6] *Department of Pathology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

[7] *Transplantation Research Center, National Research Institute for Tuberculosis and Lung Disease, Department of Pathology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[8] *Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[9] *Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, The Netherlands*
Email: elham.salehi64@yahoo.com

Introduction: Formalin-fixed paraffin-embedded (FFPE) tissues obtained from patients with proven invasive aspergillosis infections (IA) are frequently used to detect the etiology of invasive mycoses. While histopathology can prove invasive fungal infections (IFIs) by the demonstration of fungal elements in tissue specimens, genus or species level identification due to morphologic characteristics is limited. Apart from this, despite detection of fungal elements in the specific stained histological samples, fungal cultures from tissue biopsies often remain negative in a substantial number of cases. In the present study, we developed and evaluated a real-time quantitative polymerase chain reaction (PCR) assay targeting the multicopy internal transcribed spacers (ITS) region of the ribosomal DNA (rDNA) to detect and identify genus and species of *Aspergillus*, *Zygomycetes*, and *Fusarium* directly from FFPE tissue specimens obtained from patients with histologically proven IA.
Materials and Methods: In a retrospective multicenter study, tissue samples from 59 FFPE specimens with histopathology results were tested. Two 4-5 µm FFPE tissue section from each specimen was digested with proteinase K followed by automated nucleic acid extraction. A specific quantitative real-time quantitative PCR (qPCR) assay targeting the internal transcribed spacer (ITS2) region of ribosomal DNA, using fluorescently labeled primers, was performed to identify clinically important genus and species of *Aspergillus*, *Fusarium*, and Mucormycetes. The molecular identification was correlated with results from histological examination. The qPCR procedure evaluated and identified a range of fungal genera/species, including *A. fumigatus, A. flavus, A. terreus, A.niger, F. oxysporum*, *F. solani*, and *Rhizopus oryzae*.
Results: *Fusarium oxysporum* and *F. solani* DNA was amplified from five specimens that were initially diagnosed by histopathology as aspergillosis. R. oryzae was detected from histopathological aspergillosis samples. In addition, the most frequently fungi causing aspergillosis belonged to the *Aspergillus flavus* species, which represented 34% of the proven samples.
Conclusion: Our results indicated that histopathological features of molds could easily be confused in tissue sections. The qPCR assay used in this study is a reliable tool for rapid and accurate identification of the genus and species levels of fungal pathogens directly from FFPE tissues.
Keywords: Real time qPCR, Panfungal PCR, Internal transcribed spacer (ITS2), Paraffin embedded tissue, Mucormycosis, Aspergillosis, Fusariosis, Scedosporiosis

P-55

Comparison of antifungal effect of thiazole derivatives and silver nanoparticles: An in-vitro study

Behzad Ghasemi[1], Ghasem Sanjarani[2], Zahra Sanjarani[2], Farshad Shahkhah [2]
[1] *DVM, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran*
[2] *Student of Veterinary, School of Veterinary Medicine, University of Zabol, Zabol, Iran*
Email: gh.sanjarani@gmail.com

Introduction: Along with increasing antifungal resistance in the recent years, the use of novel antifungal compounds is on a growing trend and researchers are investigating new antifungal compounds. Thiazole derivatives and silver nanoparticles are chemical compounds with antifungal effects, which have recently attracted considerable attention from researchers. In this study, the inhibitory effects of four thiazole-thiazolidine derivatives were studied on *Aspergillus niger*, *Candida albicans*, and *Fusarium solani*.
Materials and Methods: For the purpose of this study, thiazole derivatives and silver nanoparticles were prepared as solution. Then, disk diffusion was used for calculation of growth inhibition zone diameters, and serial dilution method was applied to assess minimum inhibitory concentrations (MICs) in microplates.
Results: The analyses exhibited that 6a-c derivatives of thiazole and silver nanoparticles had no significant inhibitory effect on the fungi; however, growth inhibition zone diameter and MIC were reported to be 16 mm and 64 µg/ml, respectively, for thiazole derivative 6d on *Aspergillus niger*.
Conclusion: In this study, only existing of thiazole ring do not have inhibition effects but the inhibitory effects of these compounds depend on cross linking to this ring, that is, connection of oxygen to thiazole ring in 6d derivative is a proof to this claim, and also diameter and number in silver nanoparticles have important role in antifungal effect.
Keyword: Antifungal effect, Thiazole derivative, Silver nanoparticle

P-56

Aspergillus infection in burn wound diabetic patients: two case reports

Zahra Seifi[1], Nahid Gholineghad[1], Tahereh Shokohi[2], Seyed Reza Aghili[2], Seyed Zahra Nouranibaladezaei[3], Zeynab Bandalizadeh[1], Mostafa Roudbary[3], Fatemeh Nourollahi[3]
[1] *Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*
[2] *Invasive Fungi Research Center, Mazandaran University of Medical Sciences, Sari, Iran*
[3] *Burn Center of Zare' Hospital, Mazandaran University of Medical Sciences, Sari, Iran*
Email: shokohi.tahereh@gmail.com

Introduction: Burn wound fungal infection is mainly caused by *Aspergillus* species. In addition, burn is associated with decreased phagocytic activity of neutrophils and macrophages. Cutaneous aspergillosis associated with such injuries had > 70% of the total body surface area (TBSA) affected by burn and the infection usually occurred within 10–35 days of burns. Occasional infection may occur even at 50–60 days after burn. Isolation of *Aspergillus* from a burn wound or devitalized tissue is rarely colonization and often carries a grave prognosis. In this study, we aimed to describe burn wound infection in two diabetic patients caused by *Aspergillus* species with early fatal outcomes.
Case presentation: In this study, we described the case of two male patients aged 32 and 34 years with a history of type II diabetes and consumption of opium and psychiatric drugs, respectively. Patients were hospitalized after attempted suicide by self-immolation. TBSA and burn sizes were high in both cases, who were suffering from massive respiratory injury and were administered with broad-spectrum antibiotics and mechanical ventilators. The burn wounds were infected and the progressive necrotic lesions were resistant to broad-spectrum antibiotics. Sample were aseptically obtained from deep burn wounds and stained by calcofluor white for direct microscopy. Dichotomous branching septate hyphae were observed in direct examination. Culture on Sabouraud dextrose agar (SDA) plates after five days at 30°C revealed fungal colonies in repeated sampling, followed by the identification of *Aspergillus fumigatus* based on morphological characterization. Multiple sampling and repeated isolation of identical colonies were performed to confirm the pathogenic role of isolated species and rule out colonization. Molecular detection was carried out through the evaluation of sequences for polymerase chain reaction primers (ITS1 and ITS4), and *Aspergillus fumigatus* was confirmed after the comparison of the sequence with database of National Center for Biotechnology Information. Nevertheless, treatment failed and the patients died 10 and 7 days after hospitalization due to the severe injuries.
Conclusion: The present report highlighted *Aspergillus* infection in burn wounds of diabetic patients resistant to broad-spectrum

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

antibiotics. It is recommended that physicians pay more attention to the possibility of invasive fungal skin lesion and early diagnosis to improve the outcomes.

Keywords: *Aspergillus*, Burn wound infection, Diabetic patients

P-57

A report of an otitis media caused by *Aspergillus flavus* in Iran

Majid Seilsepour[1], Hossein Zarrinfar[2, 3], Mohsen Rajati Haghi[4], Majid Alizadeh[3], Alireza Esmailzadeh[1]

[1]*Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran*

[2]*Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[3]*Department of Medical Parasitology and Mycology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[4]*Department of Ear, Nose and Throat, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Email: silispourm931@mums.ac.ir

Introduction: Otitis media (OM) is defined as the inflammation of the middle ear, which could be caused by a variety of fungal agents. OM is highly prevalent in children and may lead to fidgeting. This study aimed to describe the isolation of *Aspergillus flavus* from OM in Iran.

Materials and Methods: Biopsy specimens of the middle ear were processed for microscopy, culture (Sabouraud dextrose agar) and histopathology. In addition, DNA of the fungi colony was extracted, and molecular identification was performed via PCR sequencing. The entire sequence of the beta-tubulin gene was compared with the GenBank sequence database.

Results: Direct microscopic examination in 20% potassium hydroxide and the histopathological slide stained with H&E showed branched, septate hyphae *Aspergillus flavus*. Identification of the isolate was confirmed by DNA sequencing of the beta-tubulin gene of the rDNA.

Conclusion: According to the results, *A. flavus* plays a key role in the occurrence of OM in Iran. Therefore, mycological examinations are recommended for patients with OM.

Keywords: Otitis media, *Aspergillus*, PCR

P-58

Beta-D-Glucan In Cerebrospinal Fluid (CSF) as A Biomarker For Aspergillosis In Pediatric patients

Behnam Sobouti[1], Shahrzad Fallah[2]

[1]*Associate Professor of Pediatric Infectious Diseases, Ali-Asghar Children Hospital, ran University of Medical Sciences, Tehran, Iran*

[2] *Assistant Professor Of Pediatrics, Mofid Children Hospital , Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Email: behnamsobouti@gmail.com

Introduction: Fungal infections of the central nervous system (CNS) are important causes of mobidity and mortality among immune compromised pediatric patients. Morbidities associated with Aspergillosis of the central nervous system include hemorrhagic infarction, ventriculitis, meningitis and subarachnoid hemorrhage.

Conventional culture-based approaches to testing CSF Lack sensitivity for diagnosing and therapeutically monitoring these life-threatening disease. Current molecular methods remain investigational, however fungal cell wall biomarkers such as (1→3)- Beta -D-Glucan(BDG)may provide an important approach for the detection and therapeutic monitoring of Aspergillosis of CNS.

Conclusion: The carbohydrate polymer (1→3)- Beta-D-Glucan which is expressed in the cell walls of *Aspergillus* spp., has been widely used for diagnosing invasive Aspergillosis, respectively. However, little is known about the potential utility of BDG in diagnosing aspergillosis of CNS.

P-59

A rare, fatal case of invasive aspergillosis of spinal cord caused by *Aspergillus nidulans* in a child with chronic granulomatous disease from Iran

Mahin Tavakoli[1], Setareh Mamishi[2], Hossein Mirhendi[3], Sadegh Nouripour-Sisakht[4], Mohammad T. Hedayati[1]

[1] *Department of Medical Mycology and Parasitology, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Department of Infectious Diseases, Children Medical Center, Tehran University of Medical Sciences, Tehran, Iran*

[3] *Department of Medical Mycology and Parasitology, Isfahan University of Medical Sciences, Isfahan, Iran*

[4] *Department of Medical Mycology and Parasitology, Yasuj University of Medical Sciences, Yasuj, Iran*

Email: mtavakoly85@yahoo.com

Introduction: Invasive aspergillosis (IA) of the central nervous system (CNS) is a rare, but devastating complication.

Case presentation: This is a case report of a rare and fatal IA with spinal cord involvement in a child with chronic granulomatosis disease, presented with lethargy, coryza, and progressive weakness in the lower limbs. No sign of spinal cord involvement was observed in the patient. A previous history of tuberculosis abscess in the groin and axillary, *Aspergillus* abscess in the left side leg, cervical vertebrae and splenic abscess was reported. Broad-spectrum antibiotics and antifungals were prescribed for the patient, and CT scan revealed the apical opacity of mediastinum associated with pulmonary tuberculosis and extradural involvement with erosion of the vertebral bodies in the lower cervical and thoracic areas and ribs. Moreover, magnetic resonance imaging depicted diffuse abnormal signals of the vertebral bodies in the lower cervical and thoracic areas, with cord compression and signal distortions of the T2 and T3 vertebral bodies. Our patient underwent a decompression laminectomy for the removal of the epidural mass from the spinal cord. The radiological features mimicked a highly malignant lesion. Following that, the histopathological examination of extradural spinal lesion at T2/T3 and cervical bone tissue suggested invasive aspergillosis. Given the histopathological implications of *Aspergillus* infection, initial therapy with voriconazole was started for the patient; nevertheless, he died within a year.

Conclusion: In this study, fungal culture was used, which led to the identification of *Aspergillus nidulans* and polymerase chain reaction. It is suggested that IA be considered in differential diagnosis among chronic granulomatous diseases in patients with non-specific, focal CNS symptoms. Early diagnosis and aggressive antifungal therapy are also recommended to achieve better outcomes for patients.

Keywords: invasive aspergillosis**,** spinal cord, CGD, *Aspergillus nidulans*

P-60

Azole resistance in *Aspergillus fumigatus* in fields supplemented with pesticide

Afsane Vaezi[1, 2], Hamid Badali[2], Hamed Fakhim[1, 2]

[1] *Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Department of Medical Mycology and Parasitology, Invasive Fungi Research Center (IFRC), School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

Email: vaeziiafsane@gmail.com

Introduction: Azole resistance in *Aspergillus fumigatus* is an emerging public health concern, mainly leading to treatment failure and increased mortality. Given the widely protectant fungicide use in agriculture, environmental route may be the main source for acquired azole resistance. This study aimed to evaluate the prevalence and potential mutations of triazole resistance in *A. fumigatus* isolates obtained from fields.

Materials and Methods: A total of 108 soil samples were collected from four different locations in Mazandaran (Ghaemshahr, Neka, Joybar and Sari), among which 31 (28.7 %) samples harbored *A. fumigatus* and 11/31 of *A. fumigatus* isolates grew on Sabouraud dextrose agar (SDA) supplemented with itraconazole and/or voriconazole at 48°C. Afterwards, the isolates were confirmed through partial sequencing of the β-tubulin gene.

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

In vitro antifungal susceptibility testing of 11 isolates was performed against seven antifungal agents.

Results: In this study, posaconazole and voriconazole were 2 log ^{2}-dilution steps and 3 log ^{2}-dilution steps less active than luliconazole and lanoconazole, respectively. Luliconazole was the most active agent against azole-resistant isolates of *A. fumigatus*, followed by lanoconazole, posaconazole, caspofungin and amphotericin B. Moreover, minimum inhibitory concentration values of five isolates were higher, compared to the epidemiological cut-off values in both itraconazole and voriconazole. Among the four azole-resistant isolates of this study, only two *A. fumigatus* isolates harboured TR34/L98H variants, whereas other point mutations were not detected.

Conclusion: According to the results of this study, alternative treatment strategies, such as the use of new medications and combination therapy, must be assessed to accurately manage diseases caused by *Aspergillus*.

Keywords: *Aspergillus fumigatus*, Azole-resistant, Triazole, Fungicide agriculture, Cyp51a

P-61

Antimicrobial activities of seven essential oils from Iranian aromatic plants against *Aspergillus* spp.

Kamiar Zomorodian[1], Seyed Jamal Saharkhiz[2], Mohammad Reza Moein[3], Somayeh Yazdanpanah[4,5], Keyvan Heshmati[4], Zohreh Emeripour[4], Zahra Mousavi[4], Saeideh Alipour[1]

[1] *Basic Sciences in Infectious Disease Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[2] *Department of Horticultural Sciences, Faculty of Agriculture, Shiraz University, Shiraz, Iran*

[3] *Department of Pharmacognosy and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

[4] *Department of Medical Mycology and Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[5] *Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran*

Email: zomorodian@sums.ac.ir

Introduction: Recently, investigation of natural products have been increasingly assessed to discover active compounds with antimicrobial properties from plants. Seven aromatic plants used in this study are among the popular traditional Iranian medicinal herbs with potential application in modern medicine as antimicrobial agents.

Material and Methods: Chemical compositions of essential oils (EOs) distilled from plants (e.g., *Satureja khuzestaniea*, *Satureja bachtiarica*, *Satureja rechingeri*, *Artemisia sieberi*, *Ferula assa-foetida*, *Nepeta cataria*, and *Olveria decumbens*) were analyzed using gas chromatography/mass spectrometry (GC/MS). The antifungal activities of the essential oils were evaluated using 96-well broth microdilution plates, as recommended by the Clinical and Laboratory Standards Institute method.

Results: In this study, growth inhibition of the tested *Aspergillus* spp. by the evaluated EOs at concentrations of 0.015 to 16 µL/mL was observed. Moreover, among the examined EOs, *Satureja* spp. revealed the highest antifungal properties, while *Artemisia sieberi* exhibited the lowest antifungal activities.

Conclusion: According to the results of this study, antifungal activities of the evaluated EOs might be due to their major phenolic or alcoholic monoterpenes with known antimicrobial properties. In addition, it was indicated that the assessed EOs could be used as alternative antifungal agents or food preservatives to extend the shelf time of food products.

Keywords: Essential oil, Antifungal activity, Medicinal plants

P-62

Surface-modified superparamagnetic nanoparticles by PEG-400 for embedding Ag and Au nanoparticles against *Aspergillus* spp.

Kamiar Zomorodian[1], Hamed Veisi[1], Somayeh Yazdanpanah[2,3], Mahmoud Sadeghi[1], Mahmoud Mousavi[2], Jafar Bagheri[1], Hasti Nouraei[1]

[1] *Basic Sciences in Infectious Disease Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[2] *Department of Medical Mycology and Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*

[3] *Student of research committee, Shiraz University of Medical Sciences, Shiraz, Iran*

Email: zomorodian@sums.ac.ir

Introduction: Intelligent drugs such as those controlled by magnetic forces could open a new horizon to overcome drug resistance. Ag and Au nanoparticles have been shown to have significant antimicrobial activities. Therefore, combination of these nanoparticles with iron molecules could guide the molecule in infected areas. This study aimed to carry out the chemical synthesis and characterization of Fe3O4@PEG-Ag and Fe3O4@PEG-Au nanocomposites, which were prepared as core-shell nanostructures. Moreover, these molecules were evaluated in terms of antifungal activities against *Aspergillus* spp.

Materials and Methods: Fe3O4 nanoparticles were synthesized through the coprecipitation of bi and trivalent iron ions. Polyethylene glycol (PEG) was added as a shell onto nanoparticle surfaces. Ag and Au ions reduced on nanoparticles for the synthesis of Fe3O4@PEG-Ag and Fe3o4@PEG-Au, respectively. Nanostructures were characterized by FT-IR, FESEM-EDS, VSM, TEM, and AFM. In addition, compounds were evaluated in terms of antifungal activity against *A. fumigatus*, *A. clavatus* and *A. flavus* in accordance with the M38-A protocol as recommended by the Clinical and Laboratory Standards Institute (CLSI).

Results: Newly synthesized compounds exhibited favorable antifungal activities as they were observed to inhibit *Aspergillus* spp. growth at the concentration of 16-64 µg/mL. In this regard, activity of Ag particles was superior to that of Au particles.

Conclusion: According to the results, concentrations of Ag and Au nanoparticles could increase by magnetic forces. Therefore, antifungal properties of these novel compounds render them effective in the treatment of localized infections and invasive aspergillosis in a specific location or tissue by magnetic fields.

Keywords: *Aspergillus*, Fe3O4@PEG-Ag, Fe3o4@PEG-Au, Antifungal, Superparamagnetic, Nanoparticles

P-63

Identification of *Aspergillus* isolated from patients with weakened immune system having pulmonary disorders using bronchoalveolar lavage specimens

Elnaz Zanganeh[1], Hossein Zarrinfar[2,3], Fariba Rezaitalab[4], AbdolMajid Fata[3], Majid Alizadeh[3], Mohammadali Ghanbari[3]

[1] *Department of Microbiology, Islamic Azad University of Damghan, Damghan, Semnan, Iran*

[2] *Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[3] *Department of Medical Parasitology and Mycology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[4] *Department of Internal Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

Email: elnaz_zanganeh@yahoo.com

Introduction: Due to increased number of patients with weakened immune system, fungal infections, particularly pulmonary *aspergillosis* (PA) is increasing. Lack of accurate and timely diagnosis of PA is one of the causes of death in these patients. Bronchoalveolar lavage (BAL) fluid is one of the helpful specimen for diagnosis of PA. In this study, PA was examined in patients with weakened immune system suffering from pulmonary disorders, using BAL specimens.

Materials and Methods: A total of 150 BAL specimens were collected from the patients with weakened immune system having pulmonary disorders through bronchoscopy. All the specimens were examined by direct examination (15% KOH) and culture on Sabouraud dextrose agar. Finally, the *Aspergillus* grown in the

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3

culture medium were identified, using beta-tubulin gene amplification and sequencing techniques.

Results: According to the results, out of 150 specimens, 12 (8%) samples were positive in direct examination, and 20 (13.3%) samples had positive culture including 15 (75%) *A. flavus*, 3 (15%) *A. tubingensis*, and 2 (10%) *A. fumigatus*.

Conclusion: In this study, the incidence of PA in patients with a weakened immune system was relatively high and the patients with malignancy had the highest incidence. Moreover, *A. flavus* was the most dominant species isolated from these patients.

Keywords: Pulmonary aspergillosis, *Aspergillus*, Immune system, Bronchoalveolar lavage

P-64

Role of *Aspergillus* in rhinosinusitis biofilms of patients with nasal polyposis

Hossein Zarrinfar[1,2], Akram Adabizadeh[3], Navid Nourizadeh[4], Mohammadreza Majidi[4], Amirhossein Jafarian[5], Mohammadtaghi Shakeri[6], Mohammad Javad Najafzadeh[2], Elnaz Zanganeh[7]

[1] *Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[2] *Department of Medical Parasitology and Mycology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[3] *Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran*

[4] *Department of Ear, Nose and Throat, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[5] *Department of Pathology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[6] *Department of Social Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

[7] *Department of Microbiology, Islamic Azad University of Damghan, Damghan, Semnan, Iran*

Email: zarrinfarh@mums.ac.ir

Introduction: Chronic rhinosinusitis (CRS) affects paranasal sinuses, concomitantly involving the nasal cavity. Fungal agents may cause inflammation in the nose, leading to allergic nasal polyps. *Aspergillus* species appear to possess the classical elements of fungal biofilm growth in nasal polyps. This study aimed to evaluate the frequency of *Aspergillus* biofilms in CRS patients with nasal polyposis.

Materials and Methods: This study was conducted on 60 sinonasal specimens, classified as the patient (n=31) and control groups (n=29). Histopathological examination was performed on each specimen using the H&E stain. Samples were directly examined on 20% potassium hydroxide and cultured on Sabouraud dextrose agar. Colonies were identified using beta-tubulin gene amplification and sequencing.

Results: Out of 60 specimens, four cases (12.9%) were positive in direct examination, all of which were obtained from patients with sinonasal polyposis. Moreover, cultures were positive for *Aspergillus flavus* in five CRS patients (16.1%) with sinonasal polyposis and one control subject (3.4%). Although no specific pattern of fungal biofilm was detected in the histopathological assessment, degenerated mycelium under a necrotic area was observed in one case. Moreover, polymerase chain reaction (PCR) analysis of the specimens showed no positive results for *Aspergillus*.

Conclusion: According to the results, although *Aspergillus flavus* was the most dominant fungal species isolated from the cultured specimens, it had no fundamental role in the etiopathology of polyp formation in CRS patients. In addition, PCR results were indicative of no significant concordance with other methods in CRS.

Keywords: *Aspergillus*, Rhinosinusitis, Polyposis, Biofilm, PCR

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)

DOI: 10.18869/acadpub.cmm.2.S1.2016.3