Oral Presentation Abstracts

(Presenters' last names alphabetically sorted)

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O-01

Aspergillosis in ICU

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The prevalence of Invasive Aspergillosis (IA) at the Intensive Care Unit (ICU) is not well known, but as shown in several studies, it could emerge as a life-threatening opportunistic infusion in various conditions. It should be emphasized that about 70% of these patients had no predisposing factors for IA. Moreover, in ICU patients, IA may potentially affect multiple organs and evolve into a disseminated disease, which remains largely under diagnosed and associated with poor outcome.

IA predominantly is associated with immune comprised conditions including neutropenic patients, hematopoietic stem cell or solid organ transplantation, those with solid tumors, and in patients receiving corticosteroids. Besides these patients, IA can cause invasive disease in other subsets of patients admitted to ICU. Chronic Obstructive Pulmonary Disease (COPD) is one the most common condition, accounting for almost one–third of all cases.

Other risk factors for IA in ICU include liver cirrhosis, sever sepsis, and Acute Respiratory Distress Syndrome (ARDS). In these cases, various diagnostic radiological and microbiological methods have low sensitizing and specificity, and may have been masked by the underlying acute process.

O-02

Diabetic foot infection and Aspergillosis

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Diabetes is now a worldwide epidemic problem. Diabetic foot infections (DFIs) are the most common infection in diabetic patients that needs hospitalization and surgical intervention and it occurs in 10% of all patients with diabetes. DFIs complication rate in diabetes is increasing variety of factors; inefficient treatment of osteomyelitis and its chronic trend, resistant organisms commonly seen in DFIs. Most of the infections in diabetic foot are aerobic and anaerobic bacterial origin, and in most cases polymicrobial. Many studies show that diabetes mellitus is an important underlying disease for opportunistic invasive fungal infections. Diabetic patients are vulnerable to fungal infections, which can be chronic and generalized and barely respond to antifungal therapy. Among fungal infections, candidiasis, mucormycosis and aspergillosis are the most common agents and usually do not respond to antibiotic therapy so the disease becomes chronic. Aspergillus conidia are known to be ubiquitous in nature and are the commensals in the respiratory tract. In patients with immunosuppression, aspergillus can multiply and cause widespread infection involving respiratory system and sometimes even skeletal system. Aspergillus can involve bony tissue through vascular system, direct infection, and trauma. There are many types of complications in DFI patients. That is present in approximately 20% of cases of foot infection in persons with diabetes and greatly increases the likelihood that the patient will require a lower-extremity amputation. One of the most controversial issues confronting the DFIs is lack of widely agreed guidelines for its diagnosis, treatment, and management. Almost all DFIs cases result from contiguous spread of infection from adjacent soft tissue. The soft-tissue infection usually starts as a complication of a neuropathic ulcer but can result from penetrating injury or ischemic soft-tissue loss. Arterial insufficiency may be present but tends to play a less important role than neuropathy. As foot ulcer in diabetic patients is formed at the site of pressure such as great toes, metatarsal heads, and calcaneus, thus osteomyelitis most commonly affects the underlying bone of these sites. Aspergillus can easily infect damaged foot tissue in diabetic patients through contaminated shoes and clothes. So using proper shoes and clothes and also the periodical examination of the feet for detecting skin and nail lesion, sensation of foot, anatomical changes, and vascular circulation of foot can be useful for prevention of infection. To appropriate treatment and prevent amputation, it is recommended in initiation treating of the patients with DFIs, for biopsy and culture and diagnostic tests to determine of the pathogen agents, fungal diagnostic tests should be considered addition to bacterial diagnostic tests.

O-03

Current status of antifungal susceptibility profiles and resistance rates of *Aspergillus terreus* species complex isolates in Iran

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Aspergillus terreus is emerging as an etiologic agent of invasive aspergillosis among patients undergoing cancer chemotherapy, haematopoetic stem-cell transplantation, or solid organ transplantation worldwide. Infections caused by *Aspergillus terreus* has become important due to less susceptibility to antifungal drags, resistant to amphotericin B and therefore, poor clinical outcome and higher mortality compared with infections by other *Aspergillus* species. Thus, the aim was to determine in vitro susceptibility of twelve antifungal agents against clinical and environmental isolates of *A. terreus* and screen the possible emergence of strains with less antifungal activity. The *in vitro* activities of 12 antifungal agents against clinical (n = 29) and environmental (n = 45) strains of *A. terreus* were determined. The Clinical and Laboratory Standards Institute (CLSI) provides no specific guidelines for testing the *in vitro* antifungal susceptibility of this fungus. No other testing protocol has also been validated for testing the susceptibility of *E. dermatitidis*. MIC results revealed that all echinocandins (micafungin, anidulafungin and caspofungin) and azoles (voriconazole, posaconazole, isavuconazole, luliconazole and lanoconazole) demonstrated potent activity against all the isolates. Only five *A. terreus* isolates showed high amphotericin B MICs (4 µg/ml), while, other isolates revealed Amphotericin B MICs ranging from 0.125-2 µg/ml.

Intrinsic resistance to amphotericin B in *A. terreus* is known, but some isolates with low

Amphotericin B MICs have been previously reported, suggesting a possible genetic difference that may exist in this Terrei section complex. In conclusion, focusing on epidemiology, on clinical courses of infections and to investigate mechanism behind differences in amphotericin B and azole susceptibility is highly recommended.

O-04

Voriconazole therapeutic drug monitoring

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Fungal infections are frequent life-threatening complications in immunocompromised patients.Voriconazole (VRC) is a second-generation triazole used for the treatment of aspergillosis, candidiasis, and other mold infections. The VRC steady-state level is achieved in 3 days with two loading doses of 400 mg for the first day followed by a maintenance dose of 200 mg every 12 h thereafter.

Accepted plasma level for VRC is 1-5.5 mg/L. There is a clear relationship between drug concentration and drug response. Low plasma levels of VRC (<1 mg/L) are associated with therapeutic failures and high plasma levels (>5.5 mg/L) are accompanied with variant and severe adverse drug reactions, such as encephalopathy, hepatotoxicity, liver enzyme elevation, visual disturbances, blurred vision, and electrolyte abnormalities. Therapeutic drug monitoring (TDM) is a tool to detect the VRC doses and may be helpful in the management of patients and prevention of drug-related side effects. High-performance liquid chromatography (HPLC) methods are useful in routine TDM for quantification of VRC concentration in human plasma or serum. Furthermore, bioassays (microbiological assays) can quantify the antifungal activities.

In conclusion, supratherapeutic and subtherapeutic VRC levels can lead to increased morbidity, mortality, and hospital stay in infected patients. Using VRC in combination with TDM is essential to the improvement of the safety and outcomes of the patients.

O-05

Nosocomial aspergillosis, drug resistance and hospital sources at the medical-educational centers, Urmia, Iran

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In spite of the low percentage (1%) of fungal hospital-acquired infections, *Aspergillus* species are the main agents of fulminate fungal infections. Invasive aspergillosis has a mortality rate of 90% among the *Aspergillus* infections. With respect to the high frequency of *Aspergillus* spp. isolated from clinical and environmental sites in the nephrology ward, this study aimed to determine the azole resistance through performing a molecular epidemiologic research to find accurate environmental sources for *Aspergillus* infections and colonization in large general hospitals. Samples included clinical specimens of cases with healthcare-associated infection (HAI), collected during 48 months (October 2012-September 2016) at the UMSU educational hospitals, Urmia, Iran. In addition, environmental specimens (sterile swabs from the surfaces of floor, walls, curtains, beds, trolleys, air conditioners, cooling systems and medical devices) were obtained, as well as some samples from the fingerprints of the staff and visitors. After the isolation of most current *Aspergillus* species from hospital clinical specimens, susceptibility and minimal inhibitory concentration tests were performed to find the most resistant *Aspergillus* isolates. The RAPD-PCR technique was also exploited to assess the hospital sources of the isolated specimens.

In total, 198 samples were obtained from cases with confirmed HAI during 24 months (August 2014-September 2016). The results of experimental studies on the specimens revealed 93 (47%) positive cases of fungal or bacterial infections from the above cases and 54 (58%) cases with fungal infections. Total isolated fungi included 36 cases of *Candida* (66.6%), 17 cases of *Aspergillus* (31.4%) and only one case of *Alternaria*. Among the *Aspergillus* isolates, *A. flavus* (47%), *A. fumigatus* (29.4%) and *A. niger* (23.6%) were the most frequent species, respectively. Just the R151 primer was able to differentiate among two collection strains (strain 2907 and 2913). Nevertheless, primer pair (R151 and UBC90) was not identified as the best primer set since only one single strain (strain 2905) could be differentiated by this set.

According to the results of this study, the most contaminated hospital indoor places were the surfaces of floors, walls and also air samples in low amounts.

O-06

In Vitro* Interaction of Micafungin and Voriconazole against azole-Resistant *Aspergillus fumigatus

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Voriconazole is the first-line therapy for invasive aspergillosis. Nevertheless, mortality remains high due to different factors. Although azoles are very active in vitro against *Aspergillus fumigatus*, several studies have reported azole-resistant *A. fumigatus* isolates in European countries, Asia, and the United States. Therefore, new approaches are needed. The aim of the present study was to evaluate the in vitro combination of micafungin with voriconazole as currently used antifungal drug against azole-resistant A. fumigatus with various point mutations from clinical and environmental sources. The in vitro interactions of combination between voriconazole and micafungin was determined by a microdilution broth checkerboard technique against 30 azole-resistant *A. fumigatus* with various point mutations (TR34/L98, TR46/Y121/T289 M220I and G54W) from different geographical origin (Netherlands, Tanzania, Romania, India and Iran). MICs were determined with a complete inhibition endpoint and partial inhibition endpoint (50% inhibition. Fractional inhibitory concentration (FIC) indices were calculated, and drug interactions were defined as synergistic, indifferent and antagonistic. MICs and MEC range of voriconazole and micafungin alone were 1->16 µg/ml, and 0.031-<0.016 µg/, respectively. Indifferent interactions obtained based on measuring the FIC index were dependent on the MIC endpoint (FIC indices between 0.5 and 2) by using a complete inhibition (MIC, 0) and partial inhibition endpoint (50%), in which FIC index were related to voriconazole and micafungin MICs and were influenced by the CYP51A genotype with different geographical origin. However, no antagonism was found. The combination of micafungin with voriconazole was not strongly synergistic against various triazole-resistant *A. fumigatus.* The differences between studies may be related to different methodological approaches. Therefore, clinical effectiveness in the treatment of infection remains to be determined for these promising drugs in combination.

O-07

Biomarker analysis for Invasive aspergillosis

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Invasive aspergillosis (IA) is a clinical form due to *Aspergillus* which is a major cause of morbidity and mortality in the patients with severely underlying conditions. Delayed diagnosis and therapy may lead to a poor outcome. Diagnosis of IA may be facilitated by a test for the detection of fungal biomarkers including galactomannan (GM) and β-D glucan (BDG) antigens,

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which are of increasing interest in the clinical settings. The accurate diagnosis of IA can withhold the use of expensive and potentially toxic antifungal drugs. In addition, an earlier diagnosis of IA is very important to amend the patients' survival.

In IA, the clinical manifestations and symptoms are generally non-specific. The significance of positive results of culture or positive findings of a direct microscopic examination of a respiratory specimen is greatly uncertain, because they have low sensitivity (50%) and specificity (20%–70%), depending on the underlying condition of patients. Histopathological examination as the golden standard method is not often practicable because of the patient's status that prohibits invasive procedures. Therefore due to the limitations of the aforementioned diagnostic tools, a non-culture based method with a focus on the detection of the reliable biomarkers such as GM or BDG antigens have been developed. GM is a cell wall polysaccharide component released during growth and invasion of *Aspergillus* in tissue that is detectable in patients with IA. A sensitivity of 61% to 71%, with a specificity of 89% to 93% for the detection of GM in serum samples was suggested by previous studies. BDG is a cell wall polysaccharide of most pathogenic fungi including *Aspergillus*, but not bacteria or viruses. The BDG assay is not specific to *Aspergillus* and the positive results can be observed in other fungal infections especially in infections with *Candida* species. In this review the current methods which are useful for detection of *Aspergillus* biomarkers as well as some other promising fungal targets and biomarkers for early and definitive diagnosis of IA will be discussed.

O-08

Cell Membrane Proteome of Fluconazole Resistance Gene in *Aspergillus fumigatus*

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Clinical treatments *Aspergillus fumigatus* as an opportunistic pathogen with fluconazole may fail due to the facade of resistance to this azole. This survey demonstrated that antifungals with a basic mechanism of action lead to comparable impact at the proteome level and that a proteomics approach can be used to discriminate different antifungals, with the assure to become a valuable utensil to study azole of unknown mechanism of effect to opportunistic fungi. Protein dots were in-gel digested and resulting peptides were analyzed using MALDI-TOF MS and/or nano-ESI MS/MS. MS data were aligned against the NCBInr and the Genome Therapeutics Corporation Pathogenome databases using the MS-Fit and MS-Seq and/or MS-Edman programs respectively. Probable post-translational and chemical modifications such as carbamidomethylation of cysteines, *N*-terminal acetylation, *N*-terminal pyroglutamic acid, oxidation of methionine and etc, were taken into reflection for the queries.

Circumstances of sample training and 2-DE separation were optimized in order to be able to analyze the proteome of *A. fumigatus* Fluconazole resistance gene.

567 amino acid obtained chain was matched with identity and unambiguously with an amino acid sequence present in the protein database.

Analyzing of obtained proten using bioinformatics tools showed that fluconazole resistance gene encoded a structural protein in structure of cell membrane with exact proteomic characters as shown at below diagram. This may be interpreting as a compensatory feedback in rejoinder to inhibition of (1,3) glucan synthesis that leads to an osmotically fragile cell wall. It is notable that some of the induced proteins such as enolase, HSP90p and Fbalp have been shown to be present in the cell wall of *A. fumigatus* or secreted by regenerating protoplasts in *A. fumigatus*. More expression of these proteins may reproduce stress at the cell surface and is maybe directly linked to the effect of mulundocandin.

Further studies with antifungal compounds acting at the same cellular level such as the nikkomycins, which inhibit chitin synthesis, would be helpful to confirm this hypothesis.

O-09

Chitin-induced airway epithelial cell innate immune responses are inhibited by carvacrol/thymol

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Chitin is produced in large amounts by fungi, insects, and other organisms and has been implicated in the pathogenesis of asthma. Airway epithelial cells are in direct contact with environmental particles and serve as the first line of defense against inhaled allergens and pathogens. The potential contributions of airway epithelial cells (AECs) to chitin-induced asthma remain poorly understood. We hypothesized that chitin directly stimulates AECs to release cytokines that promote type 2 immune responses and to induce expression of molecules important in innate immune responses. We found that chitin exposure rapidly induced expression of three key type 2-promoting cytokines, IL-25, IL-33 and TSLP, in BEAS-2B transformed human bronchial epithelial cells and in A549 and H292 lung carcinoma cells. Chitin also induced expression of mRNAs for the key pattern recognition receptors TLR2 and TLR4. Chitin induced expression of miR-155, miR-146a and miR-21, each of which is known to up-regulate expression of pro-inflammatory cytokines and decreased expression of SOCS1 and SHIP-1 mRNAs, which are known targets of miR-155. We conclude that direct effects of chitin on airway epithelial cells are likely to contribute to allergic airway disease and asthma

O-10

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

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Invasive fungal Infection is a major cause of mortality in patients with hematologic malignancy and hematopoietic stem cell transplant (HSCT). Invasive bronchopulmonary aspergillosis and invasive fungal sinusitis (IFS) are two important clinical presentations of *Aspergillus* in immunocompromised hosts. A descriptive cross-sectional study was performed on patients admitted to Taleghani hospital affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran during October 2012-October 2013. Clinical data of 24 patients with IFS were reviewed. All patients had hematologic malignancies, and received broad spectrum chemotherapy. Moreover, demographics data, clinical characteristics, manifested symptoms and signs, underlying diseases and outcomes of the patients were assessed.

In this study, age range of patients was 15-60 years. The IFS was proven, probable and possible in 25%, 66.7% and 8.3% of the cases, respectively. Serum galactomannan antigen was positive in 41.6% of the cases; 15 out of 24 cases with IFS had a history of antifungal chemoprophylaxis (54% fluconazole and 8.3% itraconazole) use before diagnosis. *Aspergillus flavus* (33%), *Aspergillus fumigatus* (20.8%), *Aspergillus niger* (16.7%) and *Mucor* spp. (16.7%) were responsible for the incidence of IFS. Moreover, 54% of IFS cases occurred in summer, whereas 91.6%

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happened during hospital construction, which is a risk factor in 91.6% of the cases.

Current study revealed that according to the results of this study, *A. flavus* was recognized as the most common isolated pathogen, followed by *A. fumigatus* as the second common isolated pathogen in patients with IFS. Additionally, the hospital construction was an important environmental risk factor for hospital-acquired infection in patients with hematological malignancy. It was concluded that primary disease and resistance to chemotherapy (37.5%) were the most important cause of mortality in patients with IFS, which has the most prevalence in summer.

O-11

Molecular approaches for diagnosis of invasive aspergillosis, advantages and limitations

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Traditional diagnosis of invasive aspergillosis is based on a combination of clinical, radiological, microbiological and histopathological findings. Although microbiological diagnostic methods are still the gold standards, they have low sensitivity, then there is a need for the development of new means of detecting *Aspergillus* and aspergillosis. While developed serological test (galactomannan antigen and beta-glucan) have implemented in some reference laboratories, PCR and other molecular techniques need to undergo standardization and still are under clinical evaluation. Numerous DNA amplification methods targeting ribosomal DNA genes (18S and 28S), ITS regions, β-tubulin and mitochondrial genes have been developed for the detection of *Aspergillus* DNA in blood, serum, BAL fluid, and other clinical specimens by using several amplification platforms and different amplicon detection methods. Molecular diagnostics appear promising for the rapid detection of *Aspergillus* infection directly from tissue or body fluid specimens, but until ongoing efforts to reach some form of international consensus, use of these tests in the diagnosis of invasive aspergillosis will remain investigational and continues to be a challenge.

Different diagnostic molecular approaches for invasive aspergillosis and their challenges associated with their use as a routine clinical practice, are discuss in this presentation.

O-12

Quantitative analysis of single-nucleotide polymorphism for rapid detection of TR34/L98H- and TR46/Y121F/T289A-positive *Aspergillus fumigatus* isolates obtained from patients in Iran

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We employed an endpoint genotyping method to update the prevalence rate of positivity for the TR34/L98H and TR46/Y121F/T289A mutations among clinical *Aspergillus fumigatus* isolates obtained from different regions of Iran over a recent 5-year period.

The antifungal activities of itraconazole, voriconazole, and posaconazole against 172 clinical *A. fumigatus* isolates were investigated using the EUCAST method. For the isolates with an azole resistance phenotype, the *cyp51A* gene and its promoter were amplified and sequenced. In addition, using a LightCycler 480 real-time PCR system, a novel endpoint genotyping analysis method targeting single-nucleotide polymorphisms was evaluated to detect the L98H and Y121F mutations in the *cyp51A* gene of all isolates.

The resulting MIC values for all 172 *A. fumigatus* isolates tested as follows: Itraconazole (>16 mg/liter) and Voriconazole (>4 mg/liter) were high for 6 (3.5%). Quantitative analysis of single-nucleotide polymorphisms showed the TR34/L98H mutation in the *cyp51A* genes of six isolates. No isolates harboring the TR46/Y121F/T289A mutation were detected. DNA sequencing of the *cyp51A* gene confirmed the results of the novel endpoint genotyping method. By microsatellite typing, all of the azole resistant isolates had genotypes different from those previously recovered from Iran and from the Dutch TR34/L98H controls.

There was not a significant increase in the prevalence of azole-resistant *A. fumigatus* isolates harboring the TR34/L98H resistance mechanism among isolates recovered over a recent 5-year period in Iran. A quantitative assay detecting a single-nucleotide polymorphism in the *cyp51A* gene of *A. fumigatus* is a reliable tool for the rapid screening and monitoring of TR34/L98Hand TR46/Y121F/T289A-positive isolates and can easily be incorporated into clinical mycology algorithms.

O-13

Molecular identification of fungal infection in Acute Invasive Fungal Rhinosinusitis in patients refer to ENT department in Ghaem and Emam Reza hospitals, Mashhad, Iran

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Acute Invasive Fungal Rhinosinusitis (AIFR) is characterized by invasion of the nasal cavity and paranasal sinuses with fungal organisms. Although AIFR is a rare disease, there is a rise in the incidence of AIFR due to the increased number of immunocompromised patients. Undoubtedly, rapid diagnosis of fungi in patients with fungal rhinosinusitis affects the treatment planning and prognosis of the patients. Traditional techniques for fungal identification are time consuming and some species will not be detected clearly. In this study we used ITS PCR-Sequencing for rapid and accurately identification of fungal isolates.

A total of 106 samples from patients with fungal rhinosinusitis over a period of 48 months from the ear, nose and throat of patients were collected in Imam Reza (AS) and Ghaem hospital of Mashhad. After direct microscopy identification and culture at 35°C, the fungus DNA was isolated for molecular diagnosis and then were amplified and sequenced by primers ITS1 & ITS4. Sequences were analyzed using SEGMAN software and fungal species were identified after BLAST in the Genbank.

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From 63 patients suspected of AFRS, in seven patients cultures were positive and PCR sequencing results indicated, *Aspergillus fumigatus* in 6 and *Aspergillus flavus* in one patients. In patients with clinical diagnosis IFRS culture was positive in 75% of patients and PCR sequencing results showed *Rhizopus orizaei* in 12 and *Aspergillus fumigatus* in 13 and *Aspergillus flavus* in 3 and *Lichtheimia* in 2 patients. Of the 3 patients with clinical diagnosis of fungus ball, culture was positive in one patient and PCR sequencing indicated *Aspergillus flavus* as the etiologic agent.

PCR sequencing of the fungal ITS primer is a rapid and accurate method to identify fungi in fungal rhinosinusitis patients.

O-14

Isolation, characterization and functional analysis of *Aspergillus nidulans* cotA gene using some bioinformatics tools

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Introduction: While little is known about the molecular mechanisms that establish the apical growth patterns in fungal mycelia, recent research on the genetics of *A. nidulans* and *N. crassa* has provided considerable amount information on some genes involving in polarised cell growth. *A.nidulans* is particularly amenable to experimental analysis and is importantly related to other human pathogen fungi that are not so easily studied such as *A. fumigatus*, *A. niger* and *A. flavus*.

Methods: To identify the gene, degenerate oligonucleotide primers and polymerase chain reactions were employed to generate an amplified fragment for screening *A. nidulans* cosmid library. The selected cosmid was then digested with restriction enzymes and 4.3kb *XbaI* hybridising band was cloned.

Results: We discovered *A. nidulans* cotA gene on chromosomeV. It encodes a putative serine/threonine protein kinase, predicts a polypeptide of 597 amino acids. Further database searches demonstrated that the nucleotide sequence has the following degree of identity with *N. crassa* cot-1(70%) and fission yeast *orb6* (61%). After following the molecular disruption strategy, failure to isolate the expected heterokaryons suggested that the disruption of this gene may result lethal effect in the Aspergillus conidiospores. The promoter replacement approach revealed that the homologous transformant colonies have restricted colonial phenotype in repressing condition and they are eight times smaller in colony diameter than wild type colonies.

Conclusion: Our results predicts a possible cotA protein kinase role in regulation of hyphal tip elongation and branching in the model filamentous fungus A. *nidulans*, and conservation of those kinases among pathogenic and saprophytic fungi.

Keywords: *Aspergillus*, gene, polarised cell growth.

O-15

Investigating the presence of *Aspergillus fumigatus* and *Aspergillus flavus* in clinical samples by two methods of galactomannan enzyme assay and TaqMan real-time PCR

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Invasive aspergillosis (IA) is a serious and fatal disease caused by various species of the opportunistic fungus *Aspergillus*. This agent is widely dispersed in the environment around us. Lack of early diagnosis and delay in treatment can lead to the rapid spread of the infection and relapse, increasing treatment costs and ultimately lead to death. Of the Aspergillus species, *Aspergillus fumigates* and *Aspergillus flavus* are the most common species which are isolated from mentioned disease. The objective of this study is to investigate the presence of Aspergillus fumigatus and flavus with galactomannan and Real-time PCR methods. In this cross-sectional study, Broncho alveolar lavage [BAL] fluid samples were acquired from 89 patients who were undergoing bronchoscopy in shariati hospital at risk for Invasive aspergillosis [IA] by pulmonologist to identify reports with suggestive finding of infection and lung infiltration between June 2013 and January 2014. The specimens by direct and culture methods, galactomannan EIA(GM) and Real Time PCR were tested in the laboratory of mycology in Tarbiat Modares University. The data were analyzed using XLSTAT and SPSS software by plotting ROC Curve and regression logistic. 23 samples were identified direct positive, 7 samples were *Aspergillus fumigatus* positive, 11 samples were *Aspergillus flavus* positive in Culture assays, 27 samples were EIA positive, 29 samples were positive with PAN *Aspergillus* probe and 7 samples were positive with *Aspergillus fumigatus* probe and 11 samples were positive with Aspergillus flavus probe. The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for a BAL GM level of ≥1.0 were 94.4%, 85.9%, 98.4%, and 62.9% respectively and were 100% for TaqMan Real time PCR. The results indicate that TaqMan Real time PCR have a high value in detection. Since the early detection have special place to prevent the occurrence of drug resistance and high mortality, it is necessary to consider this method directly from the BAL or blood specimens without culture which is lead to the prolongation of the diagnosis.

O-16

Airborne *Aspergillus* species: Geographical distribution and phylogenetic profiles

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Airborne *Aspergillus* species are responsible for a diverse group of human disorders from allergy to life-threatening invasive aspergillosis and mycotoxicosis. In the present study, diversity and phylogenetic relationship of *Aspergillus* species isolated from Tehran air were studied using a combination of morphological criteria and molecular analyses. The isolated aspergilli were identified based on internal transcribed spacer (ITS1 and ITS4 primers) sequencing, restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). The *Aspergillus* isolates belonged to 12 species including *A. niger* (28.94%), *A. flavus* (18.42%), *A. tubingensis* (13.15%), *A. japonicus* (10.52%), *A. ochraceus* (10.52%), *A. nidulans* (2.63%), *A. amstelodami* (2.63%), *A. oryzae* (2.63%), *A. terreus* (2.63%), *A. versicolor* (2.63%), *A. flavipes* (2.63%), and *A. fumigatus* (2.63%). The isolates were obtained by settle plate method, and they were distributed in 18 out of 22 sampling sites. RAPD and RFLP-PCR data were analysed using UPGMA software, which indicated the distribution of 12 *Aspergillus* species in up to eight major clusters. The similarity coefficient of all the *Aspergillus* isolates ranged between 0.02 and 0.40, showing high degrees of similarity and difference within and between the species. Our results revealed that various *Aspergillus* species, including some important human pathogenic ones, exist in the outdoor air in various patterns of distribution and diversity. Furthermore, we found inter- and intra-

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species genetic diversity among *Aspergillus* species by ITS sequencing, which is a rapid, sensitive, and reproducible method.

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How can the diagnostic mycology laboratory improve the outcome of Aspergillosis?

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Regarding increasing burden of Invasive aspergillosis (IA) in patients with known risk factors including; prolonged neutropenia, allogeneic hematopoietic stem cells solid organ transplantation and advanced AIDS, and even in immunocompetent patients without risk factor, early diagnosis of the disease has a significant role which can prevents mortality. IA has a myriad of clinical presentations and diagnoses mostly rely on laboratory-based results.

Unfortunately, due to insufficient evidence based education in medical school, complexity nature of disease, starting the treatment is delayed many physicians know little about fungal diseases and thus the treatment is delayed. Diagnostic tools for IA have continuously improved within the last decades. Nowadays, cultural methods, antigen testing, and molecular tests, such as polymerase chain reaction, are widely used. These methods, however, are accompanied with different limitations such lack of availability, different turnaround time and high costs. Medical mycologist with other health professionals notably infectious diseases, pulmonology, oncology, dermatology, critical care specialists can contribute to diagnose and improved outcomes. Improved clinical links can greatly empower the effectiveness of both the laboratory and patient care, but clinical experts in fungal infection covering several specialties is rare. Diagnostic mycology laboratories in well-developed countries, are supposed to be good sources of fungal infection knowledge. The center which have well-recognized clinical experts in fungal disease can deliver the best care and they can advise others. Lack of early diagnosis may cause quick dissemination and delayed treatment of infection which can increase health costs and mortality. Clinicians must be aware of the possibility of IA and consider it as the main differential diagnosis; particularly, when there is evidence of severe pulmonary, and sometimes brain, heart, liver and kidney involvement, with no proper response to antibiotic therapy. In these cases, rapid bronchoscopy and biopsy are recommended. Furthermore, *Aspergillus* antigen serology is useful.PCR methods especially Real-time offer several advantages over conventional diagnostic methods with reasonable turnaround time. This assay permits highly reproducible detection and quantification of fungal burden which is also important in monitoring the effectiveness of treatment. Real-time PCR together with the sequence-specific DNA probes allow identification of *Aspergillus* at the species level . Nevertheless, lack of standardization of the procedure is a major reason why the EORTC/MSG has not yet included in criteria for the diagnosis of IA.

Radiological evidence particularly unusual infiltration and abnormal CT findings, including nodules, halo sign and segmental infiltration are all helpful. Biopsy and/or normally sterile specimens should be obtained in accordant of the patient's situation. Observation of dichotomous branching septate hyphae and isolation of causative fungi are necessary for confirmation of diagnosis. In these circumstances, presumptive antifungal therapy is administered to achieve decisive results and to prevent death.

In this conference, we report the various cases of Aspergillosis, who referred to our lab for diagnosis and we identified causative *Aspergillus* to species level and determined in vitro susceptibility profile to antifungal that assisted clinicians in treatment decision making and as a result patients survived by prompt treatment.

This report highlights the presence of emerging *Aspergillus* and supports the necessity of careful mycological diagnosis at the species level, thereby reinforcing the physician's attention toward the possibility of invasive aspergillosis in immunocompromised patients.

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Evaluation of aspergillosis infection in solid organ transplantation

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Invasive aspergillosis is one of the leading causes of morbidity and mortality among solid organ transplant recipients, especially in lung transplantations. Diagnosis of invasive aspergillosis is one of the current challenges due to nonspecific clinical symptoms and broad differential diagnosis. Definitive diagnosis usually requires invasive procedures, including bronchoscopy and CT-guided biopsy. Despite the effectiveness of serum galactomannan and (1-,3)-beta-D-glucan in some settings, sensitivity and specificity are low in this population. Culture of bronchoalveolar lavage (BAL) for fungi is positive in approximately half of the patients. Treatment of choice for pulmonary aspergillosis in solid organ transplant recipients is voriconazole. However, combination of voriconazole and caspofungin as primary therapy might be applied in severe cases. Reduction of immunosuppression is the mainstay of treatment and prophylaxis of invasive aspergillosis, recommended for lung transplant recipients due to its considerable importance. Targeted prophylaxis could be more appropriate for other types of solid organ transplantation. Moreover, drug-drug interaction between azole antifungals and immunosuppressants must be considered prior to transplantation.

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The effect of involved *Aspergillus* species on galactomannan in bronchoalveolar lavage of patients with invasive aspergillosis

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The detection of galactomannan (GM) in bronchoalveolar lavage (BAL) is an important surrogate marker for the early diagnosis and therapeutic monitoring of invasive aspergillosis (IA), regardless of the involved species of *Aspergillus*. In the present study we compared the Platelia *Aspergillus* GM EIA (Bio-Rad) for determination of GM index in BAL of patients with proven and probable IA due to *Aspergillus flavus* versus *A. fumigatus*. In a prospective study between 2009 and 2015, a total of 116 BAL samples were collected from suspected patients to IA referred to two university hospitals in Tehran, Iran. According to EORTC/MSG criteria, 35 patients were

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classified as IA patients, of which 33 cases had positive GM above 0.5 and 22 cases with GM index ≥ 1. Twenty eight were culture positive for *A. flavus* and seven for *A. fumigatus*. The GM index for *A. flavus* cases was between 0.5-6.5 and those of *A. fumigatus* ranged from 1 to 6.5. The sensitivity and specificity of GM index ≥ 0.5 in cases with *A. flavus* were 89.3% and 100% and those of *A. fumigatus* were 100% and 100%, respectively. The mean of GM index in IA patients with *A. fumigatus* (3.1) was significantly higher than those of *A. flavus* (1.6), (P = 0.031). In the patient group selected for a high likelihood of IA, the sensitivity of GM was lower for *A. flavus* compared to *A. fumigatus.* This finding might have implications for diagnosis in hospitals and countries with a high proportion of *A. flavus* infections.

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Aspergillus spp. germ tubes induce stronger cytokine responses in human bronchial epithelial cells in comparison with spores.

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Aspergillus spp. are ubiquitous saprophytic fungi that cause a variety of diseases, ranging from hypersensitivity reactions to flu-like pneumonia and life-threatening invasive aspergillosis. As the lung is the primary site of initial infection with airborne conidia, we investigated the innate immune responses of bronchial epithelial cells against different forms of *Aspergillus spp*.

Primary human bronchial epithelial cells were treated with equal numbers of killed spores or germ tubes of either *Aspergillus fumigatus* or *Aspergillus flavus*. Analysis by real-time PCR showed that inflammatory cytokines such as IL-8 and IL-6 as well as the proinflammatory protease, caspase-5 were strongly upregulated by both treatments in a dose-dependent manner. Consistently, germ tubes induced a stronger response than spores. TNF-alpha and beta-2-defensin were induced by high a concentration of germ tubes, but not by spores. IL1 pretreatment highly induced the expression of both beta-2-defensin, IL8 and IL12.

Taken together, our results show that germ tubes of *Aspergillus fumigatus* and *A. flavus* are potent inducers of innate immune responses in human airway cells. Considering the presence of Aspergillus spores in the air, differentiation between transient spore contact and invasion, as represented by germ tube formation, is important in order to determine proper immunological response. Moreover, these results can also provide additional data in understanding pathophysiology of hypersensitivity reactions due to the aspergilli.

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