

Poster Presentation Abstracts

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P-001

Prevalence of pityriasis versicolor in kidney patients referred to dialysis center of Mazandaran.

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Introduction: Pityriasis versicolor is a fungal disease from the category of superficial fungus. This fungus causes chronic and recurrence infection of the horny layer of the skin that is created by lipophilic yeast from *Malassezia*. Human-to-human transmission is possible through direct contact. This study was performed to determine the prevalence of pityriasis versicolor in kidney transplant recipients referred to the dialysis center of Mazandaran province at Valiasr hospital in Qaemshahr.

Material and methods: In this descriptive survey, 150 people with renal disease were studied using skin scrubs, scotch glue and bulbs.

Results: Out of 150 kidney patients, 46% were male and 54% were female. The average age was between 16 and 56 years old. 28% of males and 13% of females had pityriasis. The prevalence of pityriasis was determined 14.8% in all three methods. In the presented study the most common infected parts were scaphoid and neck.

Discussion: The recent study revealed a high prevalence of pityriasis versicolor in renal patients. The prevalence of pityriasis was significantly different between males and females and it was more in males. In the two-duplicate comparison of the results, the obtained ones from all three diagnostics methods using Kappa coefficient, scotch glue method had the highest matching with the two other methods.

Keywords: Pityriasis versicolor, kidney patients, skin scrubs, scotch glue.

P-002

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Conventional identification of fungal species isolated from patients with otomycosis in Urmia, Iran

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Introduction: Otomycosis is one of the most frequently encountered fungal infections of external auditory canal, commonly seen in tropical and subtropical regions of the world. Various host (local, systemic) and environmental factors can predispose a person to otomycosis. Although clinical presentations along with otoscopic findings of the patients are well suggestive of fungal infections, proper identification of causative agents is mandatory in order to prevent recurrences and complications. The aims of this study were to determine the pattern of fungal agents, sex distribution, clinical presentation, predisposing factors, complications and treatment outcomes of otomycosis in Urmia, Iran.

Material and methods: A prospective study was conducted in the Department of Mycology and Ear, Nose and Throat (ENT), Urmia, Iran, over four months period. A total of 55 consecutive patients with clinical diagnosis of otomycosis were included in the study. Demographic profile, predisposing factors, presenting complaints and clinical findings of clinically diagnosed patients were evaluated and analyzed. Samples were collected, transported and evaluated by both direct examination and culture method for

bacteriological and mycological examination.

Results: Male to female ratio in study participants was 42%:58%. Mycological examination yielded 84.4% fungal (n = 27) and 15.6% bacterial (n = 5) isolates in 32 samples from a total of 55 clinically diagnosed cases of otomycosis. Self-cleaning (52 %), instillation of mustard oil (12%) and use of ear drops (52%) appeared to be common predisposing factors in otomycosis. The predisposing factors included frequent scratching of the external ear canal (56%), taking ototopical and/or oral antimicrobials (36%) and diabetes (8%). Significant association was observed between these practices and otomycosis. *Aspergillus* species (*A. niger*; 56.2%, *A. fumigatus*; 6.2%, *A. terreus*; 3.1% and *A. flavus*; 3.1%) were the predominant fungi followed by *Candida* species (*C. Krusei*; 16%) and *Penicillium* species (3.1%).

Conclusion: The present study highlights the highest isolation of *Aspergillus* complex especially *Aspergillus niger* complex in cases of clinically diagnosed otomycosis in a rural community with higher practice of self-cleaning and using home remedies and eardrops to get relief from sensation of blocked ear and itching. However, recurrence is not uncommon and eradication of disease can be particularly difficult in patients with diabetes and a mastoid cavity.

Keywords: Conventional identification, fungal species, otomycosis, Urmia, Iran

P-003

Interspecies differences of *Candida* species causing recurrent vulvovaginal candidiasis in response to fluconazole treatment

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Introduction: During last two decades, the recurrent vulvovaginal candidiasis (RVVC) by drug resistant non *albicans* *Candida* species has been emerged. Hence, an epidemiological study and the drug resistance of *Candida* species causing VVC and RVVC was conducted.

Material and methods: The specimens including cervicovaginal discharge were obtained from symptomatic infectious women at the Kowsar Gynecology Center, Urmia, Iran. The samples submitted to Urmia Medical Mycology Center for the direct microscopy and cultures. Identification of the species was performed using CHROMagar *Candida*, Cornmeal agar media and the PCR-RFLP assay. Drug resistance to fluconazole and Clotrimazole using disc diffusion method was determined.

Results: Commonly isolated *Candida* species included: *Candida albicans* (84%), *Candida krusei* (12%), and *Candida glabrata* (4%). Total of 27 cases of RVVC, 10 were resistant to both Clotrimazole and Fluconazole (37%). Most resistant *Candida* species were: *Candida albicans* (81.4%), *Candida krusei* (14.8%) and *Candida glabrata* (3.8%).

Conclusion: Frequency of non *albicans* *Candida* species resistant to fluconazole in this study is increasing as the other similar studies have reported.

Keywords: *Candida*, fluconazole, vulvovaginal infection.

P-004

Clinical and mycological study of vulvovaginal candidiasis (VVC)

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Introduction: Vulvovaginal candidiasis (VVC) is a common fungal infection among women worldwide. The infection is caused from the lower genital and is reported in 35%-80% of cases without any symptoms. The main risk factors are hormone replacement therapy, diabetes mellitus, antibiotic usage, pregnancy, oral-contraceptives, and insufficient therapy. *Candida albicans* is the most etiologic agent of *Candida* vaginitis capable of colonizing on the mucous membrane of genitourinary tracts of healthy humans. The aim of the present study is to identify *Candida* species obtained from patients with vulvovaginitis by Polymerase Chain Reaction-Fragment Size Polymorphism (PCR-FSP) Technique and evaluation of 3 antifungals for treatment of patients.

Material and methods: In this cross sectional descriptive study (November 2015 to April 2016), 108 suspected patients were evaluated for vulvovaginal candidiasis. Suspected patients were divided into 3 groups and each group took only 1 antifungal agent including clotrimazole, miconazole, and nystatin, respectively. Direct microscopic examination, culture, and PCR-FSP were used for identification of clinical isolates.

Results: Of the 108 patients, 59 (54.6%) had both positive culture and direct microscopic examination. The duration of disease was between 3 to 365 days. Clinical manifestations among suspected cases were pruritus (84%), burning (74%), vaginal discharge (71%), pain during or after sex (30%), and inflammatory (8%). All patients were married, however, none of the patients were pregnant. Use of antibiotics (35.6%) and diabetes mellitus (6.8%) were the most predisposing factors among patients. *Candida albicans* was the most prevalent *Candida* species isolated from patients

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(74.5%) followed by *Candida glabrata* (17%). The correlation between the kind of antifungal agents and recovery of patients was not statistically significant (P value = 0.056).

Conclusion: Resistance to various antifungal agents and emerging of non-*albicans* *Candida* species among clinical specimens are crucial affairs in the field of medical mycology. Since VVC is a prevalent and recurrent infection, controlling of predisposing factors, personal hygiene, and appropriate antifungal therapy are extremely recommended among vulnerable population.

Keywords: Vulvovaginal candidiasis, antifungal agents, molecular diagnostic techniques

P-005

A study of candiduria among kidney transplant recipients

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Introduction: Kidney transplantation, being an immunosuppressed state, put the recipient at risk of a variety of viral, bacterial, and fungal infections. Urinary tract infections (UTIs) are common throughout the first several months post-transplantation. Candiduria continue to be a significant complication for renal transplant recipients. The risk of infections depends on the amount of immunosuppression and exposure to the potential pathogens. This study aimed to investigate the *Candida* urinary tract infections in renal transplantation recipients during 5 years in Isfahan, Iran.

Material and methods: A total of 485 renal transplant recipients (849 episodes) was registered in two university hospitals (Al-Zahra and Khorshid) in Isfahan, central Iran, from May 2009 to August 2014. Tacrolimus,

mycophenolate mofetil (CellCept), sirolimus, and cyclosporin were used for patients for immunosuppression. All urine samples were examined by repeated urine culture on Sabouraud Glucose agar, and CHROMagar *Candida*. The number of yeasts in urine specimens was counted; a count of >1000 colony/mL was considered "candiduria". All isolates were identified by PCR-RFLP profiles after digestion with the restriction enzyme *MspI*.

Results: Sixty-two patients were diagnosed with candiduria. *C. albicans* (44%) and *C. parapsilosis* complex (5%) had the most and the least prevalence, respectively. *C. albicans* was the most prevalent species isolated from diabetic patients (65%), followed by *C. tropicalis* (15%), and *C. glabrata* (15%). Twenty-six patients were male (42%) and 36 (58%) were female, ranging in age from 19 to 62 years. Diabetes mellitus (DM) and high blood pressure (HBP) were the two leading causes of end-stage renal disease among patients with candiduria. Twenty-eight (45%) patients were hospitalized in ICU, 18 (29%) in transplantation ward, and 16 (26%) in general medicine ward. Fourteen (22.5%) patients had lower urinary tract symptoms (LUTS) such as dysuria, frequency, and incomplete voiding; 6 (10%) patients had upper urinary tract symptoms (UUTS) including fever, chills, pain and tenderness, nausea, and vomiting, while 42 (68%) were asymptomatic.

Conclusion: Due to the fact that candiduria is connected with increased mortality in renal transplant recipients, precise identification of *Candida* species by molecular techniques can lead to an appropriate therapy among high risk patients.

Keywords: *Candida* species, candiduria, renal transplantation

P-006

Various causative agents of otomycosis, the clinico-microbial epidemiology in Isfahan, Iran

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Introduction: Otomycosis is a common infection due to a divergent set of fungi. This study was conducted to elucidate the epidemiology of otomycosis and identify the causative agents using molecular approach in Isfahan, central Iran. From January 2016 to January 2017 all clinically suspected patients in Al-Zahra hospital, Isfahan, Iran were recruited.

Material and methods: Specimens were taken by an otorhinolaryngology specialist and subjected for microscopical examination using KOH and Giemsa stain as well as culture on Sabouraud dextrose agar plates. Isolated fungi were identified using morphological characteristics and molecular methods. Susceptibility of the isolates to itraconazole was determined using broth microdilution method.

Results: Data were analyzed using Chi-square test in SPSS version 22. Among 120 patients, otomycosis was confirmed in 97 cases (80.83%). Females (72.16%) and age group of 30-39 years (32.99%) were more commonly affected. Pruritus was the dominant symptom observed in 84.54 % of patients and the most otomycosis episodes (50.51%) were diagnosed in summer. Using molecular methods, more than 18 species of genera *Aspergillus*, *Candida*, *Penicillium*, *Cladosporium*, *Alternaria*, *Cryptococcus* and *Talaromyces* were identified. *Candida parapsilosis* (n=22, 22.68%) was the most frequent species followed by *Aspergillus tubingensis* (n=15, 15.46%). The minimum inhibitory concentrations (MIC) of itraconazole ranged from 0.125µg/ml to >16µg/ml. generally, *Candida* species had elevated MICs to itraconazole.

Conclusion: In this study, otomycosis due to a divergent set of species including rare organisms was recorded. Application of molecular approaches for identification of otomycosis-isolated fungi might change current knowledge on the causative agents of otomycosis.

Keywords: otomycosis, epidemiology, Iran, antifungal agents

P-007

Identification of *Candida* species isolated from the oral cavity of patients with head and neck cancers in Isfahan in 2017-2018

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Introduction: *Candida* species are the most common causes of fungal infections. Oral candidiasis is one of the most clinical manifestations that occurs in different immunocompromised patients by various *Candida* species. Head and neck cancers are the most common cancers in developing countries. *Candida albicans* is the most important opportunistic microorganism in human oral cavity and other mucus membranes live as normal flora.

The purpose of this investigation was carried out to determine the frequency and identification of yeast species in the oral cavity of patients with head and neck cancers.

Material and methods: This study was performed on 35 patients with head and neck cancer who referred to Seyed Al-Shohada hospital in Isfahan. Oral specimens were collected in two stages: before radiotherapy and after radiotherapy, using two wet sterile swabs. Direct examination and culture on Sabouraud Dextrose agar were carried out on all samples and colony counts were performed after growing the colonies. Individual colonies were identified by PCR-RFLP molecular method using the *MSPI* restriction enzyme. It should be noted that the second stage of sampling was performed at least one week after the first radiotherapy

Results: A total of 10 samples obtained from the patients (28.57%) were positive in direct examination for candidiasis (observing pseudohypha and blastoconidia) before radiotherapy, and in 12 samples (34.28%) after radiotherapy.

The mean of the yeast colony number in the patients before and after radiotherapy was significantly different ($p \leq 0.05$). It showed 75% of the detected yeasts from the oral cavities of cancer patients and 63% of the isolates were *C. albicans* before and after radiotherapy, respectively. In total, at the present study, 25% of the species were identified non-*albicans* species consisting of seven species of *C. tropicalis*, four species of *C. glabrata* and one species of *C. krusei* before treatment, whereas after radiotherapy, this rate increased to 36.9%. So the result showed a shift from *C. albicans* to non-*albicans* species after radiotherapy.

Conclusion: In the present study, the dominant species of yeast isolated from the oral cavity of head and neck cancer patients before and after radiotherapy was *C. albicans*. So the result showed a shift from *C. albicans* to non-*albicans* species after radiotherapy. Induction of immunosuppression or doing radiation therapy in addition of increasing the number of oral normal *Candida* flora may be altered to the non-*albicans* yeasts.

Keywords: Head and neck cancer, radiotherapy, *Candida*, candidiasis.

P-008

Prevalence of drug-resistant *Candida* species causing recurrent vulvovaginal candidiasis

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Introduction: To investigate the drug resistance in women with vulvovaginal candidiasis which has long been treated with azoles, susceptibility of *Candida* species isolated from VVC cases to clotrimazole and fluconazole and molecular screening of *ERG3* gene in the azole resistant *Candida* species was performed.

Material and methods: For the identification at the species level of isolated *Candida* species differential media, CHROMagar *Candida*, GT test and Corn meal agar were used and confirmed by PCR-RFLP. A disc diffusion method was performed based on the standard guidelines of the National Committee for Clinical Laboratory Standards to determine level of susceptibility against fluconazole and clotrimazole. The azole resistance gene, *ERG3* was detected.

Results: Among all *Candida* isolates, 76.3% (74 cases) were *Candida albicans* followed by *C. glabrata* and *C. krusei* 9 (9.3% each) and other non-*albicans* *Candida* species 4 (4.1%). From the *C. albicans* isolates resistant to Clotrimazole, 8(53.3%) had *ERG3* gene and 7(46.7%) did not. Among all isolates resistant to Clotrimazole, 40% carried *ERG3* against 60% of others that did not show the gene. Also, in 50% of the isolated *C. glabrata* *ERG3* gene was detected.

Conclusion: As a conclusion, the wild gene of *ERG3* cannot be detected in most of the azole resistant *Candida* species.

Key Words: *Candida*, RVVC, Drug resistant, *ERG* gene.

P-009

Fungal arthritis

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Introduction: Arthritis is joint inflammation with various causes and factors. This disease

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manifests its self in different forms, the most common of which is osteoarthritis. Various fungi including *Candida*, *Aspergillus*, *Histoplasma*, *Blastomyces*, *Coccidioides*, *Cryptococcus*, *Sporothrix*, etc cause fungal arthritis. Although musculoskeletal infections of fungi are rare, their prevalence has increased in recent years due to an increase in immunosuppressive factors. Factors causing susceptibility to the development of fungal arthritis include those that debilitate the immune system, such as alcoholism, cirrhosis, diabetes, tuberculosis, cancer, long-term use of intravenous antibiotics, bone marrow hyperplasia, and especially injecting corticosteroids in the joint. Joint fungal infections are not easily diagnosed and the infection-causing fungus is not recognizable in the tissue. In addition, due to the manifestation of a wide variety of clinical symptoms, its diagnosis and treatment are delayed.

Material and methods: Radiography, medical ultrasound, and laboratory methods are among the methods used for arthritis and osteoarthritis. Computed tomography (CT), magnetic resonance imaging (MRI) and radionuclide scanning can be used to help the diagnosis of vague cases of arthritis or determining the degree of bone infection as well as the surrounding soft tissue. Moreover, ultrasonography is a technique that is able to detect intra-articular disorders, which are not shown in regular radiography. Laboratory methods include direct microscopic tests, staining with fungi-specific dyes, synovial fluid culturing, performing PCR to extract fungal DNA from synovial fluid and biopsy tissue, as well as diagnostic serologic methods such as IgG titer and IgM titer which increase with chronicity and acuteness of disease respectively, CF test, sphrolin skin test, and latex agglutination test.

Results: The most common isolated and recognized fungal pathogens in fungal arthritis and osteomyelitis diseases are various species of *Candida*, *Aspergillus*, *coccidioides*, *Histoplasma*, *Blastomyces*, and *Cryptococcus*. Effective medication proposed by articles include amphotericin B, fluconazole, 5-flucytosine, ketoconazole,

itraconazole, caspofungin, and micafungin. Caspofungin and micafungin can be used as an alternative medication since they penetrate the formed biofilms more than other drugs. Imaging studies support the presence or absence of tissue damage and are considered as physical and chemical changes occur in the synovial fluid as well as changes in blood parameters such as white blood cell count, ESR, and CRP, but they cannot be regarded as definite diagnostic indicators.

Conclusion: The best treatment for fungal osteomyelitis and septic arthritis is to eradicate the infection and prevent it from relapsing. The orthopedist's cooperation with an infectious disease specialist in monitoring patients and producing an antifungal treatment program is of great importance but less focused on. In order to completely eliminate an infection, consuming a single type of antifungal drug is insufficient and can be problematic. Although selecting an effective medication for managing arthritis is simple, the duration of treatment is different for each of these medications. The release of antifungal agents is among the new methods, on which more studies are required to be conducted with regard to its efficacy.

Keywords: Fungal arthritis, Osteomyelitis, Synovial fluid

P-010

First case of onychomycosis due to *Neoscytalidium novaehollandiae* in Iran

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Introduction: Melanized fungi are a heterogenous group of molds that cause a wide range of diseases including phaeohyphomycosis, chromoblastomycosis and eumycotic mycetoma. Dematiaceous fungi which also include members of the genus *Neoscytalidium* to cause a variety of clinical conditions, including superficial and subcutaneous infections, endophthalmitis and disseminated infections. Reports of human infection by *Neoscytalidium novaehollandiae* are rare. This may be a result of difficulties with identifying the organism, or differences in its geographical distribution. To our knowledge, this is the first report of onychomycosis due to *Neoscytalidium novaehollandiae* from Iran.

Case report: We present a case of onychomycosis caused by *Neoscytalidium novaehollandiae* in a 52-year-old Iranian female without history of immunodeficiency and underlying disease who presented in June 2016 to Razi hospital in Tehran, Iran. She has blackish pigmentation in toenail and distal area of the nail was empty. The other nails and skin of the soles and interdigital webs were normal. Scrapings were collected deeply from hyperkeratotic distal areas. Examination of potassium hydroxide mounts from the samples revealed brown, septated and branching subhyaline to dark-coloured hyphae. The scrapings were cultured on Sabouraud Dextrose agar (SDA) with Chloramphenicol, at 25°C. The colony was bluish-green to dark olivaceous after 4 days. DNA extraction were performed by glass bead Phenol Chloroform method and molecular identification was performed. The entire sequence of the rDNA ITS domain was compare with the GenBank database. The nearest neighbour to our isolate within the ITS BLAST in GenBank was *N. novaehollandiae*, with 99% similarity. The ITS sequence was deposited in GenBank with accession number KY788097. *In vitro* antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) document M38-A2. The MICs of the eight antifungal drugs used in this study were luliconazole, 0.0002 µg/ml; itraconazole,

0.002µg/ml; efinaconazole, 0.063µg/ml; voriconazole, 0.125µg/ml; itraconazole, 4µg/ml; terbinafine, >0.5µg/ml; Amphotericin B 0.5µg/ml; and fluconazole, 32µg/ml.

Conclusion: Onychomycosis was considered as a fungal nail infection mainly caused by dermatophytes, sometimes caused by nondermatophyte molds such as dematiaceous fungi. In this study, we reported a case of onychomycosis caused by *Neoscytalidium novaehollandiae* that morphologically similar to the type specimen *N. dimidiatum*. The findings of this study indicated that sequencing rDNA gene is a valuable tool for identification of *Neoscytalidium novaehollandiae*.

Keywords: Melanized fungi, *Neoscytalidium novaehollandiae*, Iran

P-011

Prevalence of superficial and cutaneous fungal infections in patients referred to bu-ali clinical diagnostic laboratory in Zahedan during 2017-2018

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Introduction: Superficial and cutaneous mycoses are public health problems and remained the major causes of infections that affect the skin, hair and nails. The aim of this study was to investigate the epidemiology of superficial and cutaneous fungal infections among patients referred to Bu-ali clinical diagnostic laboratory in Zahedan.

Material and methods: In this descriptive and cross-sectional study a total of 161

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patients suspected to superficial and cutaneous mycoses. Samples were examined by direct microscopic examination of wet mount with 10% KOH (potassium hydroxide), Methylene blue stain, and Gram staining, and then the data were analyzed by SPSS-22 statistical software.

Results: We studied 74 males and 87 females. From the total of 161 suspected patients, 121 patients (75.15%) were affected by superficial and cutaneous mycoses. Frequency of Dermatophytosis was 57.76% (93 patients) included: tinea capitis 21.11% (endothrix 8.69%, ectothrix 11.80% & favus 0.62%), tinea manuum 14.26%, tinea pedis 11.16%, tinea unguium 6.2%, tinea corporis 3.72% and tinea barbae 1.24%. The Frequency of superficial and dermatomycoses was 17.36% (28 patients) in this study.

Conclusion: Our findings showed that dermatophytosis particularly tinea capitis was very popular in this area during august 2017 to September 2018.

Keywords: Superficial mycoses, cutaneous mycoses, Zahedan

P-012

Evaluation of fungal air contamination and risk of nosocomial infections in educational hospitals of Birjand University of Medical Sciences in 2007

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Introduction: Hospital environments have different types of microorganisms. The release of airborne fungi in hospitals is a health risk factor for nosocomial infections in hospitalized patients. The aim of this study was to compare the fungal contamination of different parts of the educational hospitals of Birjand University of Medical Sciences in 2017.

Material and methods: In this descriptive cross-sectional study, in 2017, different parts

of Birjand's hospitals including ICU, ENT, infectious, internal, pediatric, burn, toxic, emergency, operation room and laboratory were studied. For sampling, plates containing Sabouraud Dextrose Agar (SDA) was used. The collected samples were transferred to the laboratory after 15 minutes and then placed in the incubator. After observing the growth of colonies, they were examined for macroscopic and microscopic morphology.

Results: Of the 200 plates, 114 plates (57.0%) were positive for fungal growth. A total of 314 fungal colonies and 9 different fungal species were isolated. The most abundant fungus isolated in this study was *Penicillium* spp with a prevalence of 35.67%. Also *Cladosporium* spp (28.67%), *Aspergillus niger* (14.96%), *Aspergillus fumigatus* (8.28%), *Rhizopus* spp (3.50%), *Aspergillus terreus* (2.87%), *Aspergillus flavus* (2.55%), *Fusarium* spp (1.91%) and *Alternaria* spp (1.59%) were identified. Among the species of *Aspergillus*, the species of *niger* was dominant (14.96%). Valiasr hospital was the most infected hospital (47.13%). Among the different hospitals, the highest rates of infection in all three hospitals were in ICUs.

Conclusion: Regarding the fact that there are no specific standards for the incidence of indoor fungal contamination, especially for hospitals, it seems necessary to develop guidelines for this issue by responsible devices. Also, control measures to reduce the amount of fungi can play a significant role in improving the health of patients.

Keywords: Fungus, Contamination, Air, Hospital, Birjand

P-013

The incidence of onychomycosis among 1072 patients referred to three medical mycology laboratories in Tehran, capital of Iran

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Introduction: Onychomycosis is the fungal infection of the nails with worldwide occurrence, caused by various species of fungi. Concerning increase of onychomycosis during recent decades and effect of different climates, professional and socio-economic conditions in prevalence of onychomycosis, local investigation for defining of incidence and causative agents of onychomycosis seems necessary. The aim of this study was to determine the prevalence and mycological features of onychomycosis among patients who were referred to three medical mycology laboratories in Tehran during the period 2014 through 2015.

Materials and methods: One thousand seventy two nail samples from patients underwent screening for the presence of onychomycosis. Samples were examined by direct microscopy and cultured in Sabouraud dextrose agar and Mycobiotic agar. Differentiation of the fungal causative agents was based on microscopic observation of characteristic fungal elements in the samples and growth of a significant number of identical colonies on the culture plate. The type of fungal pathogen was recorded as a site of infection and sex.

Results: Direct microscopy of the nail clips was positive in 417 (38.7%). Fingernail and toenail onychomycoses were recognized in 164 (39.4%) and 253 (60.6%) cases, respectively. Dermatophytes were detected in 154 (36.9%), yeasts in 139 (33.4%) and non-dermatophyte molds in 124 (29.7%) patients. The results of fungal culture showed *Candida albicans* isolated in 89 (64.2%) and other *Candida* spp. isolated in 50 (35.8%) cases as

the most common agents of onychomycosis while among dermatophytes, *Trichophyton mentagrophytes* was found in 64 (41.5%) cases as the main dermatophytic agent followed by *T. rubrum* 38 (24.6%). Among the nondermatophyte molds, *Aspergillus spp.* was the most prevalent species with 71 (57.2%) cases, followed by *Fusarium spp.* with 10 (8.0%) cases. Moreover, 45 (4.1%) samples with positive direct microscopy yielded no growth and 23 (2.1%) samples with positive culture were negative whit direct microscopy.

Conclusions: The clinic epidemiology data collected can serve as reference for future researches and may be useful in the development of preventive and educational strategies.

Key word: Onychomycosis, Dermatophyte, Tehran

P-014

Onychomycosis among patients with pemphigus diseases

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Introduction: Onychomycosis a fungal infection of the nail caused by dermatophytes, or molds and nondermatophytes and yeasts. Many studies reported a higher prevalence of onychomycosis among particular patients such as those with immunosuppression. However studies of the prevalence of onychomycosis in autoimmune patients who carry many predisposing factors have been limited. Pemphigus is an autoimmune disease, incidence of nail changes in this disorder has been found to be high in few recent studies. Since no previous study on onychomycosis among patients with Pemphigus diseases exist in Iran, this study

aimed to determined prevalence of onychomycosis among

Material and methods: This study was carried out in Bullous clinic at shahid faghihi hospital in Shiraz city from May to Sep 2018. 40 patients with pemphigus diseases was examined clinically for evidence of onychomycosis, sampling from nail clippings were obtained from 12 patients with abnormal nails .The presence of fungus was confirmed in direct microscopy (KOH smear) and culture .

Results: clinical diagnosis of onychomycosis was made in 12/40 (30%) patients with Pemphigus disease .The main age affected was 47 years. A total of 9 patients (75%) had pemphigus vulgaris, 2 patients (16.6%) had Pemphigus foliaceus and 1 (8.3%) patient had IgA pemphigus. 83.3% of them were female and 16.66% had a diabetes mellitus . fingernail involvement was seen in 91.6%. Direct microscopic examination was positive in 9/12 (75%) and positive culture was obtained in 7/12 (58.3%).*Candida* species was the main isolated organism from fingernails (7/9, 77%), followed by dermatophytes (1/9, 11%) and nondermatophytes /mold (*Aspergillus spp*) (1/9, 11%).

Conclusion: This study revealed that onychomycosis was more frequent among patients with pemphigus vulgaris and *Candida spp.* was the main isolated from the fingernails. The high occurrence of onychomycosis in pemphigus patients could be explained by the fact that all of the pemphigus patients in this study were undergoing immunosuppressive treatments (prednisolon, cyclophosphamid, azatioprin and ritoximab), another hand nail changes such as paronychia, onycholysis and onychomadesis are common in patients with long-standing disease because of accumulated inflammatory effects and this can be trigger factor for onychomycosis among the pemphigus patients.

Keywords: Onychomycosis, Pemphigus, *Candida*, Autoimmune disease

P-015

The human oral mycobiome: Tiny things in our mouth with huge effect on our health

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Introduction: Like all other complex multicellular eukaryotes, our body is fighting with microbes. Although the first study of microbes has started more than 150 years ago, in the past 10 Years the study of microbiomes as a new and interesting subject in which all microbes (bacteria, Archaea, viruses and fungi) in an environment are taken into account, has got the closer look. Over 99% of microbiomes counts are bacterial constituents and less than 1% are other microbes that called rare biosphere however this very small count has a lot to do with human health and disease. The definition of the microbiome is a combination of the words mycology and microbes and it was first used to point to the fungal microbiome in 2010. Fungi is one of the most important microbiome in the rare biosphere. Even though the low abundance, the impact of this microbiome is wide-ranging on human health and disease. Because of the fungal infections the oral microbiome has received less attention, there are several reasons why uncultivable nature of many fungi in laboratory and eukaryotes complex genetic composition, in this paper we are going to discuss more on oral microbiome.

Material and Methods: Data were collected by performing searches using a specified set of Medical Subject Heading (MeSH) terms like mycobiome, oral, human and health and disease in the following databases and search engines: MEDLINE, ISI Web of Science, Ebsco, Science Direct, Scopus, and Google Scholar.

Results: The species and their percentage found in oral cavity by oral rinse sampling in a healthy human are as follows: *candida*

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(22.2%), *Cladosporium* (19 %), *Aspergillus* (11.1%), *Fusarium* (5.6%), *Glomus* (5.6%), *Penicillium* (4.2%), *Alternaria* (4.2%), *Saccharomycetales* (13.9%), *Cryptococcus* (2.8%), *Ophiosoma* (2.8%), *Phoma* (2.8%), *Schizosaccharomyces* (2.8%) and *Zygosaccharomyces* (2.8%). however in a Human with AIDS by oral mucosal swap the only fungi was *Candida* (100%). It is interesting that *Cladosporium* species, *Aspergillus* species and *Penicillium* species all dominate other fungal genera in oral cavities. The largest part of the human microbiome is unculturable fungi. Eleven out of 85 oral cavity fungal genera are unculturable in culture-independent methods we have 37 different fungal groups. However by culture-dependent methods we only identify 5 but it has led to new approaches in identifying fungi by culture-independent methods such as restriction fragment length polymorphism (RFLP) analyses, oligonucleotide fingerprinting of RNA genes (OFRG), denaturing gradient gel electrophoresis (DGGE) and in-situ hybridization. Although these techniques are useful for comparing fungal diversities between different groups, they are not useful for large-scale study.

In a recent study on matched oral samples indicate that fungal signatures are more sensitive to DNA isolation methods than bacterial signatures. HIV⁺ individuals with a progressive CD₄⁺ cell loss developed pharyngeal candidiasis. Patient with candidiasis also shows a high level of *Candida* in oral wash specimen, however, *Aspergillus* in the lungs had little detectable *Aspergillus* in oral washes.

Conclusion: However new culture-independent methods are more useful and specific, culture-dependent method are not dominated yet. *Candida* is the main fungi in both healthy and unhealthy individuals, and it has habitat overlap with *Aspergillus* and *Penicillium* in the oral cavity.

Keywords: Mycobiome, fungi, normal flora

P-016

Survey of antifungal susceptibility *Candida glabrata* isolated from genitourinary tract

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Introduction: *Candida* species are a common cause of fungal infections, which can lead to life-threatening and non-threatening diseases. Over the past decades, reports have shown that the agent of *Candida albicans* has changed to non-*albicans*. *C. glabrata* following *C. albicans* is the second and third most common cause of candidiasis infection in immunocompromised patients, leading to high mortality in these patients. It has an intrinsic resistance to azole antifungal drugs. There are many factors involved in *C. glabrata* resistance to antifungal drugs such as tolerance, environmental stress, cell density, efflux pump-mediated resistance, extracellular matrix, genetic alterations. We aimed to evaluate antifungal susceptibility of *C. glabrata* in patients with vaginal candidiasis and candiduria.

Material and Methods: In this study, a total of 30 *C. glabrata* were isolated from patients with vaginal candidiasis and candiduria. In order to identify the isolates phenotypical characteristics were considered by using CHROMagar (CHROMagar™ *Candida*, U.S.A) medium, germ tube test and presence or absence of chlamydoconidia and hyphae on corn meal agar with tween 80. We determined the minimal inhibitory concentration (MIC) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Version 9.0, valid from the 2018-02-12 reference document. All samples tested against amphotericin B, voriconazole, posaconazole, caspofungin, terbinafine, (Sigma-Aldrich, Germany), Fluconazole (Serva, USA).

Results: Our findings demonstrated that all isolates were sensitive to voriconazole with epidemiological cut off value (ECV) = 1 and 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

SDD (susceptible dose-dependent) were to fluconazole with ECV = 32. The results showed that the resistance of isolates to posaconazole and amphotericin B with ECV = 1 were 6.7%, 33.3% respectively. The MIC results for caspofungin and terbinafine were 0.032-1 ug/ml and 32-≥128 ug/ml, respectively with undefined ECV. The lowest MIC GM was found for voriconazole and posaconazole while the highest MIC GM observed for terbinafine.

Conclusion: It is important to evaluate the infections by fungi in patients. As some guideline including EUCAST and CLSI, there is no define ECV for some antifungal drugs because of not enough data from worldwide. Also, by understanding the situation of fungal infection in patients the decision to treat will be easier. Our data demonstrated that *C. glabrata* were susceptible to fluconazole and voriconazole and the highest resistance related to amphotericin B that consistent with the previous results in Iran. We suggested laboratory examination before prescription and annual the evaluation of incidence of *C. glabrata* and its antifungal resistance.

Keywords: *C. glabrata*; antifungal susceptibility, genitourinary tract

P-017

Detecting *Cryptococcus neoformans* in CSF samples suspected to be meningitis

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Introduction: *Cryptococcus neoformans* is the most common fungal pathogen to infect the central nervous system, and an effective diagnostic method is, therefore, necessary for the early diagnosis of cryptococcal meningitis. This study was designed to provide a scientific basis for detecting *C.*

neoformans in Seven Cerebrospinal fluid (CSF) samples in Golestan Province, Iran.

Materials and Methods: CSF samples suspected to be suffering from meningitis were screened for *C. neoformans*. This samples analyzed by Immuno-chromatography and whit *C. neoformans* Immunity kit.

Results: *C. neoformans* infections were identified in 1 of 7 (14.28%) patients. Of these 7 patients, 4 (57.14%) were men and 3 (42.86%) were women. The median age of patients was 37 years old.

Conclusions: The Immuno-chromatography procedure is rapid, reproducible, easy to perform and can use in the diagnosis of cryptococcal infection.

Keywords: *Cryptococcus neoformans*, meningitis, Immuno-chromatography.

P-018

Identification and antifungal susceptibility testing of *Candida* species in oral lesions of patients with cancers: *in vitro* activity of new azole luliconazole compared to fluconazole

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Introduction: Oral candidiasis is the most common fungal infection in patients undergoing chemotherapy. In this study, the *Candida* isolates from cancer patients were identified by molecular methods and the efficacy of new azol, luliconazole compared with fluconazole.

Materials and Methods: This study carried out on 385 patients with various types of cancer which were under chemotherapy. The

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clinical isolates identified using internal transcribed spacer (*ITS1/ITS4*) primers for PCR amplification and *MspI* restriction enzyme for restriction fragment length polymorphisms (PCR-RFLP) method. The minimum inhibitory concentration (MIC) values were determined using broth microdilution according to the M27-A3 protocol of the Clinical and Laboratory Standards Institute (CLSI). The modal MIC, MIC₅₀, MIC₉₀ and geometric mean (GM) values, were evaluated for all the isolates.

Results: *Candida albicans* was the most common species 26 (72.2%) detected from the oral samples, followed by *C. glabrata* 5(13.8%), *C. kefyr* 3(8.3), *C. krusei* 1(2.8) and *C. stellatoidea* 1(2.8). The *in vitro* activities of novel imidazole; luliconazole was compared to fluconazole against clinical isolates. The concentration ranges for fluconazole and luliconazole were considered (0.25-128) and (0.007-4) µg/ml, respectively. The lowest GM values was 0.85 in *C.glabrata* and 1.14 µg/ml in *C.kefyr* isolates. The GM values of both antifungal drugs showed no significant differences between the *C.albicans* isolates.

Conclusion: In this study, the most common species identified in the oral cavities of cancer patients were *C. albicans* with 26 (72.2%) isolates. luliconazole showed better activity against all of *Candida* isolates, compared to fluconazole. It should be considered that luliconazole may emerge as an effective and broad-spectrum antifungal agent in the future.

Keyword: *Candida*, luliconazole, fluconazole, Chemotherapy.

P-019

Genotyping of *Candida albicans* isolates from the oral cavity of Iranian diabetic patients

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Introduction: Diabetic patients are susceptible to opportunistic fungal infections. Oral candidiasis in diabetic population has been considered as a common mucosal infection. This study aimed to investigate the molecular typing of *Candida albicans* isolates recovered from the oral cavity of diabetic patients with oral lesions referred to Tehran Diabetic Center from April to October 2017.

Material and Methods: One hundred sixty diabetic patients and 40 healthy persons enrolled in this study. The specimens were collected from oral lesions of patients using sterile cotton swab and microscopic direct examination by KOH was performed. Samples cultured on Sabouraud Dextrose Agar (SDA) medium. The species were identified by CHROMagar *Candida*. PCR assay was carried out using the universal primers for internal transcribed spacer (ITS) region. The PCR products were sequenced. The resulting sequences were analyzed and compared with the reference data available from the Gen Bank database using the BLAST sequence search tool. Genotyping of *C. albicans* strains was performed by INT specific primers. Indeed hydrophobicity and hemolytic activity of *C. albicans* were evaluated.

Results: Out of 160 oral samples, 88(55%) strains were recovered from patients on SDA medium. Using sequencing 70(80%) of isolates identified as *C. albicans* whereas 18(20%) species were *non-albicans Candida* species including *C. glabrata*, *C. kefyr* and *C. dubliniensis*. The mean of HbA_{1c} and age of patients was 8mg/ml and 56 years old, respectively. Four genotypes include (A: 66%, B: 10%, C: 4% and D: 20%) of *C. albicans* strains was detected. Genotypic analysis indicated that 66% of *Candida* isolates belong to genotype A. It was found there is no significant difference between hemolytic activity and predominant genotype (A). Interestingly, there was found a positive correlation between hydrophobicity activity of isolates and genotype A (P<0.05).

Conclusion: Taken together, molecular typing using INT internal sequence of 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

Candida genome seems a useful molecular method for epidemiological studies. According to our finding, genotype A of *C. albicans* is a major genotype among patients causing oral candidiasis. This method is simple, economical and time-consuming to differentiate the diversity of clinical strains of *Candida* species among the population.

Keywords: *Candida* species, Diabet, Hydrophobicity, Hemolytic, Genotyping

P-020

Nosocomial fungal infections: epidemiology, diagnosis, treatment and prevention

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Introduction: Nosocomial fungal infections are one of the important causes of mortality in patients admitted to healthcare settings, especially in immunocompromised populations. The predominant pathogens include *Candida* spp., *Aspergillus* spp., *Mucorales* spp., *Fusarium* spp. and other fungi. Nosocomial fungal infections are increasing due to the underlying factors in decades ahead. One of the predisposing factors includes immune system suppressing due to the extensive use of invasive treatment modalities such as stem cell transplantation, organ transplantation, chemotherapy and the use of immunosuppressive drugs.

Material and methods: Data were collected by performing searches using a specified set of Medical Subject Heading (MeSH) terms like Nosocomial Fungal Infections, *Candida* spp., *Aspergillus* spp., *Mucorales* spp., *Fusarium* spp. and other fungi in the

following databases and search engines: MEDLINE, ISI Web of Science, Ebsco, Science Direct, Scopus and Google Scholar.

Results: *Candida* species are the most common fungal pathogens causing serious healthcare-associated infections, especially in patients admitted to intensive care units and candidemia is the third or fourth most common cause of healthcare associated bloodstream infections in the United States of America hospitals. According to recent studies, the most important risk factors for *Candida* infections include malignancy, hematopoietic stem cell transplantation (HSCT), using central venous catheters, immunosuppression, and using of broad-spectrum antibiotics. In severely immunocompromised patients, such as HSCT recipients, invasive aspergillosis is the most important cause of infection-related mortality. Aspergillosis accounted for 59% of all invasive fungal infections and is associated with a 6-week mortality of 22%. Common risk factors for *Aspergillus* infections include allogeneic HSCT, using of corticosteroids, severe graft versus host disease, neutropenia, and T-cell depleting agents. Although infection caused by *zygomycetes* is uncommon, it is often a fatal disease. Population-based studies estimate an annual incidence of 0.43 to 1.7 cases per million persons. In a recent review of 929 patients with zygomycosis, the underlying conditions were diabetes (36%), malignancy (17%), solid organ transplantation (7%), desferrioxamine therapy (6%), injection drug use (5%) and bone marrow transplantation (5%). *Fusarium* is a soil saprophyte and causes keratitis and onychomycosis in humans. Outbreaks of keratitis caused by possible contamination of contact lens solutions have been described. The invasive disease has generally been reported in patients with prolonged neutropenia, especially in HSCT recipients, and to a lesser extent in solid organ transplantation recipients. The incidence of fusariosis has been estimated to be 4 to 5 cases per 1000 HLA antigen-matched allogeneic HSCT recipients to as high as 20 cases per 1000 HLA antigen-mismatched recipients.

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Conclusion: The performance of recommended infection control methods can avoid catheter-related candidiasis and also minimize exposure to airborne *Aspergillus* spores in immunocompromised patients in hospital settings. A significant percentage of these infections can be prevented without advanced equipment and spending high costs. Therefore, it is only possible to prevent by training healthcare workers in the use of medical equipment. Treatment for these infections is costly due to increased length of stay in health care settings. Antifungal prophylaxis in patients at risk for invasive fungal infections should be considered during the periods of severe immunosuppression.

Keywords: Nosocomial fungal infection, *Candida*, *Aspergillus*, *Mucor*, *Fusarium*

P-021

***Candida auris*: An emerging multidrug-resistant fungal pathogen**

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Introduction: Recently, *Candida auris*, a multidrug-resistant fungal pathogen, has emerged as a global threat. *C. auris* was first reported in 2009 from an ear swab of a patient in Japan and subsequently from ear swabs of 15 patients in South Korea in the same year. After that in 2011, *C. auris* was described as the reason of 3 cases of fungemia in South Korea for the first time. Alarmingly, in a span of only 9 years, this pathogen has caused a broad range of invasive healthcare-associated infections in the form of individual cases or outbreaks in over 20 countries on 5 continents.

Material and methods: Data were collected by performing searches using a specified set of Medical Subject Heading (MeSH) terms like *C. auris*, emergence, multidrug-resistant and fungal pathogen in the following databases and search engines: MEDLINE, ISI Web of Science, Ebsco, Science Direct, Scopus, and Google Scholar.

Results: According to the recent studies, *C. auris* has increasingly been associated with mortality and misidentification in far-Eastern Asia, the Middle East, Africa, Europe, North and South America. Most isolates have been reported from India, the United States of America and the United Kingdom. *C. auris*, multidrug-resistant yeast, has been described as an emerging global threat. Bloodstream infections, wound infections and otitis are just parts of diseases caused by this *Candida* species. Although *C. auris* has been documented to cause infections in patients of all ages, most isolates were from male patients, patients hospitalized in the intensive care units and blood specimens. In general, patients were found to have similar risk factors for infections as those patients with other *Candida* species infections, including immunocompromising diseases, recent surgery, recent antibiotics, the presence of central venous catheters or urinary catheters, diabetes, sepsis, lung diseases, and kidney diseases. Additionally, detection of *C. auris* has been reported in patients receiving antifungals for treating the infections with other *Candida* species. Recent reports well indicate the challenges such as misidentification by available commercial identification systems, resistance to fluconazole and markedly variable susceptibility to other azoles, amphotericin B and echinocandins, high clonal inter- and intra-hospital transmission ability, and significant patient mortality. Resistance to fluconazole (44.29%), amphotericin B (15.46%), voriconazole (12.67%) and caspofungin (3.48%) are common. Commonly used diagnostic tools included polymerase chain reaction (30.38%), Bruker matrix-assisted laser desorption/ionization-time of flight mass spectrometry (14.00%), Vitek 2 YST ID (11.93%) and whole genome

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sequencing (10.04%). Two novel drugs, SCY-078, and VT-1598 are currently in the pipeline.

Conclusion: A robust response that involves laboratories and clinicians is needed to identify, treat infections and prevent transmission. Contact precautions, strict infection control, periodic surveillance and cleaning with chlorine-based detergents, efficient, faster and cheaper detection tools are necessary for prevention, containment and early diagnosis of *C. auris* infections.

Keywords: *Candida auris*, Emergence, Multidrug-Resistant

P-022

Antifungal susceptibility profile of clinically important dermatophyte species isolated from Iranian

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Introduction: Dermatophytosis, the commonest superficial fungal infection. The last few years infections caused by dermatophytes along with a concomitant increase in the number of difficult to treat cases have increasingly been recognized indicating that dermatophytosis remains a challenging public health problem. Therefore, the requirement for precise identification of causative agents of infections and antifungal susceptibility test is so necessary. This study aimed to determine antifungal susceptibility profile of clinically important dermatophytes.

Material and methods: Clinical samples obtained from patients suspected to dermatophytosis referred to the Department of Mycology of the Pasteur Institute of Iran were examined for etiologic dermatophytes. A total 97 identified dermatophyte isolates including *Trichophyton rubrum* (n = 19), *T. interdigitale* (n = 26), *T. tonsurans* (n = 29) and *Epidermophyton floccosum* (n = 23) were included in this study. Reference strains of *T.*

rubrum (PFCC 51431) and *T. mentagrophytes* (PTCC 5054) were tested in all steps. Based on our results, dermatophytosis was confirmed in 99 cases by direct microscopic examination, culture, and sequencing of internal transcribed spacer (*ITS*) region. Antifungal susceptibility testing of all isolates and two reference strains was assessed to eight antifungal agents using CLSI M38-A2 guidelines.

Results: Minimum inhibitory concentration (MIC_{50}) for luliconazole was 0.004 $\mu\text{g/ml}$, compared to 0.03, 0.06, 0.06, 0.125, 0.25, 0.25 and 0.25 $\mu\text{g/ml}$ for itraconazole, econazole, butenafine, ketoconazole, voriconazole, lanocanazole and griseofulvin respectively. The results indicated that *T. tonsurans* was the most susceptible species to luliconazole ($MIC_{50}=0.004$), whereas *E. floccosum* was the most resistant species to it ($MIC_{50}=0.02$).

Conclusion: Taken together, our results assist clinicians and prompt the current knowledge about the necessity of antifungal susceptibility testing to select effective strategies for the management of clinical cases of dermatophytosis.

Keywords: Dermatophytes, antifungal susceptibility test, dermatophytosis

P-023

Severe disseminated phaeohyphomycosis in a patient with inherited *CARD9* deficiency
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Background: The caspase recruitment domain containing protein 9 (*CARD9*) is clinically indistinguishable and patients with *CARD9* defects are susceptible to recurrent and severe fungal infections. We describe a case with progressive disseminated phaeohyphomycosis due to a melanized fungus and inherited *CARD9* deficiency to highlight the clinical presentation of this disorder.

Case Presentation: A 26-years-old healthy Iranian female was admitted to the Department of Infectious Diseases, Tehran University of Medical Sciences, Tehran, Iran, with severe dissemination of lesions to the skin of face, chest, and legs. Skin biopsies examined in KOH 10% showed septated and pigmented hyphae. Despite surgical excision and antifungal therapy, the number of the lesions increased. The tissue samples were inoculated onto Sabouraud Dextrose Agar and brain heart infusion agar with 5% sheep blood and incubated at 27°C and 37°C for two weeks but cultures remained negative. Other biopsies prepared for histopathology showed a granulomatous infiltrate with irregular branched and melanized septated hyphae. The diagnosis of disseminated phaeohyphomycosis due to melanized fungi was made on the basis of clinical and histopathological findings. *CARD9* gene was sequenced and a homozygous c.883C >T mutation in exon 6 at codon 295 was found, resulting in a mutation at position 295, Q295X. Ultimately, despite combination antifungal therapy, amphotericin B deoxycholate (0.5 mg/kg/day) and oral voriconazole (400 mg/day), the patient died 38 days after admission because of respiratory failure. To obtain an etiological diagnosis, DNA was extracted from a formalin-fixed paraffin-embedded tissue using the tissue DNA isolation kit (Qiagen) according to the manufacturer's instructions. PCR amplification and sequencing were

performed for *ITS* rDNA and *D1/D2* regions, but due to the lack of sufficient tissue for examination, we failed to identify the cause of the phaeohyphomycosis despite extensive efforts.

Conclusions: The higher incidence *CARD9* deficiency in Iran may be associated with rapid population growth, large family size, and the availability of diagnostic facilities. Although Iranian patients with *Q295X* mutation are susceptible to candidiasis and dermatophytosis, our patient is the first report of phaeohyphomycosis related to *Q295* mutation.

Keywords: Disseminated Phaeohyphomycosis, Melanized Fungi, *CARD9*, Iran

P-024

***Fusarium proliferatum* as a dominant *Fusarium* species isolated from patients with onychomycosis in the North of Iran**

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Introduction: Onychomycosis refers to any fungal infection of the nail and is usually caused by dermatophytes; however, non-dermatophyte molds (NDMs) and yeasts are increasingly recognized as the pathogens accounting for nail disease. Several studies have shown that *Fusarium* is the most common NDMs causing onychomycosis and its spread has increased in the past years.

Material and methods: The present study was conducted to describe the molecular epidemiology of *Fusarium* onychomycosis in the north of Iran. 257 nail samples collected from the Iranian patients clinically suspected

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of onychomycosis were subjected to direct microscopy, calcofluor white staining, and fungal culture. The characteristics of *Fusarium* isolates were further identified at a species level by determining multi-locus sequences for internal transcribed spacer and translation elongation factor 1 alpha.

Results: According to the results, *Fusarium* species were isolated from 27 patients with onychomycosis. Based on previous partial genes analysis, the recognized species in our study were among the members of *F. fujikuroi* species complex (n=14), *F. solani* species complex (n=12), and *Fusarium incarnatum-equiseti* species complex (n=1). In the present study, *F. proliferatum* was the dominant *Fusarium* species isolated from the samples.

Conclusion: With regard to the increased prevalence of *Fusarium* onychomycosis and the intrinsic resistance of *Fusarium* species to a broad range of antifungals, it is necessary to correctly identify them to the species level.

Keywords: Onychomycosis, *Fusarium fujikuroi* species complex, *Fusarium solani* species complex, *Fusarium proliferatum*

P-025

Molecular identification and antifungal susceptibility of clinical fungal isolates from onychomycosis (uncommon and emerging species)

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Introduction: Onychomycosis is a common nail problem, accounting for up to 50% of all nail diseases. The aim of the present study was to determine the species distribution

based on the restriction fragment length polymorphism (RFLP) and susceptibility patterns of the causative agents of onychomycosis.

Material and methods: This cross-sectional study was conducted on nail samples collected from 257 patients suspected of onychomycosis within a year. Fungal isolates was identified by RFLP with the enzymes *Msp I*, *Mva I*, *Alw I* and sequencing.

Results: According to the results, Out of the 257 patients participating in the study, onychomycosis was diagnosed in 180 (70.03 %) cases, among which 51.1% were caused by non-dermatophyte molds (NDMs), 34.4% yeasts, and 10.6% dermatophytes. Numerous cryptic species recovered from onychomycosis for the first time. In the majority of cases, novel triazoles and imidazoles (i.e., efinaconazole, luliconazole, and lanocanazole) showed potent activity in comparison to other antifungal agents. The minimum inhibitory concentration (MIC) of luliconazole and lanocanazole ranged from 0.001 to > 1 µg/ml and their geometric mean MICs were 0.0154 and 0.0309 µg/ml against all isolates, respectively.

Conclusion: It seems that obtained data will be useful to improve the knowledge of researchers, clinicians, and dermatologists about the distribution of onychomycosis agents and the species diversity for appropriate treatment.

Keywords: Molecular identification, antifungal susceptibility, Onychomycosis

P-026

Laboratory diagnosis fungal endocarditis with negative blood culture

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Introduction: Fungal endocarditis (FE) is rare and extremely disabilities disease, with an incidence 1-10% among endocarditis cases. *Candida* species and *Aspergillus* species are most commonly isolated pathogen. The epidemiology of invasive *Candida* infections is changing with a predominance of non-*albicans* *Candida* species. It is most prevalent in patients who are immunosuppressed and intravenous drug users, especially in pediatrics. Fungal endocarditis report is increased in four major groups of the native valve, prosthetic valve, endocardial surface, and indwelling cardiac device-related endocarditis. Early FE detection can be difficult because it often lacks some classic signs and symptoms found in bacterial endocarditis.

Material and methods: We describe three old female cases of the Cardiac valve, and embolic materials obtained during open heart surgery in suspected infective endocarditis patients were examined for fungal infections based on direct smear (KOH and CFW preparation), culture (S, SC, SCC, *Candida* CHROMagar,...), and PAS and H&E stain of histopathological sections. Blood culture performed for all samples. Echocardiography performed routinely for patients.

Results: In all cases, echocardiogram showed large and friable vegetation on the cardiac valve but the blood culture was negative. Fungal endocarditis was determined and reported in the cases based on direct smear and culture. The etiologic agents

were *Candida tropicalis* (two cases) and *Acremonium* (one case).

Conclusion: Fungal endocarditis is associated with a poor prognosis and high mortality (>50% of the affected population) and of optimal antifungal drugs, therapeutic doses remains debatable. Early diagnosis and differentiation of etiologic agents, and negative blood or catheter cultures, is important for more prompt and effective antimicrobial therapy to decrease mortality due to this condition.

Keywords: Fungal endocarditis, Infection, *Candida tropicalis*, *Acremonium*

P-027

Is there a relationship between age and gender with *Microsporium canis* infection?

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Introduction: Dermatophytosis is a common mycotic infection of the skin, nail, and hair associated with major public health concern worldwide. *Microsporium canis* is a worldwide-distributed zoophilic dermatophyte which causes clinical features often characterised by multifocal alopecia, scaling, and circular lesions in animal and humans. This study was conducted to determine the relationship between age and gender with *M. canis* infection in Iran.

Materials and Methods: The patients with *M. canis* infection from different provinces of Iran were obtained. Data including the age, sex, history of contact with animal and type of clinical features with *M. canis* were collected from all patients. The *M. canis* isolates were identified by molecular approach with rDNA-ITS. The obtained results were analyzed by SPSS 16 software.

Results: The Eighty-one isolates of *M. canis* identified from Tehran (n=51), Sari (n=14), Ahvaz (n=6), Urmia (n=5), Bushehr (n=3) and Mashhad (n=2) from patients. The most commonly infected age group was the 1-9 years old (29.6%), followed by 20-29. The number of affected women was more than that of men. Tinea corporis (49.4%) was the most prevalent type of clinical manifestation, followed by tinea capitis (26.4 %), tinea manuum (9.9 %), tinea faciei (6.6), tinea cruris (5.5 %), tinea pedis (1.1 %) and tinea unguium (1.1 %). 44.4 % of patients had the history of contact with animals. There was a significance difference between age and gender in occurrence *M. canis* infection (p<0.05).

Conclusion: In this present study, we found a relationship between age and gender with *M. canis* infection. This noticeable information improves our current knowledge about dermatophytosis causes by *M. canis* and assists to establish effective prevention and therapeutic strategies to overcome the disease.

Keywords: *Microsporium canis*, Relationship, age, gender

P-028

Central line associated with candidemia in open-heart surgery ICU

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Introduction: Catheter-related blood circulation infection is the most dangerous and serious side-effects of vascular catheters which leads to the enhancement of the costs, mortality, and hospital stay duration especially in the Intensive Care Unit. The aim of the current study was to identify the prevalence of catheter-induced candidemia in the Tehran Heart Center, Tehran, Iran.

Material and methods: This study was conducted on patients admitted to Tehran Heart Center for a minimum of 7 days during the months. To detect the fungal elements, blood culture and catheter culture were performed in the patients receiving central or 18 peripheral venous catheter. The polymerase chain reaction (PCR) was performed to determine the possible diagnosis.

Results: The investigation of 223 samples led to the identification of a total of 15 (6.7%) yeast isolates obtained from 9 (60%), 4 (26%) and 2 (13.4%) catheter, blood, and skin (of the catheter insertion areas) cultures, respectively. Out of nine *Candida* isolates obtained from the catheter samples, 1 (11.1%), 1 (11.1%), 2 (22.2%), and 5 (55.6%) cases were identified as *C. tropicalis*, *C.membranifaciens*, *C. glabrata*, and *C. albicans*, respectively, using the internal transcribed spacer region sequencing. Furthermore, the four yeasts

isolated from the blood culture included *C.tropicalis*, *C. carphophila*, *C.membranifaciens*, and *Cryptococcus albidus*. Additionally, one case of *C. glabrata* and one case of *C. albicans* were isolated from the skin culture of the catheter insertion areas in patients with positive catheter culture. We reported two cases of catheter-related candidemia caused by *C. membranifaciens* and *C. tropicalis* on the basis of the genetic similarity of the species isolated from the blood and catheter. These cases were treated successfully with intravenous fluconazole and catheter removal.

Conclusion: There is some evidence indicating the growing prevalence of non-*albicans Candida* infections. Many risk factors, including prior antibiotic therapy, use of a central venous catheter, surgery and parenteral nutrition, are considered to be associated with candidemia in hospitalized heart failure patients. The identification of the route of infection in candidemia is difficult. In the current study, the positive blood and catheter cultures for *Candida* isolates and the similarity of the ITS region of ribosomal DNA sequence of *Candida* isolated from two patients confirmed the diagnosis of intravenous catheter-related candidemia.

Keywords: candidiasis, catheter-related candidemia, Nosocomial infection

P-029

Prosthetic valve endocarditis caused by multidrug-resistant *Candida albicans* in patient with myelodysplasia syndrome: A case report

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Background: *Candida* endocarditis (CE) is an infrequent disease with a high fatality rate, which is commonly reported in patients with valve replacement.

Case report: We reported a 70-years-old woman with the history of severe mitral stenosis and myelodysplasia syndrome. She underwent mitral valve replacement for two times. The blood cultures were positive and phenotypic identification to the species level was performed based on microscopic and macroscopic characteristics. On second prosthetic valve replacement (PVE) the formation huge fungal white and creamy vegetation were observed which the yeast isolate was identified as *Candida albicans* using conventional and molecular methods. Amphotericin B deoxycolate, caspofungin and voriconazole together with broad spectrum antibiotic including vancomycin and gentamicin are administered. The patient presented with dyspnea, decreased consciousness, decreased blood cells and finally she went into a coma. The patient died due to sepsis probably related to the candidemia and *Candida* PVE with the multi-azole and amphotericin B resistant *C. albicans*.

Conclusion: CE is an uncommon but devastating infection that affects the elderly with a weakened immune system as a late consequence of prosthetic valve replacement. The extended follow-up visits, early diagnosis, repeating valve replacement surgeries and timely selective antifungal treatments are warranted.

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Keywords: *Candida* endocarditis, myelodysplasia syndrome, prosthetic valve replacement

P-030

Bening esophageal ulcer associated with *Candida tropicalis* infection in a diabetic patient

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Background: The most common symptom of an esophageal ulcer is burning pain in the chest. The pain can be mild or severe. Other symptoms of an esophageal ulcer included: nausea indigestion acid reflux (heartburn), bloating vomiting lack of appetite pain, swallowing dry cough and sour taste in the mouth. However, some people do not experience any symptoms at all.

Case presentation: A 67-years-old cigarette smoker man with type 2 diabetes mellitus was refer to our clinic of gastroenterology, in Tonekabon, Mazandaran, Iran, with anorexia, nausea, vomiting, acid reflux (heartburn) and pain when swallowing. He was diagnosed as diabetes 13 years ago and his blood sugar was controlled by oral hypoglycemic agents. In the initial treatment of a discomfort by a general practitioner, the patient was given Amoxicillin as antibacterial, Acetaminophen/Codeine as NSAID and Ranitidine tablet as reducing the amount of acid produced in the stomach. Endoscopic examination of the upper

digestive tract revealed 3 unclear border, 2-3 mm diameter diffuse mucosal defect at middle third of the esophagus, which was covered with yellowish plaque and exudates. Biopsy of ulcer margin was performed and examined microscopically by 10% KOH wet mount, histological examination and cultured in Sabouraud's dextrose agar containing chloramphenicol (SC) and CHROMagar *Candida* media. Histological examination of the biopsy specimens obtained from the base and the edges of the ulcer and 10% KOH wet mount examination showed the yeast cells. *Candida tropicalis* showed typical cream colored, smooth colonies on SC and typical dark blue color on CHROMagar *Candida*. Therefore, the patient was diagnosed with *C. tropicalis* infected esophageal ulcer. Administration of anti-ulcer drugs for 2 months and fluconazole 200 mg once a day for two weeks have resulted in complete patient recovery.

Conclusion: These observations suggest that benign esophageal ulcer associated with *Candida* species infection should be suspected in all patients with unclear border esophagitis.

Keywords: *Candida*-associated esophageal ulcer, esophageal candidiasis, *Candida tropicalis*, Diabetes

P-031

Black *Aspergillus* as the main causative agents of otomycosis in Yasuj south west of Iran

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Introduction: As a common condition throughout the world, otomycosis is an acute, sub-acute or chronic superficial mycotic infection of external auditory canal caused by a wide range of fungal species. In this study we aimed to identify fungal agents isolated

from patients clinically suspected to otomycosis.

Materials and methods: External ear canal samples were taken from 275 patients who referred to the outpatient department of Shahid- Mofatteh Clinic in Yasuj City, southwest Iran. The collected samples were examined by both direct microscopy and culture. DNA of all isolated fungi were extracted with glass-bead disruption followed by ITS-PCR-RFLP analysis for identification of yeasts and β -tubulin sequencing for identification of *Aspergillus* species or other molds.

Results: A total of 275 patients suspected to otomycosis were included in this study from which 128 patients were positive on KOH preparation and 144 samples consisted of 61 (42.3%) males and 83 (57.7%) females were positive in culture. Predominant predisposing factor was self-cleaning of external ear with unhygienic tools and housewives were the main occupation. The most common isolated fungi were *Aspergillus* species (n=120) including 73 isolates of *Aspergillus* section *nigri*, 43 of section *flavi*, 3 of section *terrei* and one of section *fumigati*. 34 isolates were *Candida* species including *C. parapsilosis* (n=22), *C. albicans* (n=12) and *C. tropicalis* (n=1). 8.3% of patients had mix infection of two or three species.

Conclusion: As clinical feature of otomycosis are not specific, laboratory diagnosis is important to know the exact etiology of otomycosis for appropriate antifungal therapy. Further research is needed to determine susceptibility of fungal agents to antifungals and targeted treatment.

Key words: Otomycosis, *Aspergillus*, *Candida*, Iran

P-032

The first case of fungal keratitis due to *Aspergillus minisclerotigenes* in Iran

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Background: We report the first case of fungal keratitis due to *Aspergillus minisclerotigenes*, a species phenotypically similar to *Aspergillus flavus*.

Case presentations: A 68-years-old rural woman living in Iran who carried out agricultural and livestock work visited the north-eastern ophthalmology center at Khatam-Al-Anbia Eye Hospital in Mashhad, with severe eye pain, burning, foreign body sensation and reduced vision in the right eye. This person has long-term uncontrolled diabetes and her right eye doesn't close well due to an anatomical problem with the right eyelid. This anatomical problem might be the underlying reason for the fungal infection (due to the entry of soil particles or dust in the eye). Direct microscopic analysis of smears of corneal scrapings showed branched septated hyphae. The corneal smear sample was cultured and the fungus isolated from the culture was initially identified, based on macroscopic characters, as *A. flavus*. The isolate was further studied by sequencing a part of the calmodulin gene and identified as *A. minisclerotigenes*. The patient did not respond to antifungal treatment and eventually a corneal transplantation was performed.

Conclusion: This report shows that fungal keratitis can be caused by less common species and molecular methods are needed to reliably identify these isolates.

Key words: *Aspergillus minisclerotigenes*, fungal keratitis, Iran, Cornea.

P-033

Evaluation of acquired fungal infections from 3 hospitals in Kerman

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Introduction: The fungal pathogenicity is based on the ability of the fungus to adapt to the environmental conditions and resistance to host cell defense. In this study, the frequency of acquired fungal infections from hospitals has been investigated.

Materials and methods: This descriptive study was performed on 180 suspected cases of fungal infections. Samples were identified simultaneously for direct and culture methods for mycological studies as well as by diagnostic methods such as *Candida* CHROMagar and molecular method PCR-RFLP of *Candida* spp.

Results: From 134 cases of fungal infections, 53 infectious cases (39.5%) were acquired from the hospital and 81 infectious cases (60.5%) were acquired from the community. The highest prevalence of nosocomial infection is in the parts. The only fungal agent causing nosocomial infections in this study was *Candida*.

Conclusion: There is a significant difference in the frequency of non-fungal and fungal infections among the hospital units. With regard to the possibility of dissemination of fungal colonization and septicemia caused by fungi, the prognosis of these infections, the care of patients and, if necessary, their treatment, is an important step in preventing the possible risks of these infections.

Keywords: Hospital-Acquired Infections (HAIs), *Candida* species, PCR-RFLP

P-034

Molecular detection of *candida* spp. isolated from oral mucous in patients with leukemia and lymphoma in South-eastern Iran

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Introduction: Oral candidiasis is a serious problem for immunocompromised patients, especially patients with hematological malignancies. Because after becoming a systemic candidiasis, it is difficult to diagnose, control and treat in individuals with hematological malignancies. In this study, our goal was to diagnose candidiasis in the oral mucosa of patients with leukemia and lymphoma in a timely manner in order to prevent their transmission to systemic candidiasis

Materials and Methods: In this cross sectional study, 50 samples were collected from the mouth of patients with hematological malignancies undergoing chemotherapy from the oncology units of educational hospitals in Kerman, Iran. Patients were not only from Kerman province, but also from the neighboring provinces of Sistan and Baluchestan and Hormozgan. Sampling was restricted to patients with diagnosed acute lymphoid leukemia (ALL); acute myeloid leukemia (AML); chronic lymphoid leukemia (CLL); chronic myeloid leukemia (CML); Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL).

The species identification was done by using conventional methods like color of colony on CHROMagar *Candida* medium, germ tube production and assessing the morphology on corn meal agar and their identity was confirmed by the PCR-RFLP method.

Results: A total of 50 patients participated in this study, 14 patients (28%) of which had positive oral candidiasis. *Candida albicans* (57.14%) was the most common species among them followed by *Candida glabrata* (14.28%), *Candida parapsilosis* (14.28%), *Candida krusei* (7.14%) and *Candida kefyr* (7.14%). In this study, the most commonly isolated species of oral candidiasis was

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Candida albicans and was the highest rate of oral candidiasis infection in ALL (35.71%) and then NHL (28.57%) patients.

Conclusion: The results indicated that oral candidiasis is a prevalent complication in the hematologic malignancies population, being *C. albicans* the main etiological agent, however, there is a serious participation of other *Candida species*.

Keywords: *Candida* spp, Oral candidiasis, Hematologic malignancies, Leukemia, Lymphoma, PCR-RFLP

P-035

Diagnosis of fungal infections in pediatric cancer patients

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Introduction: Pediatric cancer patients undergoing chemotherapy are at high risk of developing fungal infection which remains a major cause of morbidity and mortality. Though incidence of fungal infection in pediatric cancer patients is lower than bacterial infection but it causes higher mortality rate due to not accurate and sensitive diagnostic tests. Comparing conventional tests, identification by MALDI-TOF Mass Spectrometry of fungal elements is reliable and much quicker. Recent studies have shown that MALDI-TOF MS is potential to identify yeasts, filamentous fungi and dermatophytes accurately, providing specific standardized procedures. Moreover, MALDI-TOF MS is successful for fungal typing and identification at the subspecies level, epidemiological studies and for taxonomical classification.

Keywords: Fungal infection, Pediatric, MALDI-TOF Mass Spectrometry

P-036

Onychomycosis caused by co-infection of *Aspergillus niger*, *Penicillium* Spp. and *Alternaria* Spp.; A case report

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Background: Onychomycosis is a fungal infection of the toe or fingernails that occurs throughout the world and is caused by dermatophytes, yeasts and molds. Molds are saprophyte fungi living in the soil and some of them may behave as primary pathogens of the skin and nails. Here, we report a case of co-infection onychomycosis caused in a 60-years-old male farmer by saprophytic molds. This person was organ transplant which had been treated with Azathioprine.

Case Presentation: This 60-years-old male farmer had organ transplant had been treated with Azathioprine. Direct microscopic examination using an improved clearing preparation containing 20% KOH was performed on nail samples. The nail samples was cultured on Sabouraud dextrose agar, therefore slide culture and finally antifungal susceptibility test were done for each isolates. Direct sample examination of the nail samples revealed branching septated hyphae and dematiaceous septated hyphae. From culture the nail samples on SDA three types of colony grew up together. Based on morphological features in direct sample examination and slide culture, the isolates were identified as *Aspergillus niger*, *Penicillium* spp. and *Alternaria* spp. According to antifungal susceptibility test carried out for each one, all were resistance to fluconazole (MIC >64 µg/ml) and were susceptible to itraconazole, voriconazole and posaconazole.

Conclusion: Onychomycosis by molds is infrequent and the global prevalence varies depending on the geographical region studied and the diagnostic criteria used. According to this case, in farmers, onychomycosis can occur due to trauma by fungi, so immunocompromised patients are

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susceptible to fungal co-infection. Onychomycosis is responsible for up to 50% of diseases of the nail and it is a multifactorial disease, and age, lifestyle and immunity have important effect on this disease.

Keywords: Onychomycosis, *Aspergillus niger*, *Penicillium*, *Alternaria*

P-037

Evaluation of fungal colonization of the respiratory tract of patients in the intensive care unit in Fasa Vali-Asr Hospital

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Introduction: The incidence of invasive fungal infections has increased significantly throughout the world. In general, severely ill patients are exposed to invasive fungal infections. These diseases in the intensive care unit (ICU) with patients with underlying disease are a matter of serious concern today. Fungal infections and colonization are becoming a major challenge for ICU patients. The variety of fungi species that causes the disease are significant, most common fungi species that causes infection in severely ill patients are *Aspergillus* and *candida*.

Materials and Methods: A total of 50 clinical samples were taken from 25 patients in surgical and internal intensive care units of Fasa Vali-Asr Hospital for 10 months. Samples were collected through bronchoalveolar lavage (BAL) 48 hours after admission of patients in ICU. Initially, samples were tested by gram stain and culture in SDA medium. Then, isolates identified by PCR-RFLP and PCR amplification of *HWPI* gene, and finally, *in vitro* antifungal

susceptibility testing performed for all isolated fungi.

Results: Out of 50 samples taken from 25 patients of both lung, 23 (46 %) yeast and yeast like strains isolated which *Candida albicans* (n=11) was the most frequently isolated species followed by *C. krusei* (n=4), *C. tropicalis* (n=3), *C. glabrata* (n=2), *C. parapsilosis*, *C. guilliermondii* and *C. famata* each (n=1). So, antifungal susceptibility testing revealed that the fungi species were quite sensitive to the four medications; Fluconazole, Itraconazole, Voriconazole and Posaconazole.

Conclusion: In this study, direct evaluations of BAL samples from admitted patients in ICU showed that almost half of the patients were positive in terms of colonization and infection by different opportunistic fungi.

Keywords: *Candida*, Colonization, ICU, PCR-RFLP

P-038

Vaginal candidiasis and species identification in pregnant women in Shahid Noorani hospital in Talesh

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Introduction: Vaginal candidiasis is common in during pregnancy .It may lead to complications like abortions, premature birth, low birth weight, chorioamnionitis and systemic neonatal infection. The aim of present study is the diagnosis of vulvovaginal candidiasis in pregnant women and species identification.

Materials and Methods: This cross-sectional study was conducted on 80 pregnant women with or without clinical symptoms of vulvovaginal candidiasis at Shahid Noorani hospital in Talesh. All specimens were examined by direct microscopy and culture on CHROMagar *Candida* medium. Cultured media incubated at 35°C for 48 hours and evaluated based on color and number of growth colonies. If no

growth was observed, the media were incubated for several additional days. Subcultures were done on Sabouraud dextrose agar and Corn meal agar + Tween 80 media for further study and identification of *Candida* spp. by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: Vulvovaginal candidiasis was observed in 20(25%) patients. Twenty-two isolates were obtained from culture of specimens on CHROM agar *Candida* medium. The most common isolated species was *Candida albicans* (72.6%) followed by *Candida glabrata* (22.7%) and *Candida krusei* (4.6%). Two patients had mixed infection with 2 different *Candida* species (*C. albicans* and *C. glabrata*). Direct microscopy examination showed yeast budding cells and pseudohyphae in 8 culture positive specimens. In the present study results of routine mycological method in differentiation of *Candida* spp. was consistent with molecular results, in 80% of cases. There was also significant correlation between vulvovaginal candidiasis with clinical symptoms (P=0.0001), diabetes mellitus (P=0.014), and taking antibacterial drugs (P=0.003) in pregnant women.

Conclusion: Vaginal candidiasis during pregnancy may lead to serious complications. *C. albicans* was the most common isolated *Candida* species in this study but, infection by non-*albicans* *Candida* species also should be considered. CHROM agar *candida* is a useful medium in detection of mixed infection and identifying *Candida* species but, in some situations it is not reliable for definite diagnosis of *Candida* species and PCR-RFLP method could be considered as a reliable test in differentiation of species.

Keywords: Vulvovaginal candidiasis, Pregnancy, *Candida albicans*, CHROMagar *Candida*, PCR-RFLP

P-039

Study of clinical features and molecular identification of etiological agents in 25 cases of mucormycosis in Iran

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Introduction: Mucormycosis is a serious infection caused by fungi of phylum zygomycota, subphylum mucormycotina and order mucorales. The incidence of mucormycosis has dramatically increased due to increase of the population at risk. Early diagnosis of mucormycosis is vital for timely treatment and improves the prognosis. This study was performed for the purpose of evaluating the clinical and mycological aspects of patients suffering mucormycosis. In the present study, the clinical and mycological aspects of mucormycosis patients are reported.

Materials and Methods: In this study clinical specimens were included 25 mucormycosis cases. Direct examination and culture had been performed for all specimens and isolated fungi were identified based on their morphology and sequence analysis of ribosomal DNA.

Results: Patients include 8 (32%) males also 17 (68%) females with the mean age of 47.16 years. Rhino-cerebral mucormycosis was the most common clinical form (24 cases) followed by pulmonary mucormycosis (one case). Diabetes mellitus was the most common predisposing factor (n=17, 68%). Cultures were positive in 15 specimens and the isolated fungi were morphologically identified as *Rhizopus* spp., while using the molecular method, all the isolates were identified at the species level as *Rhizopus oryzae*.

Conclusion: In our study, regarding full agreement between the results of morphologic and molecular methods. Conventional procedures could be trusted at least for *Rhizopus* species, however, due to the high identification power of PCR-

sequencing, applications of this procedure is recommended.

Keywords: Mucormycosis, Zygomycota, *Rhizopus* spp., PCR-sequencing.

P-040

Onychomycosis prevalence in Kashan Shahidbeheshti Hospital

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Introduction: Onychomycosis is one of the most common nail disorders. It affects 10-30% of the world population and is caused by dermatophytes, non-dermatophytes molds and yeasts. The aim of this study is the survey of onychomycosis prevalence in Kashan Shahid Beheshti Hospital.

Materials and Methods: In this retrospective study, samples were taken from 110 patients. Direct microscopic examination by 20% KOH solution and culture of samples in Sabouraud Chloramphenicol (SC) agar and Sabouraud Chloramphenicol Cyclohexamide (SCC) agar was done. Results were reported after 21 days.

Results: Fungal examination showed that 80 cases were positive, 80% of them were female and 20% were male and the mean age of them was 48. 60% of cases were diabetic patients. Fungal agent isolation respectively was *candida* (95%), dermatophytes (3%) and non-dermatophytes molds (2%).

Conclusion: In this study *Candida* was the most frequent fungal agent and the major group of infected cases was diabetic. Thus, we recommend that fungal examination and surveying the prevalence of onychomycosis in diabetic patients in the other study.

Keywords: Onychomycosis, Diabetic, *Candida*, Tinea unguium

P-041

Prevalence of superficial fungal infection in diabetic patient in Shahid Beheshti Hospital in Kashan

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Background: superficial fungal diseases are common in Iran. As well as diabet is one of the most prevalence chronic, metabolic disease. The prevalence of fungal infection seems to be higher among diabetic patients than in the non-diabetic population. Thus the aims of this study were to determine the frequency of superficial fungal infection in diabetic patient.

Methods: This retrospective research was carried out on 72 diabetic patients since 1395 to 96. The epidermal scales and nails were cleared and examined microscopically by KOH 10-20% and lacto phenol cotton blue solutions and they were cultivated on sabouraud's dextrose agar (SDA) and mycosel agar (Sc).

Result: 58 cases (80.5%) of diabetic patients had superficial fungal diseases. 22cases (37.9%) were males and 36 were females and Average of patient's age was 57.2 years- old. Prevalence of superficial fungal infections respectively including : candidiasis 36 cases (62%), dermatophytosis 8 cases (13.7 %), pityriasis versicolor 7 cases (12%) , aspergillosis 5 cases (8.6 %) and erythrasma 4 cases (6.8 %). 3 patients had 2 various complication of fungal disease simultaneity.

Conclusion: Nowadays despite of control and decrease of many complications of diabet, one of the important involvements in these patients are fungal diseases. Therefore this important issue should be more studied and surveyed.

Keywords: diabet, superficial fungal diseases

P-042

Annual epidemiologic survey of onychomycosis in patients attending the mycology laboratory of Tehran University of Medical Sciences

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Introduction: Onychomycosis or fungal nail infection is one of the most common fungal infections, which is increasingly prevalent and difficult to eradicate with drug treatment. It can occur due to invasion of dermatophytes, yeasts and saprophytic molds to the nail plates. Nearly 50% of all nail disorders are caused by fungi. The purpose of this study was to identify and determine the prevalence of causative agents of onychomycosis in Tehran based on age, gender and occupational activities.

Materials and Methods: During one year, from March 2017 to March 2018, 395 patients suspected with onychomycosis, referred to the medical mycology laboratory of Tehran University of Medical Sciences, were assessed for the presence of onychomycosis. Both direct microscopy and cultures of the nail material were performed to identify the causative agents.

Results: Among specimens from 395 patients affected by nail disorders, 155 (39.2%) patients with onychomycosis including 33.6% male and 66.4 female (78 fingernails, 73 toenails and 4 both of them) via direct examination and/or culture methods were diagnosed. Yeasts were the most prevailing causative agents of finger nail onychomycosis (72.2%) and saprophytic fungi were identified as main causative agents of toe nail onychomycosis (52.1%). The most common age group infected was >60 years (27.8%). From 163 isolated fungal agents from culture 53 isolates were related to housewives job category which means

they were the most common infected population (32.5%). In this study onychomycosis of finger nails and toe nails were most prevalent in female patients.

Conclusion: Because of considerable prevalence of onychomycosis, necessity for a careful mycological examination in patients with nail disorders is highlighted. This epidemiological data may be useful in the development of preventive and educational strategies.

Keywords: epidemiologic survey, causative agents, onychomycosis, Iran.

P-043

Incidence of invasive fungal infections in transplant recipients

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Introduction: Invasive fungal infections (IFIs) are a major problem among transplant recipients. Overall, candidiasis, followed by aspergillosis is the most common fungal infections in solid organ transplant and hematopoietic stem cell transplant recipients. The epidemiology of these infections is changing due to some different reasons such as increasing in the population at risk of the infection, increased using antifungal prophylaxis, and improvements in diagnostic methods such as molecular techniques. The incidence of IFI after allogeneic HSCT is estimated to be 10-25% in high-risk patients. The mortality rate of these infections may reach to 70- 90%. Improvement in diagnosis of these infections can be helpful for physicians to give effective prophylactic agents and vigilantly monitor these patients to reduce the mortality and morbidity associated with IFIs. This review article discusses about the epidemiology, risk factors, clinical features and diagnostic methods of invasive fungal infections in transplant recipients with focus on the two most common infections, candidiasis and aspergillosis.

Results: Although *Candida* spp. have remained as the most common cause of IFIs

in SOT and HSCT recipients, *Aspergillus* and non-*Aspergillus* molds account for about 40% of IFIs in the transplant population. Non-*Aspergillus* molds have been increasing and have implications for therapy since they exhibit a variable drug susceptibility profile to the commonly used antifungals.

Conclusion: In present study, with available novel diagnostic tests and new antifungal drugs, we have already witnessed several changes in the epidemiology, mortality and outcomes of fungal infections in transplant recipients.

Keywords: Invasive fungal infections, transplant recipients, *Candida*, *Aspergillus*, antifungal agents.

P-044

Combination antifungal successful treatment without any neurosurgical debridement in a patient with

sinocerebral mucormycosis: A case report

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Background: Mucormycosis is an uncommon insidious infection that is caused by Mucorales fungi from the zygomycetes class that usually accounts for <2 % of all isolated fungal pathogens. Patients with severe immunodeficiency admitted to the hospital are at greatest risk for developing this infection. Mucormycosis usually is transmitted in humans via inhalation or direct inoculation of spores into injured skin and mucosa. Mucormycosis varies in clinical manifestations and severity. There is no pathognomonic sign for mucormycosis and symptoms are often nonspecific, complicating early diagnosis. Common

presentation of sinocerebral mucormycosis includes facial pain, headache, lethargy, visual loss, proptosis, and/or palatal ulcer. With early diagnosis and treatment of sinocerebral mucormycosis with antifungal combination therapy, fungal infections may be cured, especially in patients who have predisposing risk factors such as chemotherapy.

Case presentation: A 35- years- old woman was a new case of high grade B cell lymphoma that had been admitted to take R-CHOP chemotherapy (Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) and after that CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate/ifosfamide, etoposide and high-dose cytarabine) was prescribed. During last phase, he was feverish and neutropenic (ANC: 200 cell/ml).

After eight days her right eyelid was swollen, erythematous and she suffered headache and blurred vision. Chemotherapy was immediately stopped. Broad spectrum antibiotics meropenem, vancomycin and Amphotricin B liposomal (300mg/daily) was prescribed and para nasals CT scan and brain MRI was done. Brain Magnetic resonance imaging (MRI) showed mild mucosal maxillary thickening, intra cranial ring enhancement with dimension of 10*10 mm, left frontal cortex enhancement and left frontal dura enhancement. Ribbon like hypha that compatible with Mucormycosis was seen in the histological examination of endoscopic sinus biopsy and confirmed by culture. Syrup posaconazole (5 cc QID) was added to Amphotricin B. Due to major involvement, patient was candidate for enucleation of left eye, decompression of orbit and sinocerebral debridement, but due to dissatisfaction of patient, just multiple sinus debridement was done and antifungal combination treatment was continued. During third week after combination regimen, there was a remarkable improvement in signs and symptoms of the patient and also significant decrease in frontal lobe and orbit MRI involvement.

Conclusion: Antifungal combination therapy (Amphotericin B Liposomal and

Posaconazol) can be a promising method in treatment of patient with Sinocerebral Mucormycosis.

Keywords: Sinocerebral Mucormycosis, Combination anti-fungal treatment, invasive fungal infection

P-045

Successful treatment of postoperative sternal osteomyelitis, due to *Candida albicans*, with triazoles

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Background: Fungal osteomyelitis is a rare disease, but is challenging to manage due to the poor vascularization of bone that favors the proliferation of microorganisms. This infection can be fatal for immunocompromised patients. We report a case of post-surgical sternum infection, due to *Candida albicans*.

Case presentation: A 65-years-old man presented with post-operative wound infection after sternotomy for coronary artery by-pass grafting. Two tissue biopsies were sent for mycological tests. Yeast isolates were growth, but could not be identified to the species level using conventional methods such as germ tube formation and the use of CHROMagar *Candida*. Sequence analysis of internal transcribed spacer regions (*ITS1*) identified *Candida albicans*. Caspofungin (0.063 µg/ml), micafungin (0.063 µg/ml), anidolafungin (0.063 µg/ml) and Amphotricin B (1 µg/ml) had potent activity, while the highest MICs were consistently observed for fluconazole (≥64 µg/ml), followed by voriconazole (16 µg/ml) and itraconazole (16 µg/ml) against two *Candida albicans* isolates. Initial treatment with oral

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fluconazole (200 mg daily) failed. The dose was reduced to 100 mg per day. The fistulae gradually closed after 1 month.

Conclusion: The species level identification and antifungal susceptibility tests in patients with post-operative wound infection are important to provide proper diagnosis and treatment.

Keywords: *Candida albicans*, Osteomyelitis, Sternum

P-046

Prevalence of vaginal candidiasis among urban and rural women of Ardebil, Iran

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Introduction: Vulvovaginal candidiasis (VVC) represents the second most frequent cause of vaginal affections, after bacterial vaginosis. It is estimated that around 75% of women in their reproductive age, suffer from at least an episode of vulvovaginal candidiasis.

Material and methods: This study was a cross – sectional study which was done on 4, 287 women who were attended to Alavi hospital in Ardebil city, Iran, during 2013 – 2018. Cervical specimen were collected from all the patients and smears were immediately fixed with 95% ethanol and stained by Papanicolaou's method and examined microscopically.

Results: Of the 4, 287 women enrolled for the study, 267 (6.23%) were positive for vulvovaginal candidiasis. The age range of women was between 18 and 70 years old. The most frequently affected women were below 40 years.

Conclusion: Although VVC is a complex disease, the prevalence of cervico-vaginal infections was consistent with the results of many studies obtainable in Iran and other part of the world. The multifactorial nature of the disease shall be fully evaluated for the overall frequency among Iranian population.

Keywords: vulvovaginitis, candidiasis, candidosis, Iran.

P-047

Study on the distribution of *Candida* spp. in the mucosal surfaces of healthy persons from different age groups

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Introduction: *Candida* species are normal inhabitants of the mucosal surfaces. The importance of epidemiological monitoring of *Candida* spp. which can involve in pathogenic processes is unquestionable due to the increase of *candida* infections over the last decade. Susceptibility to such infections may be attributed to reduced host defense mechanisms and/or virulence of the organism. As we know different species belong to *Candida* genus have different pathogenicity and different susceptibility to antifungal drugs. Another important point is that *Candida* spp. distribution varies with individual's age and gender.

Materials and methods: In this study, 223 healthy people were divided to 3 age groups, including: children, adults and geriatrics. Using a cotton-tipped swab moistened with sterile serum physiology sampling from their nose, saliva and vagina was done. Samples were collected from January to July 2016 and all of them cultured on Sabouraud Chloramphenicol Agar. The *Candida* isolated species were identified by culture on CHROM-agar *Candida* and the *ITS1* and *ITS4* rDNA regions sequencing.

Results: The results demonstrate that for this test population, the frequency of *Candida* species varied as an effect of host age. The most *Candida* isolation were related to the children age group followed by the adults and the geriatrics. *C. albicans* was the predominant isolated species in all age groups. This study showed no statistically

significant effect of the subject's sex on mucosal *Candida* composition.

Conclusion: Our study showed that the highest prevalence of *Candida* isolation was related to the children's age group, which could be due to more tooth decay in this age group, because most people in this age group ranged from 3 to 12 years old. It should be noted that oral and dental hygiene in this age group is less, which can be another reason for the greater isolation of this fungus from the oral mucosa of this group. Also after this group, the highest prevalence of *Candida* isolation was related to the elderly. The main reason for this finding is the use of dentures in this age group. In this work, the cutaneous *Candida* community was similar between males and females. Similar life style and diet could be the reason for the observed result. We found that *C. albicans* is the predominant *Candida* species resident on mucosal surfaces. It means among *Candida* species, *C. albicans* is still the most common infectious agent isolated from clinical samples in Iran.

Keywords: age groups, DNA-sequencing, gender, Microbial epidemiology, mucosal *Candida* composition.

P-048

Causative agents, underlying diseases and treatment outcomes of aspergillosis: our experience with 29 cases

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Introduction: Infections due to *Aspergillus* species are a big threat for immunocompromised patients including those with leukemia or transplant recipients. The mortality of invasive aspergillosis in 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

untreated patients could rise up to 100%. So, timely diagnosis and proper treatment of these infections have a great importance. Generally, *Aspergillus fumigatus* is the most common cause of invasive aspergillosis while in cases of sinus involvement, *A. flavus* is more frequent.

Materials and Methods: In this cross-sectional study, all clinically suspected patients from Nov. 2017 to Oct. 2018 were enrolled. Clinical and radiographic examinations, as well as galactomannan test and mycological examination were done and the isolated fungi were identified morphologically. The prescribed drugs and the treatment outcomes were also recorded.

Results: During the present study among about 300 under study patients, a total of 29 patients were confirmed for aspergillosis. The mean age and F: M ratio of the patients were 41.14 ± 18.6 years and 10:19, respectively. Leukemia was the leading underlying disease (51.72%) followed by transplantation (17.24 %). Bronchoalveolar lavage galactomannan test was done for 14 patients with positive result in all cases (mean galactomannan level: 2.67 ± 1.55). Serum galactomannan test was done for 21 patients with positive results in 15 cases (71.42%). *A. flavus* was the dominant species (n=24, 82.76%) followed by *A. fumigatus* (n=3, 10.35%) and *A. niger* (n=2, 6.96%). Initial treatment with amphotericin B and subsequent shifting to voriconazole were the most common approach of treatment. Generally, 15 patients (51.72%) were died.

Conclusion: In this study, *A. flavus* was the leading causative agent of aspergillosis which is not in line with the global pattern.

Keywords: Aspergillosis, *Aspergillus flavus*, leukemia, transplant recipients

P-049

Candidemia due to non-albicans Candida species: incidence rates, risk factors, and antifungal susceptibility profile at tertiary care academic hospitals in Tehran

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Introduction: The emergence of new species of *Candida* as potential pathogens is a reflection of the changing scenario in candidemia. A number of risk factors have been identified for candidemia. However, the search through available literature has revealed paucity of data regarding differences between the *C. albicans* and non-*albicans Candida* (NAC) species. The aim of this study was to investigate the epidemiology of candidemia and further analyze the risk factors, incidence rates and antifungal susceptibility profile of NAC spp.

Materials and methods: This study was conducted on a total of 106 patients with bloodstream infections in two tertiary care academic hospitals at Tehran. The patients were categorized according to the referenced diagnostic criteria. The identification of *Candida* species was accomplished based on conventional examination, assimilation profile test, and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The minimum inhibitory concentrations (MICs) were determined and interpreted based on the guidelines of Clinical and Laboratory Standards Institute, M27-A2 procedure.

Results: During the study period 83 episodes of non-*albicans Candida* species including *C. glabrata* (45.7%), *C. parapsilosis* (27.7%), *C. tropicalis* (14.4%), *C. lusitaniae* (4.8%), *C. guilliermondii* (3.6%), *C. kefyr* (1.2%) and *C. krusei* (1.2%) were identified.

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Central venous catheterization (54.7%) was the major risk factor associated with candidemia. Cancer (67.7%) and major surgery (56.5%) were identified as significant risk for candidemia due to NAC spp. Azole resistance was significantly high in *C. tropicalis* and *C. glabrata* species.

Conclusion: The emergence of NAC spp. highlights the importance of species identification along with antifungal susceptibility testing for institution of most appropriate antifungal drug.

Key words: non-*albicans Candida* , Candidemia , antifungal susceptibility testing

P-050

Species distribution and antifungal susceptibility profile of invasive *Candida* isolates from paediatric ICUs in Tehran, Iran

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Introduction: As paediatrics candidaemia data and the antifungal susceptibility patterns of isolated strains are scarce in Iran, this study aimed to determine species distribution and antifungal susceptibility profile of *Candida* species isolated from Iranian hospitalized paediatrics with invasive candidiasis.

Materials and methods: A total of 235 yeast strains recovered from normally sterile body fluids of patients admitted at the intensive care units of Children's Medical Centre, Tehran, Iran, were identified to the species level using CHROMagar *Candida*, molecular methods including PCR-RFLP and sequencing and were reconfirmed by MALDI-TOF. Minimum inhibitory concentrations (MICs) of amphotericin B, fluconazole, voriconazole, micafungin, and anidulafungin were determined using broth microdilution test, according to European Committee on Antimicrobial Susceptibility testing reference method (EUCAST E.Def 7.3.1).

Results: *Candida albicans* (53.6%), *C. parapsilosis* (24.7%) and *C. tropicalis* (8.5%) were the most common species, followed by *C. lusitaniae* (4.3%), *C. glabrata* (3.0%), *C. guilliermondii* and *C. orthopsilosis* (each 1.7%), *C. kefyr* (1.3%), *C. dubliniensis* (0.8%), and *C. intermedia* (0.4%). Amphotericin B MICs were ≤ 1 mg/L for all *Candida* isolates. *C. albicans* isolates were susceptible to all five antifungal agents. All *C. parapsilosis* isolates categorised as intermediate to micafungin and anidulafungin, except two isolates that had the MICs >2 mg/L for micafungin. MIC₅₀ and MIC₉₀, and MIC range for fluconazole were 0.25 mg/L, 1 mg/L, and 0.125 - ≥ 32 mg/L, respectively. Fluconazole and voriconazole showed 100% activity against main *Candida* species.

Conclusion: Although no resistance to amphotericin B was observed, the desirable susceptibility to fluconazole makes it a reasonable choice for treatment of invasive candidaemia.

Keywords: *Candida* species, antifungal susceptibility, Paediatric ICU, Iran

P-051

New treatments of toe nail onychomycosis

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Introduction: Toe nail Onychomycosis is a prolonged disease with many failure in treatment. In this mini review we have overviewed new different treatments introduced for last 10 years. Treatments have a varieties of new azoles, laser therapy, limited surgery & others.

Conclusion: Although the topical and systemic medications as a classic treatment are available composition or not with new methods described in different studies. Methods like Non-thermal laser therapy, using the nanocapsule formulations prolonged release of azole, providing new pathways and opportunities for drug access to targets within and beneath the nail plate by nail penetrating. Also nail alterations caused by onychomycosis investigation aided to find new treatments opportunities.

Keywords: onychomycosis, treatment, toe nail

P-052

Molecular characterization and antifungal susceptibility profile of dermatophytes isolated from scalp dermatophyte carriage in primary school children in Arak city, Center of Iran

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Introduction: Asymptomatic carriage is a condition of positive dermatophyte scalp culture without signs and symptoms of tinea capitis. Carriers are the source of dermatophytes that are able to transfer fungal agents to other people. The aim of this study was evaluating asymptomatic dermatophyte scalp carriage among students of primary schools in Arak city.

Materials and methods: Sampling by a sterilized hairbrush from scalp was performed among 3174 students. Hairbrush was inoculated onto Mycosel agar plates. Dermatophyte isolates were identified by PCR-RFLP using *MvaI* enzyme. In vitro antifungal susceptibility test was done according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 protocol. The antifungal drugs used included griseofulvin (GRZ), terbinafine (TER), itraconazole (ITC) and fluconazole (FLU).

Results: A total of 3174 school children were screened, 15 cases (0.48%) had a positive culture for dermatophytes. Asymptomatic carriers including 11 (73.3%) boys and 4 (26.7%) girls and their age range was between 7-12 years. *Trichophyton tonsurans* (80%), *T. interdigitale* (13.3%) and *T. rubrum* (6.7%) were the most common isolated dermatophyte. Based on the obtained antifungal susceptibility results, terbinafine had the lowest and fluconazole had the highest (Minimum Inhibitory Concentration) MIC values for all of the tested dermatophyte isolates.

Conclusion: In the study, *T. tonsurans* was the most common species isolated from asymptomatic carriers and of the four antifungals tested, terbinafine had the most active antifungal in vitro against all isolates. Identifying and treating of scalp dermatophyte carriers can prevent the spread of tinea capitis in the community.

Keywords: Antifungal susceptibility test, Asymptomatic dermatophyte scalp carriage, Molecular method, Primary school children, Iran

P-053

The survey of *Tinea Versicolor* prevalence in patients referring to the Farabi laboratory in Ardabil between 1395-96.

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Introduction: *Tinea Versicolor* as a fungal infection was caused by the lipophilic yeast called *Malassezia furfur*. The important factors that lead to tinea versicolor infection are lack of individual hygiene, stress, chronic infections, excessive sweating or hyperhidrosis, malnutrition, genetic causes. The use of broad-spectrum antibiotics, tight and nylon coatings, and the long-term use of steroids are related to known epidemiology of this type of disease. The use of preventive measures helps to save the cost of treatment. The purpose of this study is to investigate the prevalence of tinea versicolor among patients referred to Farabi's laboratory in Ardabil by two methods; skin scraping and scotch test between 1395-96.

Materials and methods: This descriptive cross-sectional study was performed on 686 suspected fungal infections at Farabi laboratory of Ardabil in 1395-96. Out of the total number of patients, 24 cases were collected through skin scraping and Scotch tape and diagnostic tape were confirmed by direct microscopic examination. Then, the results were analyzed by SPSS software.

Results: From 686 patients with suspected fungal infections, 322 females and 364 males were studied. A total of 24 (3.5%) patients with a mean age of 23 years were infected with *Tinea Versicolor*. 10 cases (41.7%) were women with an average age of 21 years and 14 (58.3%) of men with an average age of 25 years, and the most common site for breast, neck and shoulders

Conclusion: According to the results of this study, the prevalence of this type of fungal infection in men with an average age of 25

years is higher than the women, in some cases with lifestyle and non-observance of personal hygiene and living in areas with wet weather.

Keywords: Tinea Versicolor, *Malassezia furfur*, Scraping, Farabi Lab

P-054

Occurrence and distribution of pathogenic *Mucorales* in hospital environmental soil samples from Urmia, Iran

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Introduction: Mucormycosis is an emerging life threatening invasive fungal disease that affects both immunocompetent and immunocompromised patients. Although there are some cases of healthcare-associated mucormycosis, the majority of infections are supposed to be acquired from the environment. *Mucorales* are mostly opportunistic pathogens originating from air, soil, decaying organic matter and composting vegetation. There are currently few data on prevalence of this group of fungi in the environment. The aim of the present study was to assess the prevalence and diversity of species of *Mucorales* from soil samples collected in Urmia, Iran.

Materials and methods: A total number of 174 soil samples were collected in sterile tubes in seven different hospitals in Urmia, Iran between 2016 and 2017. Two grams of soil were homogenized in sterile saline and plated on Sabouraud dextrose agar (SDA) and RPMI agar supplemented with 4 mg/l of Itraconazole or 1 mg/l of Voriconazole. Both media contained Chloramphenicol and Gentamicin. The plates were incubated at 35 ± 2 °C and checked daily for fungal growth for a maximum of 7 days. *Mucorales* were subcultured to purity on SDA. All *Mucorales*

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isolated from both SDA and RPMI agar media were identified microscopically.

Results: 73 isolates of *Mucorales* were retrieved from 348 culture samples. All *Mucorales* species belonging to three species were isolated. Among the recovered isolates, *Rhizopus* species (n = 54), *Mucor* species (n = 19), and one *Cunninghamella* species were found. Highest positive soil samples were from Motahari (n = 24), followed by Taleghani (n = 18), Emam Reza (n = 12), Artesh (n = 9), Arefiyan (n = 4), Emam Khomeini (n = 3) and Shohada (n = 3) hospitals. *Mucorales* were retrieved from samples obtained from hospital environmental soil samples of Urmia, Iran from different season. Voriconazole and Itraconazole-containing medium improved the recovery of *Mucorales* compared with other media.

Conclusion: The present study showed that pathogenic *Mucorales* are frequently recovered from soil samples in Iran. Species diversity should be further analyzed on a larger number of soil samples from different geographic areas in Iran and in other countries. In summary, this study demonstrated that pathogenic *Mucorales* could be frequently detected in soil samples across Iran. Due to fragmentary data in the literature, large sampling in various geographical areas in Iran and in the rest of the world is warranted for a better understanding of the ecology of this important group of fungi.

Keyword: Conventional identification, Soil, *Mucorales*, Urmia, Iran

P-055

Common fungi and major factors of the contaminations at the indoor of student dormitories

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Introduction: Microbiological quality of environments is an important criterion that must be taken into account when indoor workplaces are designed to provide a safe environment. Therefore, the purpose of this study is to provide insight into how students are exposed to fungal contamination of the dormitory indoor and to figure out the major possible factors that govern the contamination levels.

Materials and methods: The study samples were obtained from two female dormitories of UMS University. The specimens were collected by using sterile swabs from indoor environments such as rooms, kitchens, washrooms /bathrooms, corridors and study rooms. A morphologic identification was performed using colony features and microscopic characteristics for the fungal isolates, and the findings were confirmed by PCR-RFLP molecular method.

Results: Molds and yeasts were recovered from the indoor places including rooms, study room, kitchens and bathrooms from student living areas of the dormitories. A total of 160 swab samples yielded fungal growth. The number of fungal colonies recorded was 458 cps (colony per swab) included common mold: *A. flavus* (31.7%), *A. fumigatus* (28.7%) and *A. niger* (5.8%) and yeasts: *Candida albicans*, *C. dubliniensis*, *C. krusei*. The black fungi (dematiaceous fungi) were also isolated 67 (11.5%). Other molds included *Penicillium* spp(9.5%), *Rhizopus* spp (4.3%), *Scopulariopsis* spp (0.5%), *Pseudoallescheria* spp and *Fusarium* spp (0.35% each).

Conclusion: Our findings show that *Aspergillus* species are most common fungi contaminant in the dormitories indoor and kitchens contain most species of mold isolated.

Keywords: Fungi, Contamination, Dormitory, Indoor environment.

P-056

The frequency of vaginal Candidiasis in the city of Kermanshah

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Introduction: Candida vaginitis is one of the most common opportunistic fungal infections and 75% of women have struggled at least once with this infection during their lifetime. The aim of this study was to determine the most common of *Candida* species in patients referred to clinics of Kermanshah University of Medical Sciences.

Materials and methods: In this study, 125 vaginal sampling was done by two sterilized swabs from suspected patients to vaginal candidiasis. The direct experiment performed by preparing two staining slides, one with Giemsa staining and the second for 10% KOH. For culturing, Sabouraud Dextrose agar with Chloramphenicol (SC) and CHROM agar candida were used. At first, the isolates were identified by microscopic observation and secretion of specific color on CHROM agar. For more accurate determination, molecular methods (PCR-RFLP) using *MspI* enzyme was used.

Results: Out of the 125 samples, 53 (42%) were positive for vaginal candidiasis. Isolated species were as follows: *C. albicans* 46 isolates (87%), *C. glabrata* 5 isolates (9%) and *C. krusei* 2 isolates (4%).

Conclusion: In present study *C. albicans* like most studies, diagnosed as the most frequent species. This study shows the high frequency of vaginal candidiasis in women in the west of the country. Therefore, accurate identification of these species can be useful and effective in controlling and using specific treatments to prevent recurrence of disease in this group of people.

Keywords: Vaginitis, Candidiasis, *Candida* species, PCR-RFLP.

P-057

Drug resistance and hospital sources of *Aspergillus* species causing HAI at a medical educational center in Urmia

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Introduction: In spite of a low percent (1%) of fungal hospital acquired infections (HAIs), *Aspergillus* species are the main agents of fulminate fungal infections. Invasive Aspergillosis has a mortality rate of 90%, among other *Aspergillus* infections. Regarding high frequently of *Aspergillus* spp. isolated from clinical and environmental sites in the Nephrology ward, we tried to determine the Azole resistance and to perform a molecular epidemiologic study, to find the accurate and exact environmental sources of *Aspergillus* infections and colonization in the large, general hospitals.

Material and methods: Our subjects included clinical specimens of case with HAI which collected during 48 month from October 2012 to September 2016 at the UMSU educational hospitals, Urmia, Iran. Also, environmental specimens including sterile swabs from surfaces of floor, walls, curtains, beds, trolleys, air condition and cooling systems, medical devices were obtained as well as some samples from finger tips of the visitors. The MICs for the Azole family (Fluconazole and Ketoconazole) were described as the lowest concentration of the drug that could reduce 50% of fungal growth. The molecular method RAPD-PCR using six random *Aspergillus* primers was performed to study the hospital sources of the isolated *Aspergillus*.

Results: During 24 month, August 2014 to September 2016, totally 198 samples were obtained from cases with proven HAI. The results of experimental studies on the specimens showed 93(47%) positive for a fungal or bacterial infections from the above

case, 54(58%) had a fungal infection. Among the isolated *Aspergillus*, *A. flavus* (47%), *A. fumigatus* (29.4%) and *A.niger* (23.6%) were the most frequent. Some were commonly isolated from clinical and environmental sources. Using RAPD-PCR, two clinical-environmental sets including *A. niger* (sinus mass -floor) and *A. flavus* (BAL-air conditioner) were matched. All *Aspergillus* commonly clinical and environmental isolates were susceptible in MIC test.

Conclusion: The most contaminated hospital indoor places in our study were the surfaces of floors, walls and also air samples in low amounts. As expected, all three of the isolates obtained from one patient showed identical patterns (RAPD combined type A/D-12). However, the same pattern was found in two environmental isolates obtained from the wards.

Keywords: *Aspergillus*, Nosocomial, Hospital, Source

P-058

Role of Moth fly (Diptera: Psychodinae) as a mechanical vector of *Mucor* and *Fusarium* in hospitals

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Introduction: Some of arthropods, such as the house fly, cockroach and moth flies, could act as mechanical vectors for pathogenic agents, including fungi, bacteria and helminthes. The worldwide distribution and the presence of moth flies in healthcare services such as hospitals make it as a potential mechanical vector of nosocomial infections especially fungi. The objective of this study was the isolation and identification of pathogenic fungi on external and internal

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surfaces of the moth fly collected from hospitals of Babol city.

Materials and methods: From May to October 2016, 121 adult moth flies were collected directly from their resting sites, without contacting the substrate, using sterile test tubes. Flies were collected in different areas of three hospitals in Babol city, North of Iran. Adult flies were immediately transported alive to a laboratory for Identification. All flies were identified to species level using a standard taxonomic key. Samples were isolated from the cuticular surface and the gut of the flies, and were cultured in Sabouraud Dextrose agar medium supplemented with Chloramphenicol and incubated at room temperature up to 2 weeks. Identification of *Mucor* and *Fusarium* were performed based on dark, chained spores on conidiophore presentations (tree like).

Results: Totally, 242 specimens were cultured from 146 adult moth flies. All moth flies were identified as *Clogmia albipunctata* (Diptera: Psychodinae). *Mucor* and *Fusarium* were isolated from the external and gut of the flies. In total, 14% flies were contaminated for *Mucor* (4%) and *Fusarium* (10%). Moreover, infection rates in the external and gut were determined for *Mucor* 100%, 0% and for *Fusarium* 90%, 10%, respectively.

Conclusion: The present has shown that moth flies carry pathogenic fungi in the hospital environments and play a role as mechanical vectors of nosocomial infections. Therefore, control of *Clogmia albipunctata* in hospitals is essential in order to control the nosocomial fungal infections.

Keywords: Moth fly, *Mucor*, *Fusarium*, Babol, Fungi.

P-059

Distribution of *Histoplasma* spp. And *Aspergillus* spp. in Iran: Acontemplated review

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Introduction: *Histoplasma* is found in soil that contains large amounts of bird or bat droppings. Principally the disease is confined to USA, particularly *H. capsulatum* which is endemic in central and eastern states however, certain cases have also been reported from Europe, Africa and Asian countries including, Iran. Renal transplant cases have been mostly documented in the literature. Aspergillosis is another clinical disease caused by diverse fungal species. There is wide array of diseases caused by this fungus however, the clinical spectrum has been grouped as classical diseases, mostly in patients with liver failure, chronic pulmonary disease, and metabolic syndrome. The other set comprises of immunocompromised patients or those having high risk of getting the disease. We aimed to study the prevalence of histoplasmosis and aspergillosis by reviewing past literature.

Material and methods: To review prevalence of *Histoplasma* and *Aspergillus* infections in Iran from January, 2000 to December, 2017, a Google scholar, Pubmed, SID and Medline literature search was performed.

Results: Both the mycological diseases are being reported sporadically from Iran, and mostly the literature is in the case report profile. *Histoplasma* spp. has been isolated from soil and bats residing in caves and few clinical cases comprising hemodialysis patient and kidney transplant have been reported but the prevalence is too low. *Aspergillus* has been isolated from Iranian soil (22.5%). Emergence of Azole resistant *Aspergillus fumigatus* has become a clinical concern since last few years.

Conclusion: The number of cases of fungal diseases is low but therapeutic approach needs attention.

Keywords: *Histoplasma*, *Aspergillus*, Infections, Prevalence, Antifungal resistance, Review

P-060

Molecular characterization of environmental *Alternaria* species isolated from Iran

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Introduction: *Alternaria* is a ubiquitous fungal genus that includes saprobic, endophytic and pathogenic species. It is associated with a wide variety of substrates including seeds, plants, agricultural products, animals, soil and the atmosphere. Species of *Alternaria* are known as serious plant pathogens, causing major losses in a wide range of crops. Because of the significant negative health effects of *Alternaria* on humans and their surroundings, a correct and rapid identification of *Alternaria* species

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would be of great value to researchers, medical mycologists and the public alike. We aimed to study the diversity of *Alternaria* species from environmental sources through DNA sequence analysis.

Materials and methods: Airborne samples were collected using the settle plate method, and soil samples were obtained from a depth of 5-10 cm of the superficial soil layer. Samples were cultured on Sabouraud Dextrose agar (SDA) plates, incubated at 25°C, and examined daily for fungal colonies for two to three weeks. Isolates were identified as *Alternaria* species according to the macroscopic and microscopic criteria. For species differentiation, DNA from 33 isolates was extracted and subjected to amplification of the internal transcribed spacer (*ITS*) region followed by sequencing.

Results: A total of 145 samples were collected from various environmental sources, of which 65 strains of *Alternaria* species were isolated. The most frequent species was *A. alternata* (46.6%), followed by *A. tenuissima* (33.4%), *A. malorum* (6.6%), *A. clamydospora* (6.6%), *A. japonica* (3.4%), and *A. rosae* (3.4%).

Conclusion: The collected data can build a foundation for future research and may be useful in the development of preventive and educational strategies. Epidemiological investigations should be performed in multiple areas of the country and compared to data from clinical samples in order to determine the relationship between environmental and clinical strain isolated.

Keywords: *Alternaria* species, Sequence analysis, Iran

P-061

Study on *Candida* infection in patients with type 2 diabetes mellitus in Sari, Iran

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Introduction: Most physicians believe that patients with type 2 diabetes mellitus are predisposed to various infections. Candidiasis is one of the most common

infectious diseases can complicate the control of the diabetes. *Candida* species are the most important commensal yeasts on the skin and mucosal surfaces of the 20-50% humans. In patients with type 2 diabetes mellitus, these fungi can cause various infections including: oral, vaginal and urinary tract candidiasis. The aim of this study was to determine the prevalence of candidiasis in patients with type 2 diabetes mellitus.

Materials and methods: A total of 88 patients with type 2 diabetes mellitus have participated in this study. Enzyme-linked immunosorbent assay was used for detection of IgG, IgM, and IgA antibodies against *C. albicans* in sera of participants. The serum total cholesterol, triglyceride, lipoproteins, and glucose levels were measured by an enzymatic method with standard kits made of Pars Azmun Co. Iran.

Results: Chronic candidiasis (IgG level more than 30U/ml) and acute candidiasis (IgM level more than 10U/ml) were seen in 63.6% and 17% of the patients, respectively. The percentage of patients with IgA level more than 10U/ml was 2.3%. Statistically, a significant inverse relationship was observed between the levels of IgG and IgM antibodies against *Candida* and HDL-C level, $P=0.038$.

Conclusion: The results of this study proved that a large percentage of patients with type 2 diabetes mellitus suffering from chronic candidiasis and acute candidiasis. Moreover, HDL-C may have a role in preventing candidiasis.

Keywords: Antibody, Candidiasis, Diabetes mellitus

P-062

Study of the prevalence of fungal flora in Zabol city in 2018

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Introduction: Fungi are the air contaminating organisms that can cause many diseases, including superficial diseases, opportunistic and systemic

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infections, and allergic reactions. Regarding the importance of diseases caused by airborne fungi, this study aims to identify the diversity and frequency of fungal flora in the province of Zabol in 1396.

Materials and methods: In this descriptive and cross-sectional study, sampling with 540 plates containing Sabouraud Dextrose agar containing Chloramphenicol medium from five areas of Zabol in two seasons of spring and summer was carried out from active and out-of-field environments. For detection of colony fungal culture was applied using culture method and the results were analyzed using Fisher test.

Results: In this study the most common fungi which were divided from the internal and external environment in the spring and summer were *Aspergillus fumigatus* (20.8) and *Mucor* spp (19.5). The highest fungal concentrations in the indoor and the outdoor environment of *Aspergillus fumigatus* (Cfu: 285.37) and *Mucor* spp (Cfu: 289.15) were as in the external and internal media of *Aspergillus fumigatus* (Cfu: 275.45) and *flavus* (Cfu: 265.45) fungi. There was a significant difference between the prevalence of fungi in both summer and spring in both indoor and outdoor environments ($P=0.000$). The concentration of fungus flora with temperature and wind speed was inversely correlated with moisture content.

Conclusion: The results of this study showed that the air of Zabol city contains different types of fungal spores, therefore, considering that fungi can cause various diseases in humans and also are important causes of pathogenicity and mortality in immunocompromised individuals. Therefore, knowing the diversity of fungal flora in different places and identifying the environment from the point of view of fungal flora to infectious specialists, skin, doctors, etc. will be helpful in preventing, treating and reducing the mortality of diseases caused by human contact with fungi.

Keywords: Normal Flora, Zabol, Air

P-063

Clinical characteristics and in vitro susceptibility profiling of Candidemia in

febrile neutropenic patients; a brief report in Tehran, Iran

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Introduction: Febrile neutropenic patients are at risk of serious infections. Generally, bacteria are responsible for bloodstream infections (BSIs), but, 8% of all cases of infectious agents are *Candida spp.* Species of *Candida* that cause candidemia are the fourth most common agent of BSIs and the fifth most common cause of nosocomial infection. *Candida albicans* is the most important cause of BSIs; however 45% are caused by non-*albicans* species. The aim of the present study was to identify the frequency, species, and susceptibility patterns of candidemia in febrile neutropenic patients.

Materials and methods: This cross-sectional study was conducted on febrile neutropenic patients suspected with candidemia who had been referred to 3 educational hospitals during 9 months of the study.

Results: The blood samples of 80 febrile neutropenic patients with the mean age of 48±16.6 years were studied (60% female). Five (6.25%) episodes of candidemia were identified. The underlying disease was acute myeloid leukemia in 4 (80%) cases and all 5 (100%) cases had central venous catheter and were receiving prophylactic ciprofloxacin and acyclovir. 100% of isolates were found to be susceptible to

voriconazole, 80% to caspofungin, 60% to Amphotericin B, and 40% to fluconazole.

Conclusion: The frequency of candidemia among the studied febrile neutropenic patients was 6.25%, with 80% mortality rate, and the most frequently identified yeast was *Candida albicans* (100% susceptible to Voriconazole).

Keywords: Candidemia; Febrile neutropenia; Antifungal agents; Drug resistance

P-064

Molecular identification of *Candida* species isolated from oral cavity of Pemphigus vulgaris patients and evaluation of antifungal activity among the isolates

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Introduction: Pemphigus vulgaris is an autoimmune disease that mostly affects oral cavity. *Candida* species are opportunist fungi that could affect mucosa and cause oral candidiasis. The aim of this study was to identify the fungal agents isolated from lesions of oral cavity and evaluate antifungal activity profile against the isolates.

Materials and Methods: Among 40 pemphigus vulgaris patients, a total of 25 patients with active oral lesions were included in this study. Identification of the fungal isolates was performed based on conventional methods and DNA sequence analysis of internal transcribed spacer (*ITS*) rDNA gene region. Antifungal activity of Fluconazole, Itraconazole, Ketoconazole, Psoconazole, Econazole, and Amphotericin

B against the isolates were evaluated based on CLSI M-44 A protocol.

Results: Oral candidiasis was detected in 20% of the patients. By results of molecular method *Candida* species were identified as *Candida albicans* 22/25, *Candida glabrata* 2/25, and *Candida dubliniensis* 1/25. All of the isolates were sensitive to Amphotericin and Econazole, 96% to Fluconazole and Posaconazole, and 92% to Ketoconazole and Itraconazole. One patient showed resistant profile to Fluconazole, Psoconazole and Ketoconazole, simultaneously. One case had hairy tongue oral manifestation.

Conclusion: In this study, *Candida albicans* was the most prevalent isolate in pemphigus vulgaris patients with oral lesions. Amphotericin B and Econazole were the most effective antifungals against the isolates.

Keywords: Pemphigus vulgaris, *Candida*, Antifungal, *ITS*

P-065

Distribution of *Candida* in the oral cavity
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Introduction: Oral candidosis is one of the most common human opportunistic fungal infections of the oral cavity. In this study, our aim was to identify *Candida* species isolated from mouths of different persons.

Materials and Methods: This study was conducted on 100 individuals referred to a pathological laboratory at the following stages. Sampling by two sterile swabs from the oral cavity was performed and then direct microscopic examination, culture on Sabouraud dextrose agar containing chloramphenicol and CHROM agar and other differential tests (germ tube test, morphology on corn meal agar and API ID 32C) were used. The results were analyzed by SPSS v.16 data with chi-square and Kruskal-Wallis test.

Results: In this study, 31 positive cases were reported. Most species belonged to *C. albicans*, followed by *C. glabrata*, *C. tropicalis*, and *C.krusei*. In Smokers, *C. glabrata* was the most observed species and *C. albicans* was the most widely observed species in non-smokers. In the underlying diseases group, the most species was *C. albicans*, followed by *C. glabrata* and in the non-diseased group, *C. albicans* and *C.glabrata*, were respectively seen.

Conclusion: An increased incidence of the infections by *Candida* species is associated with some predisposing factors. The most important of these species is *C. albicans*, which is most commonly isolated from the oral cavity and is believed to be more virulent in humans.

Keywords: Oral candidiasis, *Candida* species, Opportunistic infections

P-066

Evaluation of Drug susceptibility of *Aspergillus* species isolated from ICU of Hospitals in Invitro

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Introduction: Invasive aspergillosis is the most threatening disease in immunocompromised patients. It has the highest morbidity and mortality rate amongst invasive fungal infections in hospitals. The aim of present study was to perform antifungal susceptibility testing in *Aspergillus* spp isolated from the hospital's environment.

Materials and Methods: After collecting 160 plates containing Sabouraud Dextrose agar from the air and the environment of hospital's intensive care units (ICUs), the phenotypic and molecular identification of the colonies was performed in order to identify *Aspergillus* spp. After DNA extraction, the molecular identification was carried out using universal fungal primers

internal transcribed spacer (*ITS*) region and DNA sequencing. Antifungal susceptibility testing was performed using the CLSI broth microdilution (M38-A2) method for *Aspergillus* isolates.

Results: Out of 160 hospital environmental samples, 11 *Aspergillus* species were obtained. The eleven *Aspergillus* spp. were identified by sequencing as 5 *A. flavus*, 3 *A. sydowii*, 1 *A. fumigatus* and 2 *A. Oryzae*. Our antifungal susceptibility testing results indicated that *A. sydowii* and *A. fumigatus* were sensitive to amphotericin and voriconazole but were resistant to itraconazole. *A. sydowii* was resistant to caspofungin while *A. fumigatus* was sensitive to this drug. *A. flavus* was susceptible to all the drugs.

Conclusion: There were a number of reasons including delayed diagnosis, lack of appropriate curing, and the existence of various diseases and neutropenia which could lead to the high mortality rate of patients with Aspergillosis, especially in patients admitted in ICUs of hospitals.

Keywords: Drug susceptibility, *Aspergillus* spp. ICU

P-067

Causative pathogens of urinary tract infection and their antimicrobial susceptibility patterns in patients with mucormycosis

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Introduction: Mucormycosis is a life-threatening fungal infection which mostly affects immunocompromised patients worldwide. Therefore, every co-infection should be considered seriously and treated carefully, considering that the causative pathogens and their antimicrobial resistance patterns may differ from others.

This study was conducted to evaluate urinary tract infections (UTIs, as an example of co-5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

infection), the related uropathogens, and their antibiotic resistance patterns in patients suffering from mucormycosis.

Materials and methods: An almost 6-year study was performed among the mucormycosis patients hospitalized in the Loghman hospital and had UTI at the same time. The isolates were selected from urine cultures and subjected to antibiotic susceptibility test using disk diffusion method. Multi-drug resistance (MDR) was defined based on a proposal published by a group of international expert from the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention in 2012. Extended-spectrum beta-lactamase (ESBL) pattern was tested and defined according to the Performance Standards for Antimicrobial Susceptibility testing published by the Clinical and Laboratory Standards Institute in 2018.

Results: Nine isolates were recovered from 7 patients (4 women, 3 men; 28-73 years old) who were mostly hospitalized in the Infectious Diseases ward. Unlike the usual pattern in which *Escherichia coli* is the dominant pathogen, *Pseudomonas aeruginosa* was the main uropathogen (33.3%), followed by *E. coli* (22.2%). The rest consists of some other Gram-negative and positive bacteria. All the *E. coli* isolates showed the ESBL pattern. Meanwhile, there was only one *P. aeruginosa* isolate derived from a senior male patient (66 years old) that showed the MDR pattern. One adult female patient (62 years old) had two episodes of recurrence (relapse and re-infection). In her case, while the causative pathogen in either acute or relapse episodes was *E. coli*, the isolate recovered in re-infection episode was *P. aeruginosa*.

Conclusion: It seems that UTI in immunocompromised conditions like mucormycosis patients need a more precise attention on the causative pathogens and their antimicrobial susceptibility patterns instead of initiating empirical antibiotic therapy.

Keywords: Mucormycosis, urinary tract infection, uropathogen, antimicrobial susceptibility

P-068

Drug azole resistance of *Aspergillus fumigatus* strains isolated from compost in Iran

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Introduction: In recent years, Azole resistance has grown in *Aspergillus fumigatus* and has become a global public health concern. There are two hypotheses in relation to the azole resistance in *A. fumigatus*. One, the azole resistance may occur during the treatment of azole in some patients, and the other is the use of azole compounds in the environment. It seems to be one of the main ways of the azole resistance is wide application of azole in crop protection, material preservation. Compost (decaying plant waste material) is believed to be an important biological niche for *A. fumigatus*, with high densities of conidiospores, where azole residues from agricultural waste may accumulate. The aim of this study was to determine the epidemiology of triazole-resistant *A. fumigatus* in compost material in Iran.

Materials and Methods: In this study, 185 compost samples prepared by composting companies, glasshouse, compost from gardens, agricultural land and hospital gardens from Mazandaran and Tehran province were processed and screened in terms of azole resistance (4 and 1 mg/L of itraconazole and voriconazole, respectively), using selective plates. According to the conventional techniques, *A. fumigatus* isolates were identified based on growth and standard morphological characteristics.

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Finally, the isolates were confirmed by partial sequencing of the b-tubulin gene. Afterward, *in vitro* antifungal susceptibility tests against itraconazole and voriconazole agents were performed based on the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document. Conventional PCR assay was carried out to determine the presence of the TR34/L98H mutation in the *CYP51A* gene of triazole-resistant *A. fumigatus* isolates (minimum inhibitory concentration (MIC) > 2 µg/ml).

Results: Out of 185 compost samples, 51 samples (27.56%) *A. fumigatus* isolates were detected. According to *in vitro* antifungal susceptibility tests against itraconazole and voriconazole, the MIC values of 42 samples (82.35%) and 4 samples (7.84%) were reported above the cut-off points, respectively. The MIC range of itraconazole and voriconazole are 0.25 - 16 µg/ml and 0.125 - 16 µg/ml, respectively. In addition, some strains of *A. fumigatus* showed high MIC value for voriconazole (>16 µg/ml) in contrast this isolates had low itraconazole MICs (<2 µg/ml). Among resistant samples, TR34/L98H mutations in the *CYP51A* gene were the most prevalent detected.

Conclusion: Our study indicates that the antifungal azoles used in the compost were not suitable and a very serious threat to the use of triazoles in medicine. We also reported that the rate of resistant *A. fumigatus* isolates is very high in the compost. Therefore, compost should be considered as an important niche of *A. fumigatus* resistant isolates, which may conidia migrate through the air and cause disease in people with an impaired immune system. Nevertheless, we found a significant number of isolates for which no TR34/L98H mutation in the *CYP51A* gene could be identified (4 out of 51, 7.84%). Therefore, other mutation such as (F46Y, G54W, Y121F, G138C, M172V, F219C, M220I, D255E, T289F, G432C and G448S mutations) should be considered.

Keywords: Minimal Inhibitory Concentration (MIC), *Aspergillus fumigatus*, Drug resistance, compost, Voriconazole, Itraconazole, TR34/L98H, TR46/Y121F.

P-069

Identification of *Penicillium* species isolated from food using the ITS1 region

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Introduction: *Penicillium*, ascomycetous fungi are of major importance in the natural environment as well as food and drug production. *Penicillium* Link is one of the most common fungal genera occurring in diverse environments. Therefore, this study was conducted to identify strains of *penicillium* isolated from food using the internal transcribed spacer (ITS1) region.

Materials and methods: In order to study the genetic diversity and phylogenetic relationship of *penicillium* isolates, 28 samples were collected. The DNA of the whole *Penicillium* fungus was extracted and the 463 paired region of the penny mushroom was propagated by the ITS1 region. 28 isolates were sequenced from the samples. The sequences were compared with 13 sequences of the GenBank (NCBI).

Results: According to this experiment, the isolates of the fungus were classified into 7 groups. Ten samples of food isolates were placed in a genetic similarity closely related to *ML332*, *G9*, *Penicillium ulaiense*, H1 outgroup and G2, and remained in the remaining six other gene sequences.

Conclusion: The ITS region can well differentiate levels of similarity of fungal species from food, and is an important element in identifying and evaluating different fungal species in foods.

Keywords: *Penicillium*, ITS1 region, Corrosive Food, Phylogeny Tree.

P-070

Identification of *Aspergillus* species from food material control center of Hamedan (Iran) by sequence of β -tubulin gene

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Introduction: *Aspergillus* is one of the most abundant organisms in our environment. Due to the importance of this fungus in the industry and medicine need to accurately identification of species. The traditional method based on macro and microscopic characters requires skilled technicians and takes a lot of time. The present study aimed to use the β -tubulin (*BT2*) gene sequencing for the identity of some isolate of *Aspergillus* from food materials in Iran.

Materials and Methods: Totally, about 100 *Aspergillus* culture, isolated from food material samples of Hamedan food material control center (tea, rice, bread, soya, olive salad, hazelnut and salty cucumber). Slide culture was prepared for all samples for primary identification. Then *Aspergillus* samples were cultured on Sabouraud Dextrose agar for macroscopic characters of the colony, 13 different colony sample shape were selected.

Genomic DNA was extracted from selected strains by Cinagene DNA extraction kit and then β -tubulin gene was amplified by polymerase chain reaction (PCR) from each sample. The obtained data was analyzed via comparison with sequences existed in the GenBank database.

Results: Among the 13 *Aspergillus* sequenced isolates, 4 isolates were identified as *Aspergillus oryzae*, 2 as *Aspergillus tritici*, 2 as *Aspergillus tennesseensis*, 1 as *Aspergillus luchuensis*, 1 as *Aspergillus amoenus* and 1 as *Aspergillus versicolor*.

Conclusion: The findings of this study indicated that sequencing *BT2* gene is needed to support by another valuable for species identification of *Aspergillus* isolates. It seems that needs to other genetic markers and molecular DNA-based procedures on discrimination of *Aspergillus* species are

recommended especially for samples of the same section group.

Keywords: *Aspergillus*, β -tubulin, Iran

P-071

Hospital sources of common *Candida* infections at the educational hospitals

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Introduction: Fungi especially *Candida* species are sixth common agents among the pathogens in hospitals acquired infections. From fungal agents, yeasts the fourth prevalent agents of healthcare-associated infections (HAIs), second in Urinary tract infection (UTI) and fourth in bloodstream infections. The aim of this study is determination of hospital sources for *Candida* infection by the identification of *Candida* species isolated from Urmia educational hospitals using restriction fragment length polymorphisms (PCR-RFLP) methods.

Material and methods: During six month, clinical samples of HAI cases transported to Medical Mycology Center, Urmia Medical School. At the mycology lab, a rapid examination was performed for the detection of fungal elements, if positive result was seen, immediately, a full sampling was run from hospital indoor by using sterile swabs. All environmental samples and the culture media were transported to medical mycology lab. By using morphology and molecular (PCR-RFLP) methods identifications at the level of species for *Candida* and other fungi performed.

Results: From a total of 99 clinical samples 58% of colonies belonged to fungi. The positive cases included *Candida* 66.6%, *Aspergillus* 31.4% and other fungi 0.085%. Among *Candida* yeasts, *Candida albicans* 64%, *Candida glabrata* 16.6%, *Candida*

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krusei 13.88%, *Candida guilliermondi* 2.77% and *Candida tropicalis* 2.75% were detected.

Conclusion: Our findings showed that the sources of *Candida* contamination are the patient, personels and visitors. Against it, *Aspergillus* contaminations source by surroundings and indoor places.

Keywords: *Candida*, hospital, infection sources

P-072

Identification and isolation *Cladosporium* fungi from Forests and Farms of Mazandaran Province

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Introduction: *Cladosporium* is one of the most commonly known pathogens of chromoblastomycosis that often causes fungal infections in humans and animals due to fungal infections. The fungus is present in the soil of forest areas and farms, and the fungi are from the family of advanced fungi called fungi demitasse is known.

Materials and Methods: In the research, soil samples were collected from forest areas and farms. In the lab was cultured in a culture medium Sabouraud dextrose agar (SDA) and by microscopic and macroscopic identification we had found difference fungus colony that isolated in soil farmland and forest Mazandaran province.

Results: The samples were collected from different soils by isolation method, microscopic and macroscopic examination of fungi in the fields and soils of the province that finally, in the laboratory examination, *Cladosporium* was detected, and 3.9% of the fungi identified belong to the *Cladosporium* fungus.

Conclusion: Obviously, by identifying the fungi present in a region, the Ministry of Health and the Veterinary Organization can take appropriate and effective action

regarding the correct strategy for handling these fungal diseases.

Keywords: Forests, Farmland, Identification and Separation, *Cladosporium* fungi

P-073

Prevalence of noninfectious (allergic and irritant contact dermatitis) and infectious cutaneous lesions of construction industry workers in the Mashhad -2017

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Introduction: One problem within the construction industry workers, non-infections lesions (allergic and irritant contact dermatitis) and infectious diseases can cause significant morbidity in construction industry workers.

Materials and methods: This study was a cross-sectional conducted to investigate the prevalence of skin lesions among a group of 750 randomly selected in the Mashhad.

Results: Noninfectious lesions (allergic and irritant contact dermatitis) 79 cases (10.53%), infection lesion 48 cases (640%), candidiasis 15 (31.25%), dermatophytosis 14 (29.16%), pityriasis versicolor 11(22.91%), erythrasma 5 (10.41%), and bacterial infection, *Staph aureus* 3(6.25%).

Conclusion: Fungi are important sources of allergens which could trigger cutaneous inflammation industry workers with atopic dermatitis therefore, we suggest clinicians pay more attention to screening and treatment of fungal hyper colonization reaction to fungi industry workers with atopic dermatitis.

Keywords: Allergic, Dermatitis, Infectious cutaneous, construction industry workers.

P-074

Azole Resistance of Environmental and Clinical *Aspergillus terreus* Isolates from Iran

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Introduction: *Aspergillus terreus* infections are difficult to treat because of the intrinsic resistance to amphotericin B, and higher mortality compared to infections caused by other *Aspergillus* species. Although, azole resistance among *A. terreus* isolates are rare, the percentage of azole-resistant isolates remains to be studied. Therefore, the aim of the current study was to screen the presence of point mutations in the *cyp51A* gene among clinical (n = 36) and environmental (n = 45) *A. terreus* isolates.

Materials and methods: In all, 81 clinical and environmental of *A. terreus* isolates was molecularly identified (beta-tubulin gene sequencing) and tested for *in vitro* antifungal susceptibility using the CLSI M38-A2 procedure against itraconazole, voriconazole and posaconazole. *Aspergillus terreus* isolates with reduced susceptibility underwent *Cyp51A* gene sequencing and the point mutations in the *cyp51A* gene were screen (using MEGA version 5).

Results: Molecular identification showed that 66 (81%) and 15 (19%) isolates were *A. terreus sensu stricto* and *A. citrinoterreus*, respectively. 24 out of 81 *A. terreus* isolates had MICs of ≥ 1 $\mu\text{g/mL}$ for voriconazole and itraconazole or 0.5 $\mu\text{g/mL}$ for posaconazole. Two all tested isolates with high MICs for posaconazole (0.5 $\mu\text{g/mL}$) showed *M217T* alteration (nucleic acid change T650C) in *cyp51A* gene. Point mutation was detected in two *A. terreus sensu stricto* and no point mutation was observed in *A. citrinoterreus* isolates.

Conclusion: Since the *A. terreus* is intrinsically resistant to amphotericin B, the emergence of azole resistance can be a serious concern to the therapeutic option for *A. terreus* infection. Knowledge about molecular mechanisms and epidemiology of azole resistance against *Aspergillus terreus* complex is limited and future studies are needed.

Keywords: *Aspergillus terreus* complex, azoles, *cyp51A* mutation, susceptibility profiles

P-075

Occurrence of asymptomatic candiduria in hospitalized patients with heart failure and indwelling urinary catheter in a tertiary care center

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Introduction: Heart failure (HF) is a leading cause of hospitalization among the elderly patients, and infection in these patients is the main co-morbidities and substantial mortality rates in the world. Most risk factor associated with nosocomial urinary tract infections (UTIs) is urinary catheterization (>97%). This study was aimed to determine the prevalence of candiduria in HF patients, determine their etiologic agents, related risk factors and antifungal susceptibility pattern of these isolates which help the clinicians in the better management of candiduria.

Materials and methods: This prospective, descriptive cross-sectional, laboratory-based surveillance study was investigated 305 hospitalized patients with HF to identify asymptomatic candiduria during July 2016 to

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December 2017 in super-specialty Heart Center of Mazandaran University of Medical Sciences, Sari, Iran. A total of 580 urine samples were collected and cultured on Sabouraud dextrose agar with chloramphenicol and brain heart infusion agar with chloramphenicol and incubated at 37°C for maximum one week. *Candida* species cultivated on culture plates with colony count >104 CFU/ml associated with pyuria were considered significant. Species identification was done based on PCR-RFLP method by using the *ITS1* and *ITS4* primers and the *MspI* restriction enzyme.

Results: In our study, asymptomatic candiduria rate was 18.8%, more common in 51-65 and 66-80 years age groups and women (70%) compared to men (30%). History of surgery (62.0%), use of broad spectrum anti-bacterial antibiotics (60.4%), diabetes (58.6%) and admitted more than 7 days (44.3%) were major underlying conditions in these patients. *Candida glabrata* (n=27, 40.3%) and *C. albicans* (n=27, 40.3%) were the most common cause of candiduria in patients. Non-*albicans Candida* was found in 59.7% cases while *C. albicans* was found in 40.3% cases of candiduria. There was no significant difference between genders in terms of the frequency of different *Candida* species (P< 0/05).

Conclusion: Candiduria is an emerging problem in elder population especially in hospitalized patients with HF. Asymptomatic Candiduria due to non-*albicans* species is an increasingly difficult problem for clinicians to recognize and manage in HF patients. In order to manage of these patients, accelerating in the identification of species by molecular approaches should be considered.

P-076

Polyphasic approach: the final concept in classification of important *Aspergillus* species

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Introduction: *Aspergillus* is one of the most important genus of fungi. Members of the genus *Aspergillus* are highly aerobic growing on carbon rich substrates with monosaccharide and polysaccharides. They are causative agents of a wide spectrum of clinical manifestations, including: Allergic bronchopulmonary aspergillosis (ABPA), fungal asthma (SAFS) and Invasive aspergillosis. Identification of *Aspergillus* has been based on morphological and microscopic characteristics of the grown colonies that have provided a broad concept of the species. Therefore, in this study we reviewed different methods used in differentiation of *Aspergillus* species for accurate identification of the isolated species from clinical setting for an appropriate treatment.

Materials and methods: The Scopus, PubMed and ScienceDirect databases were searched for relevant articles using terms such as *Aspergillus* species, taxonomic position, classification, morphological and microscopic characteristics and polyphasic approach.

Results: Recent advances have developed many useful molecular techniques for differentiation of *Aspergillus* species targeting including the house-keeping genes, 26S, 28S, β -tubulin and ITS rDNA sequences. Currently, based on the polyphasic approach, the genus *Aspergillus* is divided into 8 subgenera (*Aspergillus*, *Fumigati*, *Circumdati*, *Candidi*, *Jerrei*, *Nidulantes*, *Warcupi* and *Ornati*) and 22 sections.

Conclusion: Different studies have confirmed that the species within a section are very similar in aspect of morphological and microscopic characteristics; therefore to 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

make an appropriate therapy it would be needed the identification of *Aspergillus* at species level by polyphasic approach. It is also being increasingly recognized that comparative sequence of different genes loci can offer a better discrimination of species within *Aspergillus* genus.

Keywords: *Aspergillus* species, fungal classification, polyphasic approach

P-077

Candida urinary tract infection in patients with renal failure undergoing hemodialysis

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Introduction: Uremia due to renal failure causes defect of immune systems and increases susceptible to infections. Hemodialysis by the reversal of uremia is therapeutic strategy in these patients. These patients require frequent hospital or dialysis centers visits, mainly 2-3 times a week. Unfortunately, the chronic hemodialysis increases the problem of infection. Urinary tract candidiasis is known as the most frequent nosocomial fungal infection worldwide.

Materials and methods: We determined the incidence of asymptomatic candiduria in 253 patients with renal failure undergoing hemodialysis in Imam Reza hospital, Amol, Iran between September 2017 and July 2018. Urine samples were cultured in SDA containing chloramphenicol and CHROMagar *Candida* media. *Candida* species cultivated on culture plates with colony count $>10^4$ CFU/ml were considered significant. Species identification was

confirmed based on PCR-RFLP method by using the ITS1 and ITS4 primers and the *MspI* restriction enzyme.

Results: In our study, among 253 patients (128 female, 125 male; age range: 35-83 years, mean age: 60.5 ± 2.8 years, dialysis ranges: 7-98 months, mean after start of dialysis: 41.9 months) a high incidence ($n=40$, 15.81%) of *Candida* urinary tract infection (Candiduria) was observed. 11.1% of the patients were taking antibiotics ceftriaxone and vancomycin at the time of sampling. *Candida glabrata* (47.1%), *C. albicans* (17.6%), *C. tropicalis* (15.7%), *C. parapsilosis* (15.7%) and *C. kefyr* (0.4%) were identified as candiduria agents. There was no significant difference between the two genders in terms of the frequency of different *Candida* species; however, there was a significant difference between candiduria and mean month after start of dialysis ($P < 0/05$).

Conclusion: Candiduria is an important infection in patients who underwent hemodialysis for renal failure. Length of time and frequency of dialysis increase chance of *Candida* urinary tract infection. *Candida glabrata* was considered to be the most common agent of candiduria in these patients. So, identification of candiduria and species of agent of candidiasis and use of appropriate treatment protocol in order to resistant of non-*albicans Candida* to some antifungal drugs are important issues in these patients.

Keywords: Hemodialysis, dialysis, *Candida* urinary tract infection, candidiasis, *Candida glabrata*

P-078

Molecular Identification and Antifungal Susceptibility Profile of Clinical *Candida* Species Isolates

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Introduction: Candidiasis as a major challenge associated with increased morbidity and mortality rates among immunocompromised patients. Fluconazole is regarded as the dominant therapy for invasive candidiasis. The emergence of fluconazole resistance is an important factor of treatment failure and therapeutic options available for the treatment of these infections have become limited. The aim of this study was to determine the molecular identification and antifungal susceptibility pattern of clinical *Candida* species.

Material and methods: A total of 213 episodes of candidiasis infection were identified in patients from 2012 to 2017. The patients were diagnosed based on clinical examination and *Candida* infection confirmed by the conventional examination, assimilation profile test and DNA sequencing method. MICs of amphotericin B, fluconazole, itraconazole, voriconazole, caspofungin and anidulafungin were performed based on CLSI M27-A3 protocol.

Results: *Candida albicans* was the most frequently isolated *Candida* species (48.3%, $n = 103$) followed by *Candida glabrata* (19.2%, $n = 41$) *Candida parapsilosis* (16.9%, $n = 36$), *Candida tropicalis* (12.2%, $n = 26$), *Candida famata* (1.4%, $n = 3$), *Candida kefyr* (1.4%, $n = 3$) and *Candida lusitanae* (0.46%, $n = 1$). Fluconazole resistance was found in 23 isolates comprising *C. tropicalis* ($n = 10$), *C. albicans* ($n = 6$), *C. glabrata* ($n = 6$) and *C. parapsilosis* ($n = 1$). Anidulafungin exhibited the lowest MICs (MIC range, 0.031-1 $\mu\text{g/mL}$; MIC₉₀, 0.25 $\mu\text{g/mL}$), followed by caspofungin (MIC range, 0.031-2 $\mu\text{g/mL}$; MIC₉₀, 0.5 $\mu\text{g/mL}$).

Conclusions: This study confirms that knowledge of the local epidemiology is important for conducting surveillance studies to prevent and control candidiasis and will be of interest for antifungal stewardship programs.

Keywords: Molecular characterization, susceptibility profiles, *Candida* species

P-079

Prevalence of Allergic Bronchopulmonary Aspergillosis in Iranian Cystic Fibrosis Patients by Two Different Diagnostic Criteria

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Introduction: Allergic bronchopulmonary aspergillosis (ABPA) is an immunological disorder caused by a hypersensitivity reaction to allergens of *Aspergillus* species especially *A. fumigatus*. ABPA is a frequent event in patients with asthma and cystic fibrosis (CF). CF is an autosomal recessive disorder that is caused by mutations in the CF transmembrane conductance regulator (CFTR) protein. There are different diagnostic criteria for the diagnosis of ABPA in patients with CF. In this present study we evaluated the prevalence of ABPA in Iranian CF patients by two more usual diagnostic criteria as ISHAM working criteria (A) and CF Foundation Consensus Conference criteria (B).

Materials and methods: Eighty six CF patients with a positive sweat and chlorine

test and probable family history of the CF were screened for ABPA. All CF patients underwent for *Aspergillus* skin prick test (AST), *Aspergillus*-specific IgE (positive: > 0.35 kUA/L) and *Aspergillus*-specific IgG (positive: \geq 26.9 kUA/L), total IgE (positive: \geq 1000 kUA/L), pulmonary function tests, chest X-Ray and/or high-resolution computed tomography scan (HRCT), eosinophil count of peripheral blood and also direct microscopy of sputum and culture for detecting of *Aspergillus* species. The ABPA prevalence in Iranian CF patients was estimated as per two diagnostic criteria, A and B, and compared.

Results: 86 CF patients with mean \pm SD (range) of age 16.14 \pm 7.21 (0.6-34.0) years were included. The mean \pm SD (range) of total IgE and specific IgE to *Aspergillus* were 373.3 \pm 352.1 (2.3-1200.5) kUA/L and 4.4 \pm 8.1 (0.1-44.5) kUA/L in patients with CF, respectively. The frequency of positive AST, total IgE, *Aspergillus*-specific IgE and IgG were 47 (54.7%), 9 (10.5%), 42 (48.8%) and 63 (73.3%), respectively. Blood eosinophil count >500 cell/ μ l was observed in 37 (43.0%) patients. Seventy eight (90.7%) of patients with CF had evidence of bronchiectasis in HRCT. The prevalence of ABPA, using A and B criteria were same (10.5%). Using kappa test revealed, both of diagnostic criteria A and B were agreement in number of diagnosed ABPA patients.

Conclusion: According to our results, the prevalence rate of ABPA in Iranian CF patients in line with other previous studies from different countries was considerable. No differences exist between the number of diagnosed ABPA patients using the two criteria A and B.

Keywords: Cystic fibrosis, Allergic bronchopulmonary aspergillosis (ABPA), Diagnostic criteria, *Aspergillus* sensitization, prevalence

P-080

Is there relationship between isolated *Aspergillus* species and severity of asthma?

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Introduction: Asthma is a heterogeneous and chronic inflammatory disease. Sensitivity to fungal allergens may be one of the risk factors associated with enhanced severity of asthma. Colonization of fungi in the tracheobronchial tree of patients with asthma lead to release of various fungal antigens which can increase severity of asthma symptoms. Therefore in the present study we evaluated the correlation between *Aspergillus* species colonisation in asthmatic patients and severity of asthma.

Materials and methods: During 13 months, 216 patients with mild to severe asthma and 30 healthy controls referred to two university hospitals from Tehran (Masih Daneshvari) and Sari (Imam) were included in the study. All included participants underwent pulmonary function tests to record FEV1 and

FVC parameters and collection of sputum samples. Each collected sputum sample was underwent for direct microscopic examination mounted with 20% potassium hydroxide (KOH) and fungal culture. The samples for fungal culture were inoculated into malt extract agar (QUELAB, Canada). The cultured plates were incubated at 27°C for 7 days and examined daily for fungal growth. Each grown *Aspergillus* colonies were identified at species level by molecular methods. Finally, all achieved data from sputum culture and spirometry test were analysed by SPSS software.

Result: Out of 216 asthma patients, 145 (67.1 %) cases were positive for fungal growth in sputa samples. Of 264 isolated fungal colonies, 137 (51.9%) were *Aspergillus* species belong to 7 section. *Aspergillus* (51.9%, 137/264) was the most frequent isolated fungi followed by *Candida* spp. (29.5%, 78/264), *Penicillium* spp. (3.4%, 9/264). Among *Aspergillus* genus isolates, *Aspergillus flavus* (29.2%, 40/137) was the most prevalent species followed by *A. fumigatus* (27.7%, 38/137), *A. niger* and *A. tubingensis* with equal prevalence (11.7%, 16/137). Furthermore, the distribution of *Aspergillus* species were different in the mild, moderate and severe asthma groups. In the mild and severe asthma groups, the majority of species were *Aspergillus fumigatus* 26.1% (10/38) and 36.8% (14/38) which, followed by *Aspergillus flavus* 12.5% (5/40) and 30.0% (12/40) respectively, but in the moderate asthma group, the most common species was *Aspergillus flavus* 57.5% (23/40) followed by *Aspergillus fumigatus* 36.8% (14/38). The mean of FEV1 value had no significant decline ($P>0.05$) in different type of asthma and there was no significant effect on FEV1 value by *Aspergillus* isolates. We found the similar results for mean of FVC value in each groups of mild, moderate and severe asthma patients ($P>0.05$). In total, there was no significant changes of FEV1 and FVC values in patients with a culture positive sputum samples compared with those of negative results ($P>0.05$).

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Conclusion: Our result showed that *A. flavus* was the most prevalent species of *Aspergillus* airways of asthma patients. Although, *Aspergillus* colonisation in airways of asthma patients had no significantly effect on FEV1 and FVC value, the severity of asthma symptoms especially in severe asthma patients were increased.

Keywords: Asthma, *Aspergillus* colonisation, Fungi, *Aspergillus flavus*, *Aspergillus fumigatus*

P-081

Molecular identification and *In vitro* antifungal susceptibility of clinical and environmental isolates of *Aspergillus nidulans* complex collected from different countries

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Introduction: *Aspergillus* species are globally as one of the most important fungal pathogens caused a wide spectrum of infections, especially at high risk patients, ranging from allergic to invasive aspergillosis (IA). Among the pathogenic aspergilli, *A. fumigatus*, ranks the first etiologic agent implicated in aspergillosis. In the last two decades, however, a marked shift in the etiology of aspergillosis occurred led to emerge less common pathogens in diverse clinical settings including *A. niger*, *A. terreus*, and *A. nidulans*. The main reasons for this shift seems to be the frequent use of empirical therapy or antifungal prophylaxis. *A. nidulans* has been currently reported as a common etiologic agent of IA in patients with chronic granulomatous disease (CGD). The use of amphotericin B (AMB) as empirical and the first line treatments in high risk patients and its low intrinsic susceptibility may be the main causes for increased incidence of infection by this species. The aim of this study was to assess the *in vitro* antifungal susceptibility of *A. nidulans* strains collected from different countries against nine antifungal agents.

Materials and methods: A total of 28 clinical and environmental isolates of *A. nidulans* collected from Iran and European countries including, The Netherlands, Portugal, and Greece were studied. These strains, which were previously determined morphologically, were subjected to molecular analysis of β -tubulin gene. Subsequently, the minimum inhibitory concentration (MICs) of various antifungal agents including azoles (itraconazole (IZ), posaconazole (PZ), voriconazole (VZ), and isavuconazole (ISV), AMB, terbinafine (TB) and the minimal effective concentration (MECs) of the echinocandins (anidulafungin (AFG), caspofungin (CPF), and micafungin (MCF) was evaluated against these strains using of EUCAST guidelines of filamentous fungi. There is no ECV (epidemiological cutoff value) for aforementioned antifungals against *A. nidulans*, exception for IZ and ISV.

Results: The results of sequence data due to β -tubulin gene confirmed that all 28 isolates

belonged to *A. nidulans* complex. According to the EUCAST-proposed breakpoints, a high MIC values for AMB (MIC \geq 16 mg/L) was found in 25 % of the clinical and environmental isolates of *A. nidulans*. The remaining isolates (67.9%) showed the MIC values of 1 mg/L (17.8%), 2 mg/L (35.7%), 4 mg/L (10.8%), and 8 mg/L (3.6%), respectively. Moreover, 25% of the isolates were resistant to IZ with MIC values between 2 mg/L and \geq 8 mg/L. TB exhibited a high MIC (>16.0mg/L) against 14.3% of the isolates. The MICs of >8.0 mg/L and >1.0 mg/L for VZ were also found in 10.7 and 3.6% isolates, respectively. Of note, 64.3 % of all isolates were resistant to ISV with a MIC value of >0.5 mg/L. Compared to MCF and AFG, CPF showed higher MICs of 1.0 mg/L and 4.0 mg/L against 35.7% of the isolates.

Conclusion: Our finding revealed a high rate of resistance of *A. nidulans* against main antifungal agents in treatment of aspergillosis. Our study also indicates that *in vitro* antifungal susceptibility, is necessary for establishing a response profile against the different classes of antifungals, in order to prevent the spread of antifungal-resistance isolates.

Keywords: *Aspergillus nidulans*, susceptibility testing, antifungal agents

P-082

Detection of azole resistance *Aspergillus fumigatus* strains from compost

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Introduction: *Aspergillus fumigatus* is one of the most prevalent airborne fungal pathogens causing infections worldwide. *Aspergillus fumigatus* strains generally are susceptible to itraconazole and voriconazole as a major drug of choice for treatment. While, the acquired resistance to triazoles has been recently described, azole fungicides widely used for crop protection and have been reported to be linked to azole-resistant *A. fumigatus* development in the environment. The aim of this study to detect the presence of azole resistance *A. fumigatus* in market compost.

Materials and methods: One hundred and two compost samples were collected from market compost. *Aspergillus fumigatus* isolates were screened for azole resistance using agar medium supplemented by itraconazole and voriconazole.

Results: 15 out of 102 azole-resistant *A. fumigatus* were isolated and showed resistance to itraconazole and voriconazole. The TR34/L98H mutation was the only resistance mechanism in our samples.

Conclusion: Recovery of *A. fumigatus* azole resistance from the environment suggests an environmental route of resistance selection. As exposure of *A. fumigatus* to azole fungicides may facilitate the emergence of new resistance mechanisms over time, understanding of parameters involved in resistance, management of azole fungicides uses, and even prohibition of those able to promote cross-resistance in pathogenic fungi are necessary to preserve the efficiency of azole medical antifungal drugs.

Keywords: *Aspergillus fumigatus*, azole resistance, compost heaps

P-083

Prevalence of allergic bronchopulmonary aspergillosis in patients with allergic asthma by using various diagnostic criteria

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Introduction: Asthma is a chronic inflammatory and heterogeneous respiratory syndrome with an estimated global burden of 300 million people. Occupational exposure to allergens (including fungal allergens), stress and microbial infections increase symptoms. Allergic bronchopulmonary aspergillosis (ABPA) is an allergic reaction to *Aspergillus* antigens, which is induced by repeated inhalation of *Aspergillus* spores. An

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undiagnosed and untreated ABPA can cause progressive pneumonia, bronchiectasis, chronic production of sputum, loss of lung function, and inappropriate control of asthma and eventual respiratory failure. In the present study, we determined the prevalence of ABPA in individuals with allergic-asthma by four different diagnostic methods.

Materials and methods: Tow hundred consecutive adults patients from different places of Iran, with spirometry and clinical confirmed diagnosis of allergic bronchial asthma were evaluated in this study. All patients underwent for *Aspergillus* skin prick test, total IgE (≥ 417 KUA/L), elevated level of *Aspergillus*-specific IgE (> 0.35 KUA/L), *Aspergillus*-specific IgG ≥ 26.9 , spirometry tests, chest radiography and/or high-resolution computed tomography scan (HRCT), peripheral blood eosinophil count and also sputum direct microscopy and culture for detecting of *Aspergillus*. The ABPA in patients was evaluated by four diagnostic criteria including Rosenberg & Patterson criteria (A), ISHAM working criteria (B), Agarwal criteria (C) and Greenberger criteria (D). In final, the prevalence of ABPA was estimated as per the each diagnostic criteria. The four diagnostic criteria were compared to evaluation of their concordance and discordance, sensitivity and specificity.

Results: During the study, 200 patients with moderate (51.5%) to severe (48.5%) allergic bronchial asthma were included. Out of 200 patients, 111 (55.5%) were female and the mean (range) age of patients was 45.8 ± 13.03 (18-78) years with a mean (\pm SD) asthma duration of $10.04 (\pm 9.94)$ years. The mean (range) of total IgE and *Aspergillus*-specific IgE levels were $316.3 (6-1300)$ kU/L and $1.5 (0.1-61.3)$ kU/L in asthmatic patients, respectively. In total, 27 (13.5%), 65 (32.5%), 22 (11.0%) and 83 (41.5%) of patients were positive to AST, total IgE, and *Aspergillus*-specific IgE an IgG, respectively. Blood eosinophil count greater than 500 cell/ μ l were reported in 29.5% (59/200) of patients. Fourteen percent (28/200) of patients with allergic asthma had evidence of bronchiectasis in HRCT. Using

A, B, C, and D criteria the prevalence of ABPA were 5%, 2.5%, 2.5% and 5% respectively. Raising the total IgE cut-off value to >1000KUA/L reduced the number of ABPA as per criteria B and C, but not by criteria A and D. Five additional patients were diagnosed with ABPA as per criteria A and D, who were labelled not to have ABPA by criteria C and B. By using Rosenberg & Patterson criteria as gold standard hypothetically, sensitivity and specificity of criteria B and C were 62.5% and 100%, and criteria D were 100% and 100% like criteria A.

Conclusion: Although differences observed between the number of diagnosed ABPA patients using the four criteria but the number of ABPA patients diagnosed by criteria A and D, sensitivity and specificity were equal and criteria B and C had similarity in the number ABPA patients, sensitivity and specificity. There was discordance in 5 patients when the four criteria were compared.

Keywords: Allergic asthma, Allergic bronchopulmonary aspergillosis (ABPA), Diagnostic criteria, *Aspergillus* sensitization, prevalence

P-084

Morphological changes and induction of antifungal resistance within expression and mutation the Cyp51 A, B, C genes in *Aspergillus fumigatus* and *Aspergillus flavus* due to different CO₂ levels

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Introduction: Aspergillosis is one of the most common opportunistic fungal diseases in immunocompromised and neutropenic patients. *Aspergillus fumigatus* is the most common cause of aspergillosis and *Aspergillus flavus* is the second agent. Due to changes in the concentration of CO₂ that some pathogens encounter during the infection process and to understand the role of CO₂ as a carbon base. In this study evaluated of changes in antifungal susceptibility patterns, the expression and the mutation in the genes of intervener in *Cyp51A,B,C* in *A.fumigatus* and *A.flavus* in the effect of variable CO₂ concentrations.

Materials and Methods: *A. fumigatus* and *A.flavus* strains were cultured and incubated under the 1%, 3%, 5% and 12% of CO₂ concentrations, each time in one, two, and four weeks. The control culture were maintained for 1 week without CO₂ concentration. Morphological changes were investigated and antifungal susceptibility tests were performed according to recommendations stated in the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document and evaluated the expression and the mutation in the genes of intervener in *Cyp51A,B,C* with Real Time PCR protocols. All tests of different CO₂ concentrations were compared with control sample.

Results: We found that 1%, 3%, 5%, and 12% CO₂ concentration were associated with morphological colony changes. Macroscopically, the colonies were shallow dark green, smooth, crisp to powdery with reduced growth; microscopic examination revealed the absence of conidiation. The induction of antifungal resistance of susceptible strains to itraconazole, voriconazole and amphotericin B increased after expose with 12% concentration of CO₂ and four weeks of incubation. The MIC value for itraconazole, voriconazole and amphotericin B, were 16µg/ml, 2µg/ml and 16µg/ml, respectively in *A.fumigatus* and 8µg/ml, 2µg/ml and 16µg/ml in *A.flavus*. These values for control groups were 0.125 µg/ml, 0.125 µg/ml and 2 µg/ml, respectively in *A.fumigatus* and 1µg/ml, 0/5µg/ml and

2µg/ml in *A.flavus*. Also, were not observed significant point mutation in the sequences of gene *Cyp51A* in *A.fumigatus* and *Cyp51C* in *A.flavus*. The results of study, were showed both increase and decrease in the expression in the genes *Cyp51A,B* in *A.fumigatus* and *Cyp51C* in *A.flavus*, concomitant with B-actin test, in the compared to control cultures.

Conclusion: Exposure to different CO₂ concentrations inducted morphological changes and a significant increase the MIC values with increasing expression and without mutation in the *Cyp51A,B,C* genes of in *A.fumigatus* and *A.flavus*, as well. In parallel, resistance to both itraconazole and voriconazole was also observed.

Key word: *Aspergillus fumigatus*, *Aspergillus flavus*, Voriconazole, Itraconazole, Carbon dioxide

P-085

High Prevalence of *Prototheca* spp. in Milk Samples from Cows Suffering from Mastitis in Mashhad city, northeast Iran

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Introduction: *Prototheca* are colorless algae that can cause mastitis in dairy cattle. They are widespread in housing areas, pens and pastures used by dairy cattle. Most infections are clinical and remain as chronic infections. *Prototheca* are often associated with wet areas containing decaying manure and plant matter. So, the aim of this survey was to identify, isolate and determine the frequency of the *Prototheca* spp. in milk samples from cows suffering from mastitis in Mashhad city, northeast Iran.

Material and methods: The milk samples were obtained from 400 dairy cattle with clinical and subclinical mastitis from 10 dairy 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

cattle herd in suburb of Mashhad, Iran. All samples were cultured in Sabouraud dextrose agar containing chloramphenicol. Plates were then incubated aerobically at 27°C and examined daily for a 7-day period. *Prototheca* colonies were identified on the basis of macro- and micromorphological characteristics, and on the basis of physiological profile.

Results: Of the 400 samples, 93 (23/25%) were positive for *Prototheca* spp. Our results are considered to be the first report on the high prevalence of *Prototheca* spp. in milk samples from bovine mastitis in Mashhad, Iran.

Conclusion: Protothecosis is a zoonotic disease, which can be transmitted to the human by consuming milk and cause intestinal infections and enteritis because of its resistance to pasteurization. As a result, it is important and crucial to consider and identify these microorganisms in milk, because they can be potentially harmful to human and animal health.

Keywords: *Prototheca* spp., Colorless algae, Bovine mastitis, Protothecosis, Milk samples

P-086

Survey on prevalence rate of fungal and algal species in dairy cows with clinical and subclinical mastitis

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Introduction: Mastitis in cattle is a serious problem which causes considerable economic losses in dairy cattle herds. The most common etiological factors are bacteria followed by mycoplasmas, viruses, fungi and algae. Studies on fungal and algal infections of the mammary gland in cows are increasingly common due to their growing

incidence. So, the aim of this study was conducted on prevalence rate of fungal and algal species in dairy cows with clinical and subclinical mastitis.

Materials and methods: A total of 400 milk samples were collected from cows with clinical and subclinical mastitis from 10 dairy cattle herds in suburb of Mashhad, Iran. All samples were inoculated in Sabouraud dextrose agar supplemented with chloramphenicol at 28 °C for 10 days. The isolates were identified according to their morphological characteristics and biochemical profile.

Results: Fungal and algal contamination was detected in 35.7% examined samples. It was shown that milk samples of cows with clinical and subclinical mastitis were contaminated with 7 different fungal and algal agents. Among all fungi and alga isolated from milk samples, *prototheca* spp. (23.25%) was the most dominant followed by yeast spp. (7.5%), *Aspergillus* spp. and *Penicillium* spp. (2% each), *Cladosporium* spp. (1/5%), *Trichosporon* spp. (1.25%) and *Geotrichum* spp. (0/7%).

Conclusion: It is concluded that fungal and algal infections can occur in mammary glands of lactating Holstein dairy cows with mastitis. Good hygiene and sanitation practices of animal farm and judicious use of antibiotics will lower incidence of bovine mycotic mastitis.

Keywords: Fungal and algal contamination, Mammary gland, Milk samples, Mastitis

P-087

Distribution of airborne fungi from outdoor air of different areas of Isfahan municipality

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Introduction: Opportunistic fungi are responsible for the majority of fungal infections in human and animals. The airborne spores of some allergenic fungi such as *Aspergillus*, *Alternaria* and *Cladosporium* are found throughout the world and considered to be important causes of allergic diseases. Identification of fungi due to its close relationship with fatal disease risk factors such as lung disease is very important. The aim of this study was to investigate the environmental frequency of different saprophytic fungi in various sites.

Materials and methods: The sampling was taken for six months period. Totally 210 air samples were obtained from outdoor air of 14 municipal areas of Isfahan. An open plate method was applied for air sampling by exposing 90 mm settle plates containing Sabouraud dextrose agar and malt extract agar supplemented with chloramphenicol to the air for 30 min. All samples were inoculated at the same media and incubated at 28°C for 2-3 weeks. The isolated fungi were purified and detected at the genus level based on morphological and microscopic features according to standard methods.

Results: The genus *Aspergillus* (22.10%) was the most frequent isolate from air samples, followed by *Alternaria* (18.94%) and *Cladosporium* (14.76%). The lowest frequency was related to *Fusarium* (1.57%), *Stemphylium* (1.57%) and *Epicoccum* (1.05%).

Conclusion: Monitoring of fungal spore distribution in different places would provide valuable data to evaluate human health risk; therefore it is important to identify the different genera of airborne fungi and detection of their population in public areas.

Keywords: Airborne fungi, Opportunistic fungi, Outdoor air.

P-088

Molecular identification of *Candida* species isolated from Vulvovaginal candidiasis patients in Yasuj

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Introduction: Vulvovaginal candidiasis (VVC) is the frequent infection in women at reproductive age. Furthermore, the most common causative agent is *Candida albicans* but in recent years the incidence of non-*albicans* species have arisen. The main aim of this study was the isolation and identification of various *Candida* species isolated from vulvovaginal candidiasis patients by PCR-RFLP in Yasuj, Iran.

Materials and Methods: Three hundred and ten suspected women with vaginitis were sampled and examined. Genomic DNA was extracted from fresh colonies by phenol chloroform glass bead methods. PCR amplification was performed based on the ribosomal DNA internal transcribed spacer (*rDNA-ITS*), and specific electrophoretic patterns of PCR products after digestion with *MspI* enzyme used for species identification.

Result: The cultures were positive for 160 (51.6%) vaginal samples. *Candida albicans* 86.8% (n=139) was the most common species among the isolates followed by *C. glabrata* 3.77% and *C. krusei* (3%). Eight patients were identified as having two species of *Candida*.

Conclusion: Vulvovaginal candidiasis is more prevalent among women in Yasuj and the predominant agent is *C. albicans*. In addition correct identification of *Candida* species can play an important role in management and treatment of VVC.

Key words: Vulvovaginal candidiasis, *Candida albicans*, Infection

P-089

Evaluation of fungal culture in 123 patients with Rhino-Orbito-Cerebral Mucormycosis

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Introduction: Rhino-orbito-cerebral mucormycosis is an invasive and opportunistic fungal infection caused by fungal spores. Delay in diagnosis and treatment is associated with increased mortality. Although often described as a rare fungal infection, zygomycosis (mucormycosis) appears to be increasing in frequency. It mainly affects immunocompromized patients, patients with diabetes mellitus. Taking into consideration the challenges related to diagnosis of zygomycosis, we worked on mucormycosis in east north of Iran. We present here the results of 14 years of this effort.

Materials and methods: This cross sectional study was conducted during 14 years in patients with rhino-orbito-cerebral mucormycosis in two teaching hospital in Mashhad, Iran. The criteria for diagnosis of fungi / cancer research and treatment in Europe (EORTC / MSG) were used to define

mucormycosis cases. Factors were analyzed by COX regression model in SPSS.

Results: Of the 123 patients with mucormycosis, 92 cases were proven. The mean age was 45 ± 21 years. 61 patients (49.6%) were male. For 12 patients had done smear, 9 (75%) were positive. For 12 patients had done culture, 7(58%) were positive. For 4 cases reported slide culture, 3 (75%) were Mucor and 1 (25%) was Rhizopus.

Conclusion: Mucormycosis is an invasive fungal disease with significant mortality. Species of this family that cause disease in humans are mostly from the Rhizopus, Rhizomucor and Mucor. Symptoms and signs of the disease in the different species of these fungi are quite similar. Diagnosis of the disease is with clinical and paraclinical manifestations, direct smear, tissue sampling and culture. It is recommended that, in any invasive fungal sinusitis infection, the patient should be questioned regarding all risk factors and the use of immunosuppressive drugs. Then a careful examination is done and radiography will be performed. And then, based on pathological studies and cultures, the patient can be divided into definite, probable and possible levels. According to the patient's condition, biopsy is performed and sent for culture and histopathology.

Keywords: Mucormycosis; Rhinocerebral mucormycosis; Amphotricin B.

P-090

Investigation and identification of bacterial and fungal micro flora of educational laboratories of a higher education center and its relation with hand hygiene

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Introduction: Hand hygiene is the most important and effective criteria to control and prevent the spread of bacterial and fungal microorganisms in all societies, including in educational laboratories. However, the degree of hygiene observance is challenging by faculty, staff and students. In addition implementation of a multi-faceted hand hygiene program in the field of educational environments requires the serious effort of executive directors to support and educate all students. It also needs encouragement to reduce the incidence of infections.

Materials and Methods: Sampling from the surface of laboratory No. 1 tables, before and after disinfection of surfaces with 70% ethanol, was done by sterile swab. Then the samples were cultured in TSB medium to grow bacteria. In the next stage, Blood Agar and Chocolate Agar media were used to grow bacterial colonies. Sabouraud dextrose agar plus chloramphenicol was used for culture of fungi. Furthermore, several plates containing fungi medium were placed in different parts of the laboratory. Then the plates were incubated at temperatures of 30 and 37°C. Cultures were checked on a daily basis and specific colonies identification were done by using direct methods, staining, slide culture and experiments.

Results: The results showed that the most contamination was related to gram-positive bacilli, so that the number of colonies of these species in the culture of samples given before and after disinfection was 10 and 1, respectively. In another sample, six colonies of gram-positive cocci were observed. However, this colony was not observed in the sample taken after disinfection. Which indicates the destruction of the bacterial agent as a result of disinfection. Also, the study of fungal contamination showed that *Aspergillus flavus* and *Penicillium spp.* were present on working table and air, respectively.

Conclusion: Considering the different and diverse methods of microorganisms' transmission, the results of this study showed that the observance of hand hygiene and hand washing by students and all individuals can effectively prevent the transmission of microorganisms. The study also found that the use of alcohol quickly destroyed microorganisms and had the potential for degeneration of proteins. Alcohol effectively eliminates all types of fungi. In this regard, alcohol is an effective fungicide

Keywords: Micro flora, Bacteria, Fungi, Hand hygiene, Antiseptic

P-091

Identification of Non-dermatophyte fungi as agents of onychomycosis by mycological and molecular methods in Mashhad

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Introduction: Onychomycosis is a fungal infection of nails caused by dermatophytes, yeasts and non-dermatophyte molds. It is responsible for 50% of all nail disorders. Although dermatophyte infections are more commonly discussed in the literature, non-dermatophyte fungi have become increasingly prevalent as etiologic agents of onychomycosis. The causative agents of non-dermatophyte onychomycosis may vary based on geographic or temporal distribution. The aim of this study was to identify non-dermatophyte fungal agents of onychomycosis in Mashhad by molecular methods.

Materials and Methods: A total of 280 patients clinically suspected of having onychomycosis who were referred to medical mycology laboratories of Imam Reza Hospital, Mashhad University of Medical Sciences were prospectively studied. Nail

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clipping were collected from the clinically abnormal nails. After microscopic examination, Clinical materials were inoculated on Sabouraud dextrose agar containing chloramphenicol with and without cycloheximide. The cultures were incubated in 25°C and 37°C for 4 weeks and checked twice weekly. Initial identification was done based on conventional methods. After DNA extraction, polymerase chain reaction sequencing technique was done for identification of fungal species.

Results: Of the 280 patients examined, 112 (40%) revealed positive fungal growth. *Candida* species accounted for 52 (46.5%) of total culture positive cases. Non-dermatophyte molds 50 (44.6%) and dermatophyte 10 (8.9%). Female affected more frequently than male. Among the candida onychomycosis fingernails were affected more frequently than toenails, but in non-dermatophyte molds cases toenails were affected more frequently than fingernails. *Candida albicans* with 38.5% and *Candida parapsilosis* 26.9% were the most common *Candida* species followed by *C. tropicalis*, *C. orthopsilosis*, *C. glabrata* and *Meyerosyma (Candida) guilliermondii*. The most common etiologic agent of non-dermatophyte molds onychomycosis was *Aspergillus flavus* 19 (38%) followed by *A. terreus*, *A. tubingensis*, *A. sydowii*, *A. welwitschiae*, *A. minisclerotigenes*, *A. niger*, *A. amstelodami*, *A. jensenii*, *Penicillium citrinum*, *P. allisativii*, *P. crysogenum*, *Fusarium sudanense*, *F. proliferatum*, *F. globosum*, *Cryso sporium sp*, *Cladosporium sp*, *Acremonium sp*, *Sporothrix sp*, *Talaromyces sp*, *Preussia sp*, *Trichosporon sp* and *Debaryomyces sp*.

Conclusion: This study showed that the commonest causative agents of onychomycosis are non-dermatophyte fungi especially *Candida* and *Aspergillus* species and these non-dermatophytes have an important role in onychomycosis, therefore culture of clinical materials and definite identification of them are necessary.

Keywords: Non-dermatophyte fungi, Onychomycosis, *Candida*, *Aspergillus*

P-092

Recurrent Vulvovaginal Candidiasis; Predisposing Factors, Causative agents and Drug Susceptibility in Gonabad City, The Northeast of Iran

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Introduction: Recurrent vulvovaginal candidiasis (RVVC) is the second most common cause of genital tract infection in females, often caused by *Candida albicans*. Excessive use of fluconazole and other azoles is likely to cause the emergence of resistant species of *Candida*. The purpose of this research was to identify *Candida* isolates from RVVC, the predisposing factors and the antifungal effect of fluconazole against *Candida* isolates.

Materials and methods: In this descriptive study, 20 patients with confirmed diagnosis of RVVC were examined. Yeast isolates were characterized using mycological standard methods, including culture on Sabouraud dextrose agar medium; and identified by CHROMagar *Candida*, germ tube test and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods. The susceptibility of *Candida* isolates against fluconazole was determined by microdilution broth method.

Results: The mean age of patients was 29.43 ±4.63 years. *Candida albicans* was isolated from 100% of samples. The most common clinical sign of vaginal discharge (60%) was positive in women. There were statistically significant correlations between the frequency of childbirth and the reduction in disease, as well as the history of the use of antifungal drugs and the disease. Mean MIC and MFC values of fluconazole were determined for different strains of *Candida albicans*, about 45.38 µg / ml and 63 µg / ml, respectively.

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Conclusion: An intriguing point in our study was that *C. albicans* was isolates as the dominant species, while there was not any non-*albicans* isolates. All isolates of *C. albicans* were sensitive dose-dependent. In the present study, the majority of patients were users of contraceptive methods such as IUD and OCP, which considered be one of main risk factors for RVVC.

Keywords: Recurrent Vulvovaginal Candidiasis (RVVC), Fluconazole, Drug Susceptibility

P-093

Investigation of Frequency and Drug Resistance Pattern of Candida Species Isolated from Vulvovaginal Candidiasis in Gerash City

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Introduction: Vulvovaginal candidiasis (VVC) is an infection caused by *Candida* species that affects millions of women every year. Due to increased drug resistance, selection of appropriate drug has an effective role in controlling and improving the infection. The present study aimed to determine the frequency and drug resistance pattern of *Candida* species isolated from VVC in Gerash city.

Materials and methods: This cross-sectional study was performed on vaginal samples of 268 patients referred to Amir-al-momenin Ali hospital in Gerash city, during a six-month period from March to August 2018. All samples were examined direct microscopic and cultured on Sabouraud dextrose agar medium with chloramphenicol. *Candida* species were identified using standard phenotypic tests and sugar assimilation test (API20C). The drug resistance pattern was investigated by Kirby-

Bauer method. Chi-square test was used for data analysis.

Results: Out of 268 vaginal samples, 79 cases (29.47%) were positive for *Candida* species, among them 48 *Candida albicans* strains (60.75%), 16 *Candida glabrata* strains (20.25%), 12 *Candida parapsilosis* strains (15.18%), and 3 *Candida tropicalis* strains (3.79%) were isolated. The most and the least drug resistance of *Candida* species was observed to Fluconazole (64.55%) and Amphotericin B (6.32%), respectively.

Conclusion: The VVC caused by *Candida albicans* is more common in comparison with non-*albicans Candida* species. For the initial treatment of vulvovaginal candidiasis, the use of Amphotericin B and caspofungin drugs is recommended.

Keywords: Vulvovaginal candidiasis, Drug Resistance, *Candida Species*, Gerash

P-094

Molecular detection of *candida* spp. isolated from immunocompromised patients in educational hospitals of Kerman

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Introduction: *Candida* species are the important opportunistic fungi. The mentioned yeasts are abundant and can cause a wide variety of human infections, known as candidiasis. The goal of this study, was to identify colonized *Candida* species in immunocompromised patients. The identification of *Candida* isolates are necessary to obtain epidemiological data and avoid therapeutic failure.

Materials and Methods: In this cross sectional study, samples were collected from mouth of 43 immunocompromised patients of educational hospitals (Including Afzalipour, Bahonar and Shafa hospitals) in Kerman, Iran. A total of 43 patients participated in this study, 258 yeast strains isolated from specimen oral swabs that

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identified by conventional methods contains Sabouraud dextrose agar and CHROM agar candida medium, germ tube production and assessing the morphology on corn meal agar. Their identity was confirmed by the PCR-RFLP method.

Results: Of these 258 yeast strains isolated, *Candida albicans* was the predominant species (n=159, 61.62%) followed by *C. glabrata* (n=74, 28.68%), *C. parapsilosis* (n=18, 6.97%), *C. krusei* (n=3, 1.16%), *C. kefyr* (n=2, 0.77%) and *C. lusitaniae* (n=2, 0.77%).

Conclusion: The results showed that immune-deficiency is a favorable condition for growth of *Candida albicans* and non-*albicans Candida* species. Oral candidiasis is mainly caused by *Candida albicans*. However, non-*albicans Candida* species have been implicated in colonization of the oral cavity, eventually causing infection in 20–40% of immunocompromised individuals.

Keywords: *Candida* spp, Immunocompromised, RFLP-PCR

P-095

Evaluation of fungal air contamination in selected wards of two tertiary hospitals in Tehran, Iran

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Introduction: Fungi have a worldwide distribution which can cause a broad spectrum of disease ranging from allergic to systemic infections, particularly in immunocompromised individuals. Fungal spores are an important group of bioaerosols in hospital environment which are an emerging cause of hospital-acquired infection. Nosocomial infections cause significant morbidity and mortality as well as large financial burden on the healthcare system. This study aimed to evaluate the frequency and species distribution of

airborne fungi in selected wards of two tertiary hospitals in Tehran, Iran.

Materials and Methods: In this cross-sectional study, samples were collected during six months from July 2016 to December 2016 by using of settle plate method. Samples were collected from selected wards of Imam Khomeini Hospital and Children's Medical Center and then incubated at 28 °C for 8-10 days. Fungal isolates were identified using the macroscopic features of colony and microscopic characteristics in slide cultures. Yeast isolates were identified by CHROMagar candida medium. PCR-sequencing of *ITS1-5.8 S-ITS2* region of ribosomal DNA was used for identification of unknown isolates.

Results: A total of 202 colonies including 133 colonies from Imam Khomeini Hospital and 69 colonies from Children's Medical Center were isolated. *Cladosporium* spp. were the most common obtained fungi accounted for 30.1% and 47.8% of all isolates in Imam Khomeini Hospital and Children's Medical Center, respectively. *Penicillium* spp. and *Aspergillus* spp. were other frequent species in two hospitals. Infectious diseases ward in Imam Khomeini hospital and emergency and urology wards in Children's Medical Center had the highest rate of contamination.

Conclusion: According to the results of this study, the frequency and diversity of fungal spores in hospital wards were different. In addition, since the fungal contamination in the hospital environment are affected by various environmental factors and the efficiency of ventilation systems, some of these wards require better ventilation system as well as regular monitoring to remove these fungal bioaerosols in order to maintain the health of patients and health care workers.

Keywords: Air, *Aspergillus*, *Cladosporium*, Fungi, Hospitals, *Penicillium*.

P-096

Characterization and identification of candiduria due to *Candida* species in diabetic patients.

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Introduction: The presence of *Candida* yeasts in urine, known as candiduria, is an indicator of infection or colonization of the urinary tract by *Candida* species. This condition in diabetic patients can be hazardous due to diminished immune system response. The objective of this study was to investigate the incidence of candiduria in diabetic patients and to identify its causative agents. Furthermore, the demographic and laboratory (HbA_{1c}, urine glucose and pH, urine culture colony count, and fasting blood sugar) data and their possible associations with candiduria were investigated.

Materials and methods: This cross-sectional, descriptive study was performed on 305 diabetic patients referred to the diabetes research center, Hamedan, Iran, during April 2015 to September 2015. Urine and blood specimens were collected and urine analysis, urine culture, FBS, and HbA_{1c} tests were performed. Positive cases were subjected to colony count and the causative agents were subsequently identified through the routine identification tests, as well as colony color in CHROMagar Candida medium, and the assimilation patterns in API 20 C auxanographic method.

Results: Among the 305 cases, 38 (%12.5) were positive for candiduria. Causative agents were identified as *Candida glabrata*

(n=19, 50%), *C. albicans* (n=12, 31.6%), *C. krusei* (n=4, 10.5%), *C. tropicalis* (n=2, 5.3%), and *C. kefyr* (n=1, 2.6%). According to the results of the statistical analyses, there were significant association between candiduria and female gender, high FBS and urine glucose, uncontrolled diabetes (HbA1c \geq 8), and acidic urine pH ($P < 0.05$).

Conclusion: Considering the high incidence rate of candiduria in diabetic patients, control of diabetes, predisposing factors, and causal relationships between diabetes and candiduria should be highlighted.

Keywords: *Candida*; Diabetes; HbA1c; Urinary tract infections

P-097

An overview on epidemiology, causative agents and demographic features of onychomycosis in Iran

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Introduction: We systematically reviewed all available literature concerning the prevalence of onychomycosis and the distribution of pathogens causing onychomycosis across Iran from 2000 to December 2017.

Materials and methods: The literature search was based on keywords including “onychomycosis and yeasts and Iran”, “onychomycosis and dermatophytes and Iran”, “onychomycosis and saprophytes and Iran”, “tinea unguium and yeasts and Iran”, “tinea unguium and dermatophytes and Iran”, “tinea unguium and saprophytes and Iran”. Databases searched using data from MEDLINE (PubMed), EMBASE, Web of Science, Scopus, google scholar, ScienceDirect, the Iranian Research Institute for Information Science and Technology

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(IranDoc) and the Scientific Information Database (SID) and Cochrane Library.

Results: Literature search revealed 305 studies, of which 27 studies met the inclusion criteria. The highest prevalence of onychomycosis was related to Mazandaran and Tehran province respectively. As in the literature hypothesized shift in causative agents from yeasts to dermatophytes and/or moulds could not be confirmed. Females were affected more frequently than males and in both sexes those most infected were at the ages of >50 years.

Conclusion: The epidemiological data collected may be useful in the development of prevention and educational strategies. It seems the highest prevalence of onychomycosis in Mazandaran and Tehran provinces is due to the presence of more specialists and doing more studies concerned with detecting this disease in these areas. Therefore, further educational strategies in order to accurate diagnosis in other provinces is necessary to reduce the risk of onychomycosis in Iran.

Keywords: Onychomycosis, Epidemiology, Iran.

P-098

A one-year survey of superficial mycotic and pseudomycotic infections in patients referred to Medical Mycology Laboratory of Tehran University of Medical Sciences, Tehran, Iran

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Introduction: Superficial mycotic and pseudomycotic infections are an important public health problem. Their causative organisms include dermatophytes, yeasts and non-dermatophyte molds. Skin mycoses now affect more than 20-25% of the world's

population, making them one of the most frequent form of skin infections. The purpose of this study was to evaluate the prevalence and causative agents of Superficial mycotic and pseudomycotic infections in patients referred to medical mycology laboratory of Tehran University of Medical Sciences.

Materials and Methods: 758 patients suspected to tinea corporis, tinea cruris, tinea capitis, tinea faciei, tinea pedis, tinea manuum (other than onychomycosis), tinea versicolor, erythrasma and cutaneous candidiasis from March 2017 to March 2018 were referred to medical mycology laboratory of Tehran University of Medical Sciences for direct examination, fungal culture and identification based on conventional techniques.

Results: The results showed that from 758 patients suspected to superficial mycotic and pseudomycotic infections 234 cases (30.8%) were positive for these infections. Dermatophytosis with 150 cases (64.1%) was the most common infection among these mycosis, followed by cutaneous candidiasis with 50 cases (21.3%), Erythrasma with 20 cases (8.3%) and tinea versicolor with 14 cases (6%). In addition tinea pedis (27.3%) had the highest frequency among patients with dermatophytosis in this study. Among dermatophytes, *Trichophyton mentagrophytes* was found to be the most common etiological agent (37.3%) followed by *Trichophyton tonsurans* (18%) and *Trichophyton rubrum* (14.6%). Also this study showed that the age group of >60 years old was more affected (18%).

Conclusion: This study showed that the most common isolated agent from superficial and cutaneous infections was *T. mentagrophytes*. Since this dermatophyte has antropophilic and Zoophilic species, people should be aware of the danger of acquiring this infections from infected persons and animals. So this study suggests that further measures regarding public health and especially personal hygiene should be undertaken to reduce the risk of superficial mycotic and pseudomycotic infections.

Keywords: Superficial mycotic infections, Pseudomycotic infections, Iran.

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Candida auris, a new emerging fungal monster

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The incidence of invasive fungal infections (IFI) caused by unusual use to rise, driven in part by increased populations of immunocompromised *Candida* spp. The emerging multidrug-resistant yeast pathogen *Candida auris* (Auris means “ear” in Latin) has attracted considerable attention as a source of healthcare-associated infections. The isolates are often multi-drug resistance (MDR), with some strains having high MICs to drugs in all the three major classes of anti-fungal medication and are difficult to identify with standard laboratory methods. Many of these isolates have been misidentified as *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, or *C. haemulonii*. Identification requires specialized methods such as molecular identification based on sequencing the D1-D2 region of the 28s rDNA or Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF). Misidentification may lead to unsuitable treatment and in finally, *C. auris* has the tendency to cause outbreaks in the healthcare settings, as has already been reported from several countries worldwide.

Keywords : Fungal infections, *Candida auris*, MDR, MIC, MALDI-TOF, D1-D2 region, 28s ribosomal DNA

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Animal Dermatophytosis in Nigeria: Review of literature from 1980 to 2018

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Introduction: Zoonotic fungal infections can be naturally transmitted from animals to humans and in some cases have serious economic and public health consequences. Apart from using domestic animals to aid in farming and transportation, there is a rising interest in keeping animals as pets in Nigeria and this highlights the possibility of transmission of zoonotic infections to humans. Dermatophytosis constitutes a considerable part of cutaneous fungal infections that can easily and naturally be transmitted from infected animals to humans and this receives little attention in Nigeria.

Materials and methods: In this review we searched English electronic database (PubMed, Web of Science and Embase) and Google Scholar for publications between 1980 and 2018, on zoonotic fungal infections in Nigeria. Important keywords in the search were but not limited to 'dermatophytosis in animal', 'zoonotic fungal infection', 'animal dermatophytosis' with Boolean operator 'OR' used, while operator 'AND' was used with 'Nigeria'. The duration of publication was specified to narrow the search results. In addition, we also searched database of Nigerian local medical, dermatology and veterinary journals. The available publications were then reviewed, analyzed and summarized.

Results: Our finding revealed that the prevalence of dermatophytosis among big domestic animals (horses and cattle) was between 10.9% and 85%. The frequency among small domestic animals (goats, sheep, dogs, and pigs) and domestic birds (chicken, ducks, turkeys and pigeons) was in the range of 1.5% -13%. *Trichophyton verrucosum* was the predominant dermatophyte isolated (35.7% - 100% in various studies) from big 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

animals. *Microsporum gypseum* was the predominant isolates from small domestic animals and birds. *T. mentagrophytes* was the second leading isolates in all animals except the horses.

Conclusion: The frequency of dermatophytosis in animals is considerably high in Nigeria especially among horses and cattle. Interestingly, *T. verrucosum* was the predominant dermatophytes among horses. Minimizing public contact with animals shall be important step towards lessening zoonotic transmission of dermatophytes.

Keywords: Animal dermatophytosis, Zoonotic fungal infection, zoonosis, Dermatophytosis, Nigeria.

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Antifungal activity of ethanolic extract of propolis and *Trachyspermum ammi* essential oil on *Mep3* gene expression of *Microsporum canis* isolates

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Introduction: *Microsporum canis* is the main etiologic agent of dermatophytosis in dog and cat, also is the most common dermatophyte infection in human. The secretion of proteases by dermatophytes such as *M. canis* are important virulence factor. Dermatophyte secrete endo and exoproteases. endoproteases are member of two protein families, the subtilisin (serine proteases) and fungalyisin (metalloproteases). These proteases are responsible for adhesion to stratum corneum. *Mep3* is known as metalloproteases of fungalyisin family that secretes during dermatophyte infection due to *M. canis*. The regulation of expression of this gene (*Mep3*) is important for invasion to

target tissue. Antifungal effect of *Trachyspermum ammi* essence and ethanolic extract of propolis have been shown, because of thymol and flavenoid respectively. In this study, antifungal effect of *Trachyspermum ammi* essence and ethanolic extract of propolis carried out on 25 *M. canis* isolates. Also the level of *Mep3* expression during of the *Trachyspermum ammi* essence and ethanolic extract of propolis treatment were evaluated.

Material and methods: For determination of (Minimum inhibitory concentration) MIC and (Minimum fungicide concentration) MFC macrodilution broth method were used. On the other hand the effect of these component on changes macroconidia morphology were established by microscopic examination. *Mep3* expression was assessed before and after these component treatment by using Soy Peptone Medium as a promoter proteolytic activity and incubated at 30°C for 10 days. Then, RNA extracted by RNA extraction kit and RT-PCR was performed with gene *Mep3*.

Results: The result showed the inhibitory effect of *Trachyspermum ammi* essence and ethanolic extract of propolis on fungal growth. The MIC range of *Trachyspermum ammi* essence and ethanolic extract of propolis were 0.2-30 µg/ml and 0.2- 488 µg/ml, respectively. Statistical analysis showed a significant difference between MIC *Trachyspermum ammi* and MIC propolis ($P \leq 0.05$). RT-PCR results showed that *Mep3* gene expression in samples that was not affected by plant essential oil and propolis, were expressed, but in sample which exposed to the plant essential oil were not expressed.

Conclusion: This study reported that *T.ammi* essential oil and ethanolic extract of propolis inhibits the expression of *Mep3* gene in *M. canis* isolates in regard to considerable prevention in protease production by the fungus.

Keyword: *Microsporium canis*, Metalloprotease, *Mep3*, *Trachyspermum ammi*, Propolis

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Saccharomyces boulardii*: As probiotic for control of *Candida albicans

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Introduction: Probiotic is a product containing enough amount live and specific microorganisms that changes microbial flora through placement or colonization in the specific parts of body, and thus has beneficial effects on host health. *Saccharomyces boulardii*, non-pathogenic yeast, are used as probiotics in prevention and treatment of diarrhoea. In several studies were approved that this yeast inhibit *Candida albicans*.

Materials and methods: In this paper, previous researches on the effect of *S.boulardii* against *C.albicans* had been investigated.

Results: In 1920, when all India and China were involved with the cholera epidemic, Henri boulard noticed that some residents do not experience symptoms of cholera through chewing the skin lychee and mangosteen. This led to the isolation of the yeast from the lychee and mangosteen fruits called *Boulardii* and is currently the only commercial yeast probiotic. *S.boulardii* is similar to *Saccharomyces cerevisia* but lack the ability to penetrate tissue and invasion. *S.boulardii* is also incapable of forming spores. Hence, its transmission to other parts of the body decreases. The most significant difference between these yeasts is the super-high growth of *S.boulardii* at 37°C, which is proportional to human body temperature. Live cells and filtered culture extract of *S.boulardii* has reduced the adhesion and production of *C.albicans* biofilms on polyester plates. Realtime PCR method showed that *HWPI*, *INO1* and *CSHI* (genes associated with *C.albicans* virulence) genes expression decreased in the isolates treated with *S. boulardii* extract. Both the cells and the extract of *S.boulardii* inhibited the binding of *C.albicans* to CaCO₂ cell lines. Also, the expression of *IL8* gene in CaCO₂ infected with *C.albicans* after addition saccharomyces. *S.boulardii* reduces inflammation and colonization of *C.albicans*

in mice. Antifungal susceptibility pattern of *C.albicans* to Ketoconazole and Itraconazole changed after treatment with *S.boulardii* extract. The *SAP2* relative expression level was significantly downregulated after the exposure to *S. boulardii* extract. Live *S.boulardii* and its extract inhibited the hyphal and pseudohyphal formation in *C.albicans*. *S.boulardii* extract did not show any fungicidal and inhibitory effects against *C. albicans* isolates .

Conclusion: Since *S. boulardii* extract did not show any fungicidal and inhibitory effects against *C. albicans*, it can be considered as a suitable probiotic candidate to control and treat *C.albicans* infections.

Key words: *Saccharomyces boulardii*, *Candida albicans*, Probiotic

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Molecular characterization of *Candida dubliniensis* and *Candida albicans* in the oral cavity of drug abusers using duplex polymerase chain reaction

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Introduction: *Candida dubliniensis* is closely related to the most pathogenic and prevalent yeast, namely *C. albicans*. *Candida* species can opportunistically overgrow in vulnerable individuals and cause a variety of diseases. The current study aimed to identify and isolate *C. dubliniensis* species present in the *Candida albicans* species complex identified in the oral cavity of drug abusers.

Materials and methods: This study was conducted on 53 strains of *C. albicans*

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species complex, isolated from the oral mucosa of drug abusers in Isfahan, Iran. DNA extraction was accomplished through boiling procedure. Duplex polymerase chain reaction (PCR) was performed to amplify *ITS1-5.8S-ITS2* region using four specific primers. Fungal species were identified based on the difference in the size of the bands created in the agarose gel.

Results: Out of the 53 isolates under study, 30 (56.6%) and 14 (26.4%) samples were identified as *C. albicans* and *C. dubliniensis*, respectively. In the remaining 9 samples (17%), both types of *Candida* species were confirmed.

Conclusion: The findings of the present study revealed the presence of a noticeable amount of *C. dubliniensis* in the oral cavity of drug abusers. Therefore, the probable presence of this fungus should be considered during the examination of oral infection among this group. To date, no research has directly investigated this issue in Iran.

Keywords: *Candida albicans*, *Candida dubliniensis*, Drug, PCR, Smoking

P-104

Introduction of an *Aspergillus* PCR assay to the clinical mycology service in Iran

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Introduction: *Aspergillus* species are most 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.

Martials and methods: In this study we developed a PCR-Single Strand Conformational Polymorphism method to

identify the most common *Aspergillus* species. Our subjects included *Aspergillus* clinical isolates of an educational hospital in Urmia, Iran. Also, some *Aspergillus* standard species which obtained from Japanese Collection of Microorganisms. All *Aspergillus* isolates were identified by 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 95°C for 5 min and then moved quickly into ice bath for an immediately quenching. A vertical electrophoresis with 6%-12% Gradient Poly Acrylamide Gel was used full time cooling at 4°C.

Results: As a result, some of tested *Aspergillus* species including *A. nidulans*, *A. fisheri*, *A. fumigatus* and *A. niger* discriminated. SSCP assay enabled us to identify above *Aspergillus* species within 8-12 h after overnight incubation.

Conclusion: It is concluded that Single Strand Conformational Polymorphism is a simple and rapid method for identification of some medically important *Aspergillus* but we recommend this as a compliment test with other molecular methods such as PCR-restriction fragment length polymorphism to cover identification of more *Aspergillus* species.

Key words: Rapid identification, *Aspergillus*, Clinical source

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Evaluation antifungal activity of novel synthetic nanoparticle drug against *Candida albicans* standard species

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Introduction: The pathogenic fungi can cause superficial and systemic disease in

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immunocompromised patients. The increased drug-resistant fungi oblige an application to develop novel antifungal agents. Therefore, discovering novel synthetic nanoparticle derived from indolicidin and graphene oxide pattern with suitable antifungal activity and minimum side effects is needed. The aims of this study were to evaluate and compare the sensitivity of standard species *Candida albicans* with graphene oxide -Indolicidin conjugates and Fluconazole drug *in vitro*.

Material and methods: Cationic antimicrobial peptides (CAMPs), Indolicidin, were conjugated to graphene oxide (Hummers method aided) using EDC-NHS conjugation protocol. FTIR and X-ray EDX were taken on lyophilized free graphene oxide and conjugated with IN, and dispersion on a silicon chip for Scanning Electron Microscopy. *Candida albicans* ATCC10231 strain was used and fungal suspensions were prepared at concentrations of 1×10^5 (CFU/ml). The minimum inhibitory concentrations (MIC) of drugs for this standard species were determined by broth microdilution assays according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Ranges included (200-0.39 μ g/m) for GO-IN, (128-0.25 μ g/ml) for FLU, (100-0.19 μ g/ml) for IN and (200-0.39 μ g/ml) for GO. Each test was performed in triplicate. Moreover, negative and positive controls were studied. Hemolytic activities of the nanocomposite and other samples on human RBCs were regulated by macro dilution analysis in tubes and tested with spectrometry method. For the cytotoxicity assay, intestinal EP cell lines were saved in the flask T25 were incubated at 37°C and 5% CO₂ for 2 hours. Confluent cells counts were also confirmed by hemocytometer counter and Trypan blue method. Intestinal EP Cells seeded in 96-well plates at a concentration of 10,000 viable cells per well before treatment. Then 200-3.12 μ g/ml of the Nanocomposite was added to each well 24 hours after dispersing. Next, the activity of mitochondrial dehydrogenase enzymes of the cells was detected in 24 hours by using the MTT test.

Results: Our results indicated the cell durability percentage at that 3.12µg/ml concentration of nanocomposite (Minimum inhibitory concentrations: MIC) was 60% and hemolytic activity at this MIC was 2.73%.

Conclusion: Generally, our results showed that this nanocomposite could be used as an acceptable cytotoxicity agent in the treatment of fungal diseases. However, the efficacy of this nanocomposite *in vivo* & *in vitro* should be investigated in future studies.

Keywords: *Candida albicans*, Indolicidin, Graphene oxide, Cytotoxicity, Hemolytic activity

P-106

Increased *Aspartyl* proteinase gene expression in *Candida albicans* during recurrent vulvovaginal candidiasis (RVVC)

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Introduction: Recurrent vulvovaginal infection caused by the opportunistic yeast *Candida albicans* is a significant problem in women during reproductive ages. Several factors are recognized as crucial factors in the pathogenesis of superficial candidiasis; these factors include yeast to hyphal phenotypic switching, and the expression of virulence factors, including a 10-member family of secreted aspartic proteinases. These enzymes are major virulence traits of *C. albicans* that have been suggested as a significant factor in vaginitis.

Material and methods: In this study, the *in vivo* expression of *Candida albicans* secreted aspartyl proteinase (*SAP1* and *SAP3*) genes was analyzed in 40 women with recurrent vulvovaginal candidiasis. Total RNA was isolated from vaginal swabs, and the expression of *SAP1* and *SAP3* was evaluated

by Real time PCR using specific primer sets which was repeated 3 times for validation. Finally SPSS software was use to analyze the statics.

Results: An increased expression of *SAP1* and *SAP3* genes was observed in RVVC patients in comparison to *ACT1* gene in Persian Type Culture Collection (PTCC) as a control. The results of these genes were statistically analyzed and they were significant (P-value<0.05).

Conclusion: Since *SAPs* are known fungal virulence factors in mucosal infections, we investigated the expression of *SAP* genes in an *in vitro* model of recurrent vulvovaginal candidiasis (RVVC) and in patient specimens to study the pathogenic role of their gene products during epithelial tissue damage. The data obtained from this study provides further evidence supporting the crucial role of *SAP1* and *SAP3* in *C. albicans* vaginal infections.

Keywords: Recurrent vulvovaginal candidiasis, *Secreted aspartyl poroteinase*, Real time PCR

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Investigation of *ALS3* and *HWPI* gene expression associated with adhesion in *Candida albicans* isolated from Iranian HIV-infected patients

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Introduction: Oropharyngeal candidiasis is one of most common mucocutaneous infections in the HIV positive patients and can

be seen in more than 90% in initial stages before treatment and advanced stages of AIDS. Two important genes including *HWP1* and *ALS3* contribute to adhesion of *Candida* species to mucosal membrane and epithelial cells. This study aimed to investigate *ALS3* and *HWP1* gene expression associated with adhesion in *Candida albicans* (*C.albicans*) isolated from HIV positive patients.

Materials and methods: One hundred fifty HIV positive patients who referred to HIV centers with clinical signs of oral candidiasis included in this study. Specimens were collected from oral lesions of subjects by sterile swab and then examined for direct microscopy, finally cultured on Sabouraud dextrose agar (SDA). Species level identification of yeast was performed by using both morphological and molecular methods (PCR and sequencing). mRNA of *C. albicans* isolates was extracted, cDNA synthesized and quantitative Real time-PCR (q-PCR) was carried out for *ALS3* and *HWP1* genes expression levels using specific primers.

Results: Out of 150 oral specimens, 90 samples were positive for *Candida* strains and 102 *Candida* species were identified definitely by molecular methods. The most species was *C. albicans* 54(52.9%) followed by 16 *C. dubliniensis* (15.7%), 12 *C. tropicalis* (11.8%), 9 *C. glabrata* (8.8%), 7 *C. kefyr* (6.9%) and 4 *C. africana* (3.9%). Real time PCR analysis showed in 53.3% and 86.7% of *C. albicans* strains, *ALS3* and *HWP1* genes expression significantly increased in comparison with housekeeping gene (*ACT1*) respectively (P<0.05).

Conclusion: Finding of this study showed that *C. albicans* is a major cause of oral candidiasis in HIV positive patients. Increased level of *ALS3* and *HWP1* genes expression indicated the key role of these genes in adhesion and pathogenesis of *C. albicans* in oral mucosal membrane of HIV patients. These genes contribute to biofilm formation and improve the pathogenesis of *Candida* in oral candidiasis which can invade the deeper tissue as well as cause of disseminated candidiasis that affect the surveillance of patients

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Keywords: Oral candidiasis, HIV, *ALS3* and *HWP1* adhesion genes, Real-time PCR

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Is *MBL* serum concentration a reliable predictor for recurrent vulvovaginal candidiasis?

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Introduction: Recurrent vulvovaginal candidiasis (RVVC) is a common opportunistic, mucosal fungal infection, predominantly caused by the fungus *Candida albicans*. Mannose-binding lectin (MBL) is an acute-phase protein that plays a key role in the innate immunity defense against infectious disease. The present study was conducted to evaluate the relationship between the MBL serum level and the relative expression of MBL mRNA in RVVC using real-time PCR for the first time.

Materials and methods: The case-control study included 40 female participants suffering from RVVC and 40 healthy individuals. The MBL serum level was measured using a commercial ELISA kit. The relative mRNA expression of the *MBL* gene was quantified using real-time PCR.

Results: The mean MBL concentration was significantly higher in participants in the RVVC group compared to those in the control group (0.330ng/ml versus 0.253ng/ml). The quantitative RT-PCR results showed a low to significant expression of mRNA levels in the *MBL* gene (1-352 folds) (P<0.001).

Conclusion: The results of the present study showed a direct relationship between the MBL serum concentration and the rate of RVVC indicating the observed differences in the levels of MBL between the two study groups may be related to the genetic alterations of MBL. The ELISA suggest that the MBL serum level can be considered a positive indicator of RVVC. In the present study, 35 clinical samples were found to have low to significant upregulation of MBL mRNA expression. In the samples with significant upregulation of MBL mRNA expression, the results of the MBL serum level were open to contradictory interpretations. It is likely that protein translational failure may have caused such a difference in gene/protein expression. Interestingly, five samples with low MBL serum levels showed no differences in MBL expression in comparison to the samples from the control group, suggesting two possible scenarios: 1- Occurrence of at least one or two polymorphisms in the promoter region of the *MBL* gene may suppress MBL mRNA expression and subsequently lead to the reduction of the MBL serum level. 2- Based on the in silico study, a regulatory potential protein interaction (PPI) was identified. The PPI revealed a direct interaction between the serine protease (MASP 1/2) and MBL in the activation of the complement pathway. Therefore, the *MBL* gene expression profile does not reflect a precise phenotypic level in the serum.

Keywords: Recurrent vulvovaginal candidiasis, Gene expression, MBL

P-109

Investigation of *Candida albicans* genotypic entropy plot in patients with clinical vulvovaginitis

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Introduction: *Candida albicans* are one of the most prevalent and important pathogens in patients with vulvovaginitis. Nowadays,

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different methods are used for *Candida albicans* strains. The aim of this study is obtaining correct typing of strains by using short tandem repeat or microsatellites and using the PCR-SCCP molecular method. Finally, using genotypes entropy plotting, better understanding of the points with high genetic diversity draw up.

Materials and methods: From 350 suspected cases of vulvovaginal disease, samples were prepared and cultured in SDA-chromium agar and Corn meal agar media. PCR-RFLP reaction on *ITS1-5.8s-ITS2* fragment with *MspI* enzyme was performed to detect *Candida* species and *MboI* enzyme for confirmation of *C.albicans* and *C.dubliniensis* species, and then PCR-SSCP was amplified and DNA was extracted. Locus *CAI* microsatellite was amplified. Using the MEGA6 software, the genotype gap matrix was calculated from the equilibrated file and then the entropy plot was analyzed using a balanced file to examine the entropy with bio edit software.

Results: Out of 350 patients, 100 isolated patients (60.6%) were related to *Candida albicans*. Based on different patterns, *CAI* fragments were amplified and compared with standard strains, 26 different genotypes were identified according to the spatial configuration and Genotypes I, Q, K, A were the most frequent and considered as the dominant genotypes. In the study of entropy plot, it was determined that the sequences with the highest diversity were related to sequences 71m, 82a, 85m, 96v.

Conclusion: The analysis of molecular experiments and the evaluation of entropy plot showed a high degree of unmatched equalization of the genotypes. In other words, if the more nucleotides are non-identical, the disorder in the region of the nucleotide sequence is greater and the changes are better. This study can be recommended for small evolutionary changes in microsatellites and the next large scale epidemiological studies.

Key words: *Candida albicans*, Entropy, PCR-SSCP

P-110

Efficacy of ozonated water on the inhibition of *Candida albicans* colonization and formation of a plaque on acrylic denture plates

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Introduction: One of the underlying causes of denture stomatitis is the formation of plaque on the dentures. Finding new and effective ways to eliminate or reduce the colonization of oral cavity microorganisms that potentially contribute to the formation of dental plaque is one of the issues discussed in the dental sciences. The aim of this study was to evaluate the effects of various concentrations of ozonated water on cleansing of the formation of *Candida albicans* plaque on the acrylic resin pieces.

Materials and methods: In this study, 45 pieces of acrylic resin were contaminated by *C. albicans* suspension. Then, the acrylic pieces were randomly divided into nine groups and treated with 0.2, 0.5, 1 and 2 µg/ml of ozonated water, 25 and 12.5 µg/ml of oleozone, 100,000 units of nystatin (positive control), distilled water and olive oil (negative control). At the end of the exposure period of the drugs, the rinse solution from acrylic pieces was cultured in SDA and the average of the colonies from each group was compared.

Results: The average number of colonies obtained at concentrations of 0.2, 0.5, 1 and 2 µg/ml of ozonated water was 24, 24.6, 23.6 and 14.4 colonies, respectively, as well as the average number of colonies obtained at concentrations of 12.5 and 25 µg/ml of oleozone was zero and 2 colonies respectively, that compared to distilled water (146.6) and olive oil (98.8) had a significant

difference ($p < 0.001$). In all groups, by increasing the concentration of the ozone, the number of yeast colonies decreased. However, oleozone showed a more inhibitory effect than ozonated water, so that There was no significant difference between two concentrations of oleozone (at 25 µg/ml $p = 1$ and at 12.5 µg/ml, $p = 0.477$) and nystatin group.

Conclusion: The results showed that appropriate concentrations of ozonated water have an antifungal effect on *C. albicans*. Although future laboratory studies promise hope for ozone therapy in dentistry. There is little clinical evidence in this regard, so it is recommended that more clinical studies be conducted to standardize and elaborate the guidelines for using ozone therapy.

Keywords: Ozone, *Candida albicans*, Complete denture, Denture stomatitis, Antifungal

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Molecular typing of clinical isolates of *Candida glabrata* by MLST and determination of drug resistance profile

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Introduction: *Candida glabrata* infections are associated with high mortality rates and there is a tendency to rapidly developing resistance to azole antifungal agents, especially fluconazole. Studies to comprehend the epidemiology and population structure of clinical isolates of *C. glabrata* are required. The goal of this study was genotype characterized of clinical isolates of *Candida glabrata* by multilocus sequence typing (MLST) technique to determination of the endemic prevalent

genotypes and any association between isolation source and drug resistance.

Materials and methods: A total of 50 *C. glabrata* clinical isolates from Tehran, Iran were analyzed by MLST. Nucleotide sequences were compared to the *C. glabrata* MLST database and new allele numbers were assigned to new sequences. Phylogenetic analysis by neighbor-joining algorithm based on p-distance were conducted using MEGA, version 5.2, applied to concatenated sequence data. Also STs were also analyzed using the eBURST package. Isolates were tested for *in vitro* susceptibilities to amphotericin-B, caspofungin, fluconazole and voriconazole using clinical laboratory standards institute (CLSI) M27-A3 and M27-A4 document guidelines.

Results: Among these isolates, 16 distinct STs were identified, indicating a discriminatory power index of 0.9029. The three major sequence types (*STs*) were *ST-59*, *ST-N5*, and *ST-7* with 10, 8, and 7 isolates, respectively. Furthermore, a total of 11 new sequences was found, to which no allele numbers were assigned in the MLST database. All sequences have been deposited in the GenBank database under accession numbers KX187005 to KX187304. The 50 *STs* were classified into 16 clusters by neighbor-joining method. Further analyses with eBURST shown that all clusters correlated with eBURST data. All the isolates were susceptible to amphotericin B and caspofungin. Fluconazole resistance was shown in four isolates in the total collection. Also, one isolate was voriconazole resistant. According to Fisher's exact test results, no significant differences were observed in the distribution of drug-resistant isolates among the genotypes and clades.

Conclusion: A clear understanding of the epidemiology of *Candida* infections and colonization requires an extensive typing studies by reliable methods. This study shows that the population structure of *C. glabrata* in Iran consists of clonal groups closely related to the global database as well as some new clonal clusters and *STs*. Regarding the high prevalence of 11 new sequences found in this study, it can be concluded that, this new

alleles are among the endemic genotypes to Iran. The genotypes or *STs* were independent of drug susceptibility and anatomic sources.

Keywords: *Candida glabrata*, Candidiasis, MLST, Genotyping, Drug resistance

P-112

Survey of expression of *Candida albicans* secreted aspartyl proteinase 9 in human vulvovaginal candidiasis

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Introduction: *Candida albicans* is classified as commensal fungi that inhabit the human gastrointestinal tract and can cause oral and vaginal infections as well as systemic diseases. Vaginal candidiasis is one of the most common infections that affect women of reproductive age. Approximately 75% to 79% of women will experience at least one episode of vaginal candidiasis during their lives. *C. albicans* possesses several virulence factors like secreted aspartyl proteases (saps) enzymes that are coded for by the *SAP* gene family (*SAP1-SAP10*). The proteolytic activity of the sap proteins is involved in the adhesion to the host's cells, the degradation of the host's barriers during infection and immune response evasion. The expression and importance of *SAP (1-3)* during murine vaginal candidiasis were demonstrated by using RT-PCR but the expression and importance of *SAP9* is not clear.

Material and methods: A group of 150 women (age 19 - 53 years) with vaginal infections were evaluated. *C. albicans* was identified using PCR to amplify the rRNA internal transcribed spacer regions *ITS1* and *ITS2*. The presence of the *SAP9* genes was determined using conventional PCR, and their expression levels were determined using real-time PCR.

Results: *C. albicans* was identified in the samples from 80 women (53.3%). The genotyping frequency of the *SAP9* gene was 70%.

Conclusion: Results presented in this study showed that the *SAP9* gene was expressed with 70% frequency. This expression level suggested that the *SAP9* proteins play an important role in the pathogenesis of vaginal infection.

Keywords: *Candida albicans*, *SAP9* Expression

P-113

A review on antifungal effects of silver nanoparticles on control and prevention of fungal hospital-acquired infections

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Background: Hospital-acquired, or nosocomial, infections have proven to be a persistent and sometimes tragic problem in which their symptoms occur in 72 hours after hospitalization and they cause longer hospitalization and cost increases that also may lead to death. Fungal infections make up 9% of all Hospital-acquired infections; they live in our natural flora and if the immune system weakens, they will cause disease as pathogens. Considering the increase in silver antimicrobial effect by using it in nanoparticle size; the aim of this study is to assess the antifungal effects of silver nanoparticles on control and prevention of fungal hospital-acquired infections.

Methods: To write this review article, articles found in PubMed, Clinical key, Google scholar, SID and Magiran using terms for "Silver", "Nanoparticles", "Antifungal", "Hospital-acquired Infections" and their synonyms in Farsi were conducted to identify the relevant studies. Finally 28 full

text articles were assessed and 19 articles that had more relationship with this study were used.

Results: According to the assessments, significant antifungal effects of silver nanoparticles have been proven; for example wall paintings containing silver nanoparticles can reduce the pollution caused by hovering mushroom spores in the hospital. The mechanism of antimicrobial action of silver nanoparticles is to disturb the membrane potential of fungal cells and to create pores in their cytoplasm membrane. The antifungal effect of silver nanoparticles is heightened by the increase of silver layer thickness or by combining with gold nanoparticles.

Conclusion: Silver nanoparticles are demonstrated useful as fungal hospital-acquired infection preventers. The extensiveness of nanotechnology and the widespread possibility of using silver nanoparticles in production of medical tools can reveal the benefit of using silver nanoparticles.

Keywords: Silver, Nanoparticles, Antifungal, Hospital-acquired infections

P-114

Intra-species genetic variability and *in vitro* antifungal susceptibility of 33 clinical isolates of *Arthroderma benhamiae* in Iran

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Introduction: *Arthroderma benhamiae* is a zoophilic dermatophyte species belonging to the *Trichophyton mentagrophytes* complex, which produces highly inflammatory tinea corporis and tinea capitis on humans. During the past few years, a constantly increasing number of *A. benhamiae* strains have been isolated in Iran from patients with dermatophytosis. *A. benhamiae* was divided into two races due to the phenotypic characteristics and mating behavior: American–European race and African race. Identification of this species using conventional methods is difficult and time consuming. The aim of the present investigation was to explore the genetic diversity 33 isolates of *A. benhamiae* according to internal transcribed spacer (*ITS*) Sequence and further define profile of *in vitro* antifungal susceptibilities against itraconazole (ITR), voriconazole (VRC), posaconazole (PSC), ketoconazole (KTZ), terbinafine (TER), griseofulvin (GRZ) and caspofungin (CAS).

Materials and Methods: A total of 33 clinical strains of *A. benhamiae* were isolated from patients with dermatophytosis from five cities: Shiraz (n=10), Mashhad (n=10), Ahvaz (n=8), Yasuj (n=4), and Tehran (n=1). All strains were sub-cultured on Sabouraud's dextrose agar (SDA) and DNA was extracted. The *ITS* rDNA region was amplified using universal primers *ITS1* (5–TCCGTAGGTGAACCTGCGG–3) and *ITS4* (5–TCCTCCGCTTATTGATATGC–3). The DNA sequences were compared using Clustal W multiple sequence alignment programs and a phylogenetic tree was constructed. The *in vitro* antifungal susceptibility of a set of clinical *A. benhamiae* isolates obtained from 33 tinea patients, using the CLSI-M-38A2 (Clinical and Laboratory Standards Institute) broth microdilution method to seven antifungal agents.

Results: Four different types of *ITS* sequence were found in 33 clinical *A. benhamiae*

isolates. With respect to the type of the city, the strains from Shiraz had all four *ITS* types, Ahvaz n=3, Yasuj n=2, and Mashhad had only 1 *ITS* type. The geometric mean (GM) minimum inhibitory concentrations (MICs) for ITC, VRC, PSC, KTZ, TER, GRZ and minimum effective concentrations (MECs) for caspofungin (CAS) across all isolates were as follows, in increasing order: TRB: 0.026 mg/L, PSC: 0.030 mg/L, ITC: 0.048 mg/L, VRC: 0.057 mg/L, CAS: 0.28 mg/L, KTZ: 0.59 mg/L and GRZ: 0.78 mg/L. The MIC/MEC ranges across all isolates were as follows: TRB: 0.008-0.125 mg/L, PSC: 0.008-0.125 mg/L, ITC: 0.016-0.125 mg/L, VRC: 0.016-0.25 mg/L, CAS: 0.125-0.5 mg/L, KTZ: 0.063-4 mg/L and GRZ: 0.125-4 mg/L. No statistically significant differences in the susceptibility profiles of *A. benhamiae* were detected within the geographical regions tested.

Conclusion: Our findings showed that the Iranian clinical *A. benhamiae* isolates had intra-species variation. The variations were found from different parts of Iran. The climate, ecology, and source maybe cause this different nucleotide sequences. Furthermore, Terbinafine, Posaconazole, Itraconazole and Voriconazole were shown to be the most potent antifungal agents against Iranian *A. benhamiae* strains obtained from tinea patients.

Keywords: *Arthroderma benhamiae*, *ITS* phylogeny, *In vitro* antifungal susceptibility, Iran

P-115

Preparation, *in vitro* characterization and antifungal efficacy of posaconazole loaded phospholipid based nanomicelles for topical ocular delivery

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Introduction: In ophthalmology, fungal infections of the eye can be an intractable clinical problem because of risk of corneal

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blindness or reduced vision and the limited number of therapeutic delivery systems. Efficiency of conventional products are limited by rapid drug release, low residence time and limited ocular bioavailability. Local delivery of antifungal drugs through nanoparticulate systems offers a promising therapeutic approach to reduce infections. Posaconazole is a second generation triazole with a broad antifungal spectrum and promising results in fungal infections. The aim of this study was to achieve an ameliorated nanomicellar formulation as a potential biocompatible carrier for local posaconazole delivery to overcome the limitations of the conventional dosage forms. **Material and methods:** Micelles were prepared from egg phosphatidylcholine (EPC) and d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS) by thin film hydration method. Different approaches (bath sonication and probe sonication) were evaluated for size reduction. Nanoformulations were characterized for entrapment efficiency (EE), particle size, zeta potential, *in vitro* release profile and morphology. The influence of various formulation variables such as the loading method (addition of drug to lipid mixture vs. remote loading approach), TPGS percentage (30, 50 and 70%) as well as lipid/drug ratio (L/D) (20, 25 and 30) on %EE, size, polydispersity index (PDI) and drug release profile was also studied. Antifungal properties of the optimum formulation were evaluated against *C. albicans* by zone of inhibition (ZOI) method.

Results: Bath sonication for 10 min and addition of drug solution to lipid mixture were chosen as the best sizing (Z average = 58-61 nm) and loading (EE >81%) techniques, respectively. Enrichment of micelles with 70% TPGS resulted in higher drug entrapment. Increasing L/D from 20 to 25 enhanced drug loading from 64% to 98%. Further increase in lipid concentration was unfavorable for more drug encapsulation. Optimized nanomicelles had spherical nanostructure and controlled drug release profile over 10 h. This formulation showed

significantly higher antifungal effect when compared to the drug suspension.

Conclusion: Results of this study showed that the prepared nanomicelles can be taken into account as a suitable antifungal nanocarrier for local posaconazole delivery.

Keywords: Posaconazole, Nanomicelles, Characterization, Antifungal effects

P-116

Dihydrofolate reductase gene mutations in Iranian strains of *Pneumocystis jirovecii*

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Introduction: Trimethoprim-sulfamethoxazole (TMP-SMX) is in the first line of anti-*Pneumocystis jirovecii* drugs. TMP-SMX inhibits the activity of two important enzymes dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) that are essential for producing folate. Worldwide reports indicate that mutations in DHPS and DHFR genes result in sulfa drug resistant in *P. jirovecii*. This is the first study in Iran to investigate mutations in DHFR gene of Iranian strains of *P. jirovecii*.

Materials and methods: DNA extracted from 25 respiratory samples which have been approved for presence of *P. jirovecii* previously. The *DHFR* gene of *P. jirovecii* isolates were amplified with a nested-PCR method. PCR products were sequenced and multiple alignments were carried out on each sequence to find any mutation in comparison with wild type sequences exist in GenBank. Presence of synonymous or non-synonymous mutations was investigated.

Results: *DHFR* gene amplification was successful in 24 out of 25 samples. Non-synonymous mutations A539G, C603G, G190A, C373T, T526A and G596T which lead to amino acid substitution Tyr180Cys, His201Gln, Gly64Ala, Pro125Ser, Cys176Ser and Gly199Val respectively were observed in three samples. Synonymous

mutations 312(T to C) were observed in 13 samples.

Conclusion: A high number of DHFR mutations in Iranian *P. jirovecii* isolates may be evidence of a possible resistance to sulfa drugs against the fungus and may contribute to therapeutic failure of *Pneumocystis* infections. This is a warning which needs more studies and monitoring.

Keywords: DHFR, Mutation, *Pneumocystis jirovecii*

P-117

Discrimination of homozygous and heterozygous strains among vaginal *Candida albicans* isolates

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Introduction: *Candida albicans* is one of the most common opportunist fungus around the world. It consists of two genotype strains named homozygote and heterozygote. There is no data about frequency of these strains. The aim of this study was discrimination of homozygous and heterozygous strains from vaginal *Candida albicans* isolates by amplification of *HWPI* gene.

Materials and methods: A total of 100 *Candida albicans* species was enrolled in this study. DNA was extracted by boiling method. Hyphal wall protein (*HWP*) gene was amplified by specific primers. PCR products were electrophoresed on agarose gel and fragments size were measured for discrimination of strains.

Results: After amplification of *hwp1* gene, heterozygous strains produced two fragments of 941 and 839 base pair but homozygote produce one fragment at 941bp. By this method, 24% of isolates identified as heterozygote strains.

Conclusion: In this study frequency of heterozygous strains of *Candida albicans*

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were less than the other strain and evaluation of virulence factors between two genotype differentiations.

Keywords: Homozygous, Heterozygous, *Candida albicans*, Molecular method

P-118

Antifungal properties of Graphene Oxide Silver nanocomposite on fungi isolated from wheat and corn stored in Khorramabad silos

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Introduction: One of the major causes of food contamination such as cereals is fungal infections. The main natural fungal flora in food sources is *Aspergillus*, *Fusarium* and *Penicillium*. When cereals such as wheat and corn are infected by molds, there is a significant risk of contamination by secondary metabolites, called mycotoxins. Mycotoxins are secondary metabolites derived from toxin-causing strains of various fungi which results in the loss of nutritional value and the creation of many biological effects and cause acute and chronic diseases in humans and animals. Therefore, considering the harmful and pathogenic effects of fungi and the development of fungal toxins, the aim of this study was to identify different fungal species using internal transcribed spacer (*ITS1*) sequencing and to investigate the antifungal properties of silver graphene oxide nanocomposite against fungal species identified.

Material and methods: In this study, 30 specimens containing 15 wheat samples and 15 corn samples were collected from the silos of the city of Khorramabad in the summer of 2017. Samples were cultured on Potato dextrose agar. After purification of fungi, their identification was based on morphological characteristics. Different species of fungi were identified by multiplication of *ITS* regions and their antifungal properties using microdilution Broth and minimum inhibitory concentration

(MIC) and minimum fungicidal concentrations (MFC) in different species compared to amphotericin B were determined.

Results: Out of 30 samples, 23 isolates were isolated that were morphologically matched with *Penicillium*, *Fusarium*, *Aspergillus*, *Epicocom*, and *Trichoderma* species. Species of fungi are based on *ITS1* sequencing *A. niveus*, *A. amstelodami*, *A. niger*, *A. sydowii*, *A. clavatus*, *P. oxalicum*, *P. oslonii*, *P. polonicum*, *P. chrysogenum*, *F. verticillioides*, *F. proliferatum*, *Al. alternata*, *Al. malorum*, *E. nigrum*, and *T.longibrachiatum*.

Conclusion: The sequencing of *ITS1* can solve the problem of fungal species such as *Alternaria*, *Penicillium*, and *Fusarium*, which are morphologically similar. Given that the production of high levels of fungal toxins can threaten the health of humans and animals, Therefore, the correct identification of fungi and the good results of anti-fungal properties of silver graphene nanocomposite compared to amphotericin B can provide appropriate strategies for controlling fungal contamination and producing mycotoxins in human, animal and agricultural products.

Key words: Contamination fungi, Cereals, Microdilution broth

P-119

Evaluation of *glip* & *pksP* gene expression *Aspergillus fumigatus* after treatment with voriconazole by Real-time PCR

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Introduction: Invasive aspergillosis (IA) severe and fatal disease caused by various species of the fungus *Aspergillus* are

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opportunistic. Expressions of some genes are involved in the pathogenesis and diagnosis of this disease. That such gene can be *pksP*, and *glip* cited. Gliotoxin is the major and the most potent toxin produced by *Aspergillus fumigatus*. Gliotoxin has several roles in suppressing the immune system. The *glip* gene encodes a multi-modular non-ribosomal peptide synthetase. Another surface component of the fungi that has been associated with virulence is melanin, a pigment that protects the integrity of the genome in conidia from ultraviolet light, enzymatic lysis, and oxidation. Melanin synthesis seems to be produced in the synthesis route of melanin-1, 8 dihydroxynaphthalene (DHN-melanin) and is regulated by a cluster of six genes, *pksP/alb1*, *ayg1*, *arp1*, *arp2*, *abr1*, and *abr2*. Of all these, the most interesting, from the point of view of virulence, is the *pksP/alb1* gene which encodes a polyketide synthetase and catalyzes the first step of this pathway. The objective of this study is the evaluation of gene expression by *pksP* and *glip* after being treated with the drug voriconazole.

Materials and methods: In order to measure changes in *glip* and *pksP* genes expression, before and after treatment with voriconazole, the Real Time PCR SYBR Green method was performed by using specific primers of these genes on extracted RNA samples.

Results: In this study, the ratio of $2^{(-\Delta\Delta CT)}$ formulate obtained less than 1 (ratio <1) in both of two studied genes which is showing the decreased *glip* and *pksP* genes expression after voriconazole treatment.

Conclusion: Overall, the study results suggest that the study of gene expression due to a decrease in the observed expression levels may make this method to a preferred way in drug resistance investigation in the form of *in vitro* and the necessary prognosis can be achieved in the context of future drug resistance.

Keywords: Gene expression, *pksP* gene, *glip* gene, *Aspergillus fumigatus*

P-120

A study of the nucleotide sequence of the promoter area of the *CYP51A* gene and phospholipase B1 enzyme to sensitivity and resistance to itraconazole in *Aspergillus fumigatus* isolated from the ICU of hospitals in Northern Iran

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Introduction: Aspergillosis is the most threatening disease affecting the patients suffering from immune system defects which cause iatrogenic fungal diseases and high mortality in them. One of the main factors influencing the pathogenesis of this fungus is its capacity to produce and secrete B group phospholipases which cause tissue damages and destruction of cytoplasmic membranes of invaded cells. A survey of molecules has demonstrated that the main resistance against the azole antifungals in *Aspergillus fumigatus* is related to substitution of amino acid in *CYP51A* gene.

Material and Methods: Extraction of DNA from *A. fumigatus* was performed by use of the cTAB method, identification of molecules by use of primers for *CYP51A* gene, and the primer of B₁ Phospholipase by utilizing the PCR technique. After determination of the sequence, *A. fumigatus* was separated and the results were compared with the similar species in the gene bank. Afterward, the test of drug sensitivity to itraconazole via micro-dilution method and by use of the NLCCLS guideline was performed and its MIC rate was surveyed after incubation for 72 hours.

Results: Both *CYP51A* and *PLb1* gene segments were matched after identifying the sequences; and several mutations were observed in the various nucleotide sequences of their promoter region which demonstrated the sensitivity and resistance to itraconazole.

Conclusion: The *A. fumigatus* species may have several mutations in *CYP51A* gene

which causes resistance and sensitivity to itraconazole. *A. fumigatus* species examined were extracted from the intensive care unit (ICU) air and the presence of the wild-type of *A. fumigatus* is likely. The mutations happened in the survey of Phospholipase B1 is indicative of high virulence of Iranian *A. fumigatus* and its resistance to itraconazole.

Keywords: *Aspergillus Fumigatus*, Itraconazole, *CYP51A*, Phospholipases B1

P-121

Effect of farnesol on hyphal morphogenesis transformation and responsive gene *HWPI* & *SAP6* of *Candida albicans*

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Introduction: *Candida albicans* a polymorphic fungus can grow as yeast, pseudohyphae, and true hyphae forms. The hyphal form has a key role in the infection process during invasion of the mucosal membrane. A cluster of genes contribute to controlling of hyphae formation in *C. albicans*, include *Aspartyl Proteinase 6 (SAP6)*, *Hyphal Wall Protein1 (HWPI)* and *RIM101 (alkaline-responsive transcriptional regulator)*. Farnesol is a quorum sensing molecule which inhibits switching of yeast-to-hyphae form. In this study, we aimed to investigate the effect of farnesol on yeast-to-hyphae morphogenesis and its related gene expressions in *C. albicans*.

Materials and methods: *C. albicans* was exposed to various concentration (5, 10, 20, 50, 100, 150 and 300 μ M) of farnesol and the rate of yeast cell proliferations and germ tube formation was evaluated by different methods and microscopic examination. Real time-PCR was performed to assess the expression levels of the hyphae-specific genes *SAP6*, *HWPI* and *RIM101*.

Results: The funding showed that yeast growth reduces 5% in 300 μ M of farnesol approximately ($P < 0.05$). Germ tube formation strongly suppressed. Moreover, real time-PCR analysis showed that 300 μ M farnesol decreases *HWPI* and *SAP6* gene expressions significantly in comparison to control group ($P < 0.05$), whereas, there was no difference in the expression of *RIM101* gene.

Conclusion: These results demonstrated that farnesol exposed *C. albicans*, modulates hyphae formation through down-regulation of *HWPI* and *SAP6* genes expression. This action can reduce the pathogenicity and invasion of *C. albicans*. Also, farnesol inhibitory effects can be used on new targets and for designing natural-based antifungal agents.

Keywords: *Candida albicans*, Farnesol, RIM101, *SAP6*, *HWPI*

P-122

Production carotenoids by *Fusarium oxysporum* and its application in food industry

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Introduction: A variety of natural and synthetic pigments are available. Several species of algae, fungi, and bacteria have been exploited commercially for the production of pigments. Species in the genus *Fusarium* synthesize many secondary metabolites, including compounds of commercial importance because of their deleterious effects, e.g., Carotenoids, or because of their biotechnological applications. The aim of this study was production carotenoids by *Fusarium oxysporum* and application in food industry.

Materials and methods: In this semi-experimental research, the fungus was purchased from Persian Type Culture Collection. Carotenoids were extracted from 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

the *F. oxysporum* by Yasuji method. After that carotenoid analyzed using high-performance liquid chromatography (HPLC) technique. The purity percentage, moisture was determined. Statistical analysis was performed with SPSS 21 all data were compared with ANOVA method.

Results: In this study, the isolated Carotenoid was Beta-carotene. purity percentage, moisture, were 10/7 g/100, 21 g/100, respectively.

Conclusion: According to the results of this study, *F. oxysporum* can produce carotenoid. Therefore, further researches can provide the possibility of using *F. oxysporum* in various fields, such as pharmacy, food Industry, and cosmetics.

Keyword: Secondary metabolites, Carotenoid, *Fusarium oxysporum*

P-123

Carotenoid extracting with *Rhodotorula glutinis* and its application in food industry

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Background: *Rhodotorula glutinis* is a pigmented yeast, part of the Basidiomycota phylum, particularly important for food industries because it's biotechnological potential and safety implications. Various strains of *Rhodotorula* present important features such as the production of large amounts of carotenoids, single-cell proteins from ethanol, acetic acid and acetaldehyde. The aim of this study is carotenoid production by *Rhodotorula glutinis*.

Methods: In this semi-experimental research, yeast was purchased from Persian Type Culture Collection. Carotenoids were extracted from the *Rhodotorula glutinis* by M.M. Sheekh method. After that carotenoid analyzed using HPLC technique. The purity percentage, moisture were determined.

Statistical analysis were performed with SPSS21 and all data were compared with ANOVA method.

Results: In this study, the isolated Carotenoid was Beta carotene. The purity percentage and moisture was determined to be 12/21 g/100, 2 g/100, respectively.

Conclusion: Today there is great interest in the microbial production of various compounds that could be used in different areas, such as the pharmaceutical, cosmetic and food industries, to name a few, as these compounds have particular characteristics that increase the benefits of their consumption and it also reduces production costs and take advantage agro industrial wastes to perform this type of bioprocesses. *Rhodotorula glutinis* could be suggested to source of carotenoids and have a great industrial importance.

Key word: *Rhodotorula glutinis*, Carotenoid, Beta carotene

P-124

Comparison of the cytotoxic effects of Congo red before and after biological degradation with *Aspergillus tritici*

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Introduction: Azo dye is of widespread uses in such different industries like textile, food, print etc, and are widely used in different countries. One kind of azo dye is the Congo red (Direct Red 28) which is carcinogenic and toxic, however, a few studies exist relating to detoxicating it. The use of with fungi due to diversity of their enzymes is a new proposal for the biological degradation of azo dye.

Material and methods: In this study, cytotoxic, genotoxic and mutagenic effects of different concentrations (100, 200, 400 micrograms per liter) of Congo red, before and after the biodegradation by the fungi *Aspergillus tritici*, over meristemic cells root of *Allium cepa* were considered.

Results: Considerable frequencies form apoptosis and necrosis of cells that had increased by increased the dye concentration in dye treatments with fungus (1.85 and 2.85 percent, respectively, in 400 concentration of dye with this fungi) is indicative of high cytotoxic effects of metabolites resultant from biodegradation of Congo red by the fungus *Aspergillus tritici*.

Conclusion: Significant increase in the frequency of apoptosis and bynucleotid cells that affected by metabolites after biodegradation Congo red by *Aspergillus tritici*, indicate that fungal biodegradation metabolites are more cytotoxic than original Congo red.

Keywords: Azo dye, biodegradation, Cytotoxic, *Aspergillus tritici*

P-125

Antifungal efficacies of terbinafine and nano-liposomal terbinafine against *Microsporium canis* and *Trichophyton rubrum*

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Introduction: Dermatophytosis is one of the most common fungal infectious diseases in humans and animals. *Trichophyton rubrum* is among common types of human dermatophytes, and *Microsporium canis* is also one of the main causes of dermatophyte infections in dogs and cats and also in humans. One of the most usual drugs used to treat dermatophytosis is terbinafine. However, there are few disadvantages such

as creating resistance, side effects, long treatment period of dermatophyte lesions such as tinea unguium. Moreover, the traditional drug delivery methods could lead to disadvantages such as loss of the drug, the unwanted dose-related incidence, physicochemical incompatibilities, and also the clinical interactions of the drugs. Therefore, researchers are seeking effective and ideal drugs by using advanced drug delivery systems, which would possess all the necessary properties in terms of lacking side effects and can have a wide-range of efficacies at the target point and would enable short therapeutic periods.

Materials and methods: After sampling the isolates of *T. rubrum* and *M. canis* isolated from patients, the isolates were identified by macroscopy and microscopy as well as molecular analysis. Nanoparticles containing terbinafine were prepared by thin layer hydration method. Afterward nanoparticles were investigated for zeta potential and morphology. The anti-fungal effects of this formulation on the *T. rubrum* and *M. canis* isolates were compared to the common form of the drug by broth microdilution.

Results: The results of molecular, macroscopic, and microscopic examination indicated the confirmation of *T. rubrum* species and *M. canis*. The results of the electronic microscope and the zeta potential of the prepared nanoparticle showed that the nanoparticles were spherical. Antifungal effects of drugs demonstrated that minimum inhibitory concentration (MIC) range of nano-terbinafine against *T. rubrum* (0.0156 to 0.25 µg/ml) and *M. canis* (0.0078 to 0.125 µg/ml) was lower than that of free drug against *T. rubrum* (0.0625 to 1 µg/ml) and *M. canis* (0.0313 to 0.5 µg/ml).

Conclusion: Evaluation of antifungal activity of Nano-liposomal terbinafine compared to terbinafine showed that these nanoparticles had more effective antifungal effects on *T. rubrum* and *M. canis* in comparison with free drug formulation.

Keywords: Nanodrug, Dermatophytes, Terbinafine

P-126

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Congo red products generated by *Penicillium digitatum* degradation are more cytotoxic than the original Congo red

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Introduction: Congo red is an azo dye that is widely used in several countries for colorization of cellulosic fibres and is the subject of much anti perunic research and amyloid tissue detection.

This azo dye is carcinogenic and toxic. Due to complex chemical structure, it is resistant to decomposition. However, there are few studies on its biological detoxification. The use of with fungi due to the diversity of their enzymes is a new proposal for the biological degradation of azo dye.

Materials and methods: In this study, we investigated the cytotoxic effects of different concentration (100- 200- 400) microgram per liter of Congo red before and after biodegradation by *Penicillium digitatum* in meristemic root of *Allum cepa*.

Results: The results showed that fungal biodegradation metabolites have increased cytotoxicity in *A. cepa* meristemic cells. For example, the average of apoptosis in cells that affected by the biodegradation metabolites by *Penicillium digitatum* was 2.78% compared with 221% in cells that contact with original Congo Red (more than 10 times).

Conclusion: Significant increase in the frequency of apoptosis and bynucleotid cells that affected by metabolites after biodegradation Congo red by *Penicillium digitatum*, indicate that fungal biodegradation metabolites such as Banzidin and Aminobenifinil are more cytotoxic than original Congo red.

Keywords: Azo dye, Biodegradation, Cytotoxic, *Penicillium digitatum*

P-127

Determination of genotype *Candida albicans* isolated from oral cavity of

patients with AIDS who referred to West health center of Tehran

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Introduction: Candidiasis is a common fungal infection in the cavity of patients with AIDS.

Material and Methods: In this study, 50 AIDS patients with an average age of 16-66 years old were studied and 30 *Candida albicans* were slaughtered using Sabouraud Dextrose agar (SDA) and CHROMagar media. PCR method was used for confirmation of candida albicans and non-albicans and biofilm formation were evaluated and the data were analyzed by statistical analysis using one way ANOVA, test methods in SPSS software then after extraction of DNA from *Candida albicans* isolated, to determine the genotypes INT (F/R), PCR from samples of AIDS patients referred to West Tehran Health Center from primer gene after electrophoresis.

Results: 450 bp (genotype A) 650 bp (genotype B), 650-450 bp (genotype C) and other components (genotype D) were obtained by electrophoresis and classified according to this basis. This study showed that genotype A has 17 isolates (57%) B genotype (20%, 6 isolates), isolates C₃ genotype (10%), D₄ isolate genotype (13%). The result of the statistical analysis showed no significant correlation between *C. albicans* isolates genotyping with biofilm formation.

Conclusion: Investigation and determining the genotype yeast species obtained from the oral cavity of patients with AIDS can it is useful to adopt a therapeutic protocol and extension for a wider infection.

Keywords: *Candida albicans*, AIDS, biofilm formation, PCR, genotyping

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P-128

New formulation of Graphene oxide - fluconazole compound as promising agent against *Candida albicans*

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Introduction: *Candida albicans* belongs to opportunistic fungal pathogen, which causes a wide spectrum of infections in immunocompromised patients. Graphene oxide (GO), a biocompatibility agent, has been reported to exhibit effective antimicrobial activity. Due to emergence of azole resistant candida species as well as limitations in the efficacy of common antifungals agents, application of a novel formulation of antifungal drugs is a critical necessity.

Methods: Graphene oxide-fluconazole (Go-Flu) compound was synthesized and characterized using Fourier Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy. The antifungal activity of GO-Flu was examined through broth microdilution method, according to CLSI standard method against fluconazole resistant *C. albicans* (ATCC 10231) in comparison to compared to GO and Flu. DNA fragmentation was assessed through considering the antifungal mechanism of GO-Flu. The release of fluconazole in PBS medium was measured. Moreover, the cytotoxicity effect of GO-Flu, adhesion ability of *Candida* treated with GO-Flu to SW480 cell line.

Results: The minimum inhibitory concentration (MIC) of Go, Flu, and Go-Flu were determined to be 800 µg/ml, 16 µg/ml, and 400-9µg/ml, respectively. Notably, Go-Flu exhibited an intense antifungal activity compared to GO and Flu. In addition, GO-Flu

showed much less cell toxicity against SW480 cell line than GO and Flu ($P < 0.05$) did. The release of Flu in PBS (pH 7.4) medium was determined to be 72.42%. GO-Flu reduced the adhesion ability of *C. albicans* to SW480 cell line significantly. DNA fragmentation assay indicated that GO-Flu potentially degraded the DNA of *C. albicans* and caused a fungicidal influence. According to the findings, GO-Flu seems to be a promising finding for the development of a new approach to enhance the antifungal activity against *C. albicans* via DNA fragmentation with low cytotoxicity effect.

Conclusion: Taken together, our findings suggest that Go-flu compound exhibit appropriate antifungal activity and can be a proper candidate for therapeutic approach against candidiasis; however, comprehensive future *in vitro* and *in vivo* studies are required.

Keywords: Graphen oxide, Graphen oxide-Fluconazole compound, Antifungal agent, *Candida albicans*

P-129

Investigation of inhibitory effect of nano-graphene oxide-conjugate with indolicidine on expression of *ERG11* gene in *Candida albicans* by Real Time PCR to compare with clinical sample

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Introduction: *Candida albicans* is an important nosocomial pathogen that causes mucosal and systemic infection in immune compromised patients. Candidiasis' fungal resistance to azole agents increases due to high consumption. These problems make the researchers to design agents with antifungal properties. In this study inhibitory effect of novel nano-composite (conjugated of graphene oxide and indolicidin) on *C. albicans* was evaluated in comparison with fluconazole.

Materials and methods: The expression of *ERG11* gene was investigated in the vicinity of nano-composites. First, after preparation of peptide, nano-graphene oxide, conjugate nano-graphene and indolicidine peptide, their antifungal effects were evaluated using standard broth microdilution method. Then, to investigate the effect of conjugate nano-graphene on the expression level of *ERG11*, it was firstly adjoined at minimum inhibitory concentration (MIC) concentration of 1×10^3 cells of the clinical isolate of *C. albicans* and the standard species. It was extracted before, and after treatment with conjugated oxide nano-graphene by indolicidine peptide and RNA yeast, and then, cDNA was synthesized and Real Time PCR was performed to evaluate the gene expression level.

Results: As a result, the MIC for the conjugated nano-graphene peptide with indolicidine in the clinical isolate of *C. albicans* was 6.25 ug/ml; and it was 3.12 in the standard specimen. The expression of *ERG11* gene level was 11.9 times lower than the expression of the gene, after vicinity of conjugated nano-graphene in the clinical specimen and there was an 18 times reduction in gene expression in the standard specimen.

Conclusion: According to this study, the power of conjugated nano graphene with indolicidine peptide is efficient on the reduction of *ERG* expression, which is contributing to fluconazole resistance; so it can be described as effective antifungal compound.

Keywords: Graphene oxide, Indolicidine peptide, *ERG11*, *Candida albicans*

P-130

Evaluation of nano-capsuleted caprylic acid effect on *EFG1* gene expression in *Candida albicans* in vitro study

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Introduction: Candidiasis belongs to opportunistic and nosocomial fungal infection that is caused by *Candida* species. Recently, discovering the new antifungal agents with minimum side effects are deliberated. Using the nanotechnology approach can be used as alternative option in drug delivery system. Newly, the antifungal properties of the medium chain fatty acid include Caprylic acid have been presented. The aim of this study was to investigate the antifungal activity of Caprylic acid and nano-encapsulated Caprylic acid on *Candida albicans* as well as its effect on the expression of *EFG1* gene was assessed.

Materials and methods: Minimum inhibitory concentration (MICs) of the Caprylic acid and nano-capsulated Caprylic acid on *C. albicans* was evaluated compared to fluconazole. RNA extracted from *C. albicans* exposure to Caprylic acid and nano-capsulated Caprylic acid, then cDNA synthetized and the mRNA expression of *EFG1* genes in each group was evaluated using Real-time PCR assay.

Results: The MIC₉₀ of Caprylic acid was 500µg/ml and MIC₅₀ was 450µg/ml, whereas MIC₉₀ for nano-capsulated Caprylic acid indicated 6.2µg/ml and MIC₅₀ showed 3.1µg/ml. The minimum fungicide concentration (MFC) of Caprylic acid and encapsulation Caprylic acid determined as 600µg/ml and 12.5µg/ml respectively. The mRNA level of *EFG1* gene significantly decreased in *C. albicans* treated with Caprylic acid and nano-capsulated Caprylic acid compared to the control group. Moreover, the *EFG1* expression after exposure to Caprylic acid nanocapsulated was 4 fold lower than Caprylic acid treatment.

Conclusion: According to the obtained results, nanocapsulated Caprylic acid successfully inhibited the *Candida albicans* growth with the low MIC compared to Caprylic acid. Taken together, it is suggested Caprylic acid nano-encapsulated may be used as a suitable agent against *Candida* species.

Keywords: *Candida albicans*, Caprylic acid, Nano-Capsulation, *EFG1*

P-131

The study of clinical isolates of *Candida albicans*: biofilm formation and *ZAP1* gene expression

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Introduction: Biofilm formation is one of the most important virulent factors among the *Candida* species. This report focuses on the biofilm formation ability of *Candida* species and the zinc-response transcription factor *Zap1*, a regulator of a major biofilm matrix component, expression and identification of the relationship between *Zap1* expression and biofilm formation in clinical species of *Candida*.

Materials and methods: The clinical isolates of *Candida* were collected from oral candidiasis and determined by culturing on CHROMagar and ITS sequencing methods. Biofilm formation was examined according to standard protocol. The expression of *Zap1* was evaluated by quantitative PCR and *ACT1* gene was used as reference gene.

Results: Biofilm formation rate was evaluated in different species of *Candida* compared to standard strain. Biofilm formation ability in all strains was more than the *C. albicans* ATCC10231, standard strain. Among these strains, the most biofilm formation ability belonged to *C. tropicalis* while *C. dunlinsiensis*, *C. kefyr*, *C. glabrata* and *C. albicans* were able to form biofilm to less extent, respectively. The results of real-time PCR showed that the most gene expression was found in *C. tropicalis*, and *C. dunlinsiensis* revealed the lowest gene expression.

Conclusion: The identification of virulence factors in clinical isolates of *Candida* can be helpful to use effective strategies for antifungal treatment, prophylaxis, and preventive therapies in patients.

Keywords: *Candida spp.*, Biofilm, *Zap1*, Gene expression

P-132

First clinical isolation of a Terbinafine-resistant *Trichophyton tonsurans* harboring a Leu393Phe mutation in Tehran

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Introduction: With regard to increasing number of antifungal-resistant dermatophytes, antifungal susceptibility testing of dermatophytes serves as a useful tool in managing clinical dermatophytosis. Terbinafine resistance has been predominately attributed to point mutations in the squalene epoxidase (*SQLE*) target gene a key enzyme in the ergosterol biosynthetic pathway leading to single amino acid substitutions. Inhibition of this enzyme leads to accumulation of squalene inside the fungal cells, depletion of ergosterol, and finally causes cell death. This study aimed to determine point mutations in terbinafine-resistant isolates.

Materials and methods: Dermatophyte species (n= 99) was confirmed by sequencing of ITS region. Antifungal susceptibility testing of all isolates was assessed to terbinafine agent using CLSI M38-A2 guidelines.

Results: Based on our results, among 99 tested isolates, 5 (5%) showed reduced terbinafine susceptibility minimum inhibitory concentration (MIC>32 µg/ml), of which for two species *T. rubrum* and *T. tonsurans* were found to be related to amino acid substitution Leu393 by Phe in the squalene epoxidase protein. We reported terbinafine resistance for dermatophytes isolated from tinea pedis and tinea corporis. This is the first case of terbinafine-resistant *T. tonsurans* strain isolated from patient.

Conclusion: This increase of terbinafine resistance of dermatophyte isolates is worrisome warranting antifungal susceptibility testing and mutation analysis for monitoring this emerging resistance.

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Keywords Squalene epoxidase, Point mutation, Terbinafine resistance

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Adhesion of *Candida* species isolated from the oral cavity to acrylic discs with different silver nanoparticles in the presence of chlorohexidine mouthwash

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Introduction: Denture stomatitis is the common form of oral candidiasis and the use of antifungal agents within acrylics can play an effective role in preventing diseases. Therefore, we decided to evaluate adhesion of *Candida* species in the presence of chlorohexidine mouthwash to acrylic discs with various concentrations of silver nanoparticle.

Materials and methods: In this analytical-laboratory study acrylic discs with 0% and 2% concentration of silver nanoparticles were prepared and then in the presence of chlorohexidine mouthwash were put adjacent to the suspension of candida cells isolated from the mouths of individuals and their adhesion to discs was measured. At the end, data were analyzed by using chi-square and Kruskal-Wallis test.

Results: In this study, the average adhesion in the concentration of control (acrylic disc without silver nanoparticles with concentration 0%) and concentration 2% in the presence of chlorohexidine mouthwash was 50.52 and 34.17 respectively. Therefore, adhesion of acrylic resin control and acrylic resin with silver nanoparticles in the presence of chlorohexidine mouthwash was meaningful and significant (P-value < 0.05).

Conclusion: In the presence of chlorohexidine mouthwash, Adhesion of

Candida species to acrylic discs was specifically reduced by increasing the concentration of silver nanoparticles.

Keywords: chlorohexidine mouthwash, Denture stomatitis, Acrylic resin, Silver nanoparticles, *Candida* species

P-134

Nanoemulsion loaded with *Thymus vulgaris*: A promising agent against fluconazole-resistant *Candida* isolates

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Introduction: Azoles such as fluconazole are often preferred treatment for many *Candida* infections as it is inexpensive, exhibits limited toxicity, and is available for oral administration. However extensive documentations reported the high frequency of fluconazole resistant *Candida* isolates in the clinical setting. For this reason, there is a need for new natural-originated drugs with low side effects and minimum inhibitory concentrations (MIC). *Thymus vulgaris* is a species of flowering plant in the mint family lamiaceae, native to southern Europe from the western Mediterranean to southern Italy.

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Thymus vulgaris essential oil is a combination of monoterpenes which act as antioxidative, antimicrobial, antitussive, antispasmodic, and antibacterial agent.

Materials and methods: In the present research, the nanoemulsion-loaded *Thymus vulgaris* were prepared using probe ultrasonication techniques and the efficacy of the optimal formulation on a large number of *Candida* isolate was investigated. In total, 60 fluconazole resistant and susceptible isolate of *Candida albicans*, *Candida glabrata* and *Candida parapsilosis* were examined. To determine MIC for both Thyme oil and nanoemulsion formulation, the clinical and laboratory standards institute document M27-A3 and M27-S4 were used as a guideline. Fluconazole applied along with each test as the reference drug.

Results: The nanoemulsion-loaded *Thymus vulgaris* particles presented a spherical shape with a mean diameter, zeta potential and entrapment efficiency of 126.4 ± 15.2 nm, -35.1 ± 3.0 mV, and 93.6 ± 3.5%, respectively. The MIC₅₀ value for thyme oil was obtained 80 µg/ml against both fluconazole resistant and susceptible strains of *C. albicans*, *C. glabrata*, and 160 µg/ml for *C. parapsilosis*; while both fluconazole resistant and susceptible strains of *C. albicans*, *C. glabrata* and *C. parapsilosis* showed MIC₅₀ 5 µg/ml and 20 µg/ml, respectively.

Conclusion: This study showed the effectiveness of nanoemulsion loaded *Thymus vulgaris* as a delivery system against fluconazole-resistant *Candida* isolates. In conclusion, novel antifungal agents with natural origin might be used as part of therapeutic strategy or alternative treatment of candidiasis in the future.

Key words: *Thymus vulgaris*, Nanoemulsion, Fluconazole, *Candida*, Drug delivery

P-135

Nationwide cryptococcal antigen screening of HIV-infected in Iran

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Introduction: Cryptococcal meningitis is a life-threatening infection in immunocompromised patients caused by *Cryptococcus neoformans*/*Cryptococcus gattii* species complex. HIV positive and immunocompetent hosts are at risk for cryptococcal disease. Cryptococcal antigen (CrAg) lateral flow assay (LFA). CrAg LFA allows a simple, rapid and low cost test for the diagnosis and screening cryptococcosis using cerebrospinal fluid (CSF) and serum samples. This study aimed to estimate CrAg in patients with HIV.

Materials and Methods: 169 (152 serum and 12 CSF) samples obtained from 152 HIV positive patients with severe immunosuppression (CD4 T cell less than 200 cells/mL) both symptomatic and asymptomatic meningitis patients admitted at hospital or referred to the Infectious Disease Institute outpatient HIV clinic in different provinces of Iran between October 2016 to May 2018 were enrolled in study. All

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specimens were assessed for cryptococcal antigen using CrAg LFA (Immuno-Mycologics, Norman, OK, USA). All of the study patients were antiretroviral-naive patients or incompletely used antiretroviral therapy.

Results: 116 subjects were male and 36 patients were female, the mean age was 36 years. Prevalence of serum CrAg positive in screened samples was 6.4% (10/157). In CrAg positive patients, cryptococcal meningitis prevalence was 3.3% (5/152).

Conclusion: CrAg LFA has the potential to identify of patients with asymptomatic infection, enable using pre-emptive treatment at the very early phase and improve the prognosis of HIV-associated cryptococcal meningitis.

Keywords: Cryptococcal meningitis, Cryptococcal antigen, Lateral Flow Assay, HIV

P-136

Identification of common pathogenic and potentially pathogenic *Exophiala* species by using high-resolution melting analysis

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Introduction: *Exophiala* often cause infection in both immunocompetent and immuno-suppressed individuals. Due to variable morphology and low degree of phenotypic differentiation between species, phenotypic characteristics are inappropriate

for identification. Molecular methods have been used to identify *Exophiala* species, as a precise and rapid method. However, this identification is still a serious challenge. We aimed to develop a rapid and accurate real-time PCR-based high-resolution melting (HRM) to identify the common pathogenic and potentially pathogenic, *Exophiala* species.

Materials and methods: HRM primers was designed to amplify the conserved region of ITS1–5.8S–ITS2 region of ribosomal DNA of *Exophiala* species. To evaluate the effectiveness of this method, a total 110 potentially pathogenic of *Exophiala* species were tested in comparison with DNA sequencing, as the reference standard assay.

Result: The amplification of DNA using the HRM primers yielded products with close sizes, so that the size of PCR products was not sufficient to detect all evaluated species. In the HRM analysis, *Exophiala dermatitidis* and *E. castellanii* showed a similar peak, while other species including *E. xenobiotica*, *E. heteromorpha*, *E. oligosperma*, *E. phaeomuriformis* and *E. crusticola* had a distinct peak.

Conclusion: In this study describe the use of HRM assay for differentiating *Exophiala* species. The developed HRM assay was able discriminate 5 *Exophiala* species with high accuracy, precision, speed and throughput.

Keywords: *Exophiala* species, High-resolution melting, Identification

P-137

Screening of amino acid substitutions in *Fks1p* of micafungin resistant-*Candida parapsilosis sensu stricto*

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Introduction: *Candida parapsilosis* accounted for 24.4% of all isolated organisms from 156 episodes of invasive candidiasis referred to intensive care units from the Children's Medical Center, Tehran, Iran. Although fluconazole have been the main choice for therapy against invasive candidiasis, but several new antifungal agents (e.g., echinocandins) have emerged as therapeutic alternatives. *Candida parapsilosis* has an intrinsically reduced susceptibility to echinocandins and resistance to fluconazole has reported recently. The main aim of the study was to investigate the mechanism of drug resistance by screening the SNPs in genes responsible for drug resistance, *FKS1* and *ERG11*.

Materials and methods: One hundred and five isolates of *C. parapsilosis sensu stricto* were investigated in this study. In vitro antifungal activities of fluconazole, itraconazole, voriconazole, caspofungin, anidilufungin, micafungin, loliconazole and laniconazole were determined using the broth microdilution reference method according to CLSI M27-A3 and M27-S4 document. The *ERG11* and *FKS1* genes for resistant and susceptible isolates were sequenced and multi-aligned using MEGA6 software.

Results: Itraconazole and multi-azole resistance were observed in 89.5% and 3.8% of the isolates, respectively. Amino acid substitution Y132F, which was not observed in susceptible isolates, was identified in *ERG11* genes. The rate of resistance in echinocandin drugs was similar to the azoles, so that 3.8% of isolates were multi-

echinocandin resistance. However, anidulafungin was not as active as other echinocandins. The common P660A amino acid substitution was observed in both azole-resistant and -susceptible isolates and no more substitutions was detected. Itraconazole and multi-azole resistance were observed in 89.5% and 3.8% of the isolates, respectively. Amino acid substitution Y132F was not observed in susceptible isolates and identified in *ERG11* genes. The rate of resistance in echinocandin drugs was similar to the azoles, so that 3.8% of isolates were multi-echinocandin resistance. However, anidulafungin was not as active as other echinocandins. The common P660A amino acid substitution was observed in both azole-resistant and susceptible isolates and no more substitutions was detected.

Conclusion: Understanding the mechanisms responsible for drug resistance in *C. parapsilosis* is not only crucial for the development of new antifungals but is also important in choosing appropriate antifungals for patients at the earliest stages.

Keyword: *Candida parapsilosis*, *FKS1*, *ERG11*, Micafungin

P-138

Identification of clinical and environmental isolates of *Aspergillus flavus* by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) method

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Introduction: *Aspergillus flavus* has considered as a main causative agent of invasive and non-invasive infections in Middle East. Previous investigation has showed the difficulty in discrimination of *A. flavus* from *A. oryzae*. The aim of present study was using a matrix-assisted laser desorption ionization–time of flight mass spectrometry by Bruker database for differentiation of *A. flavus* from *A. oryzae*.

Materials and methods: We evaluated the ability of Bruker MALDI-TOF MS for identification of 200 clinical and environmental isolates of *A. flavus*. At first, all isolates were recognized by the β -tubulin sequence analysis of PCR amplification followed by MALDI-TOF MS.

Results: Our results indicated that the Bruker score of ≥ 2.0 identified 141 (70.5%) isolates as *A. flavus*. The score between 1.7 and 2.0 was only able to identify of 59 (29.5%) of the isolates in a genus level.

Conclusion: According to our data, the discrimination between *A. flavus* and *A. oryzae* by using the Bruker score database and the correct identification at species levels was relatively poor. Future developments are required to increase the discrimination power of MALDI-TOF MS for differentiation between *A. flavus* and *A. oryzae*.

Key words: MALDI-TOF MS, *Aspergillus flavus*, *Aspergillus oryzae*

P-139

Glabridin triggers over-expression of apoptosis inducing factor (AIF) gene in *Candida albicans*

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Introduction: *Candida albicans* is a prevalent human fungal pathogen that can cause a wide spectrum of diseases; from superficial mucosal infections to systemic disorders in patients with impaired immunity. Glabridin is a pyranoisoflavan which originally extracted from root extract of *Glycyrrhiza glabra*. Glabridin could also mediate apoptosis in yeast cells by changing the mitochondrial membrane potential, activation of caspase like proteases and DNA cleavage. The aim of this study was to investigate the mechanism of action of glabridin in *C. albicans*.

Material and materials: *Candida albicans* ATCC14053 was applied as standard strain. Total

total RNA was extracted from the isolate under glabridin-treated and untreated conditions. To evaluate the alternation in the *AIF* gene expression, Real-time PCR was performed and the obtained data was analyzed using REST software, afterward.

Results: Expression of *AIF* gene was represented as a ratio of expression relative to the reference gene. According to the REST output, the expression of the *AIF* gene increased remarkably ($P < 0.05$) under glabridin-treated conditions.

Conclusion: Our results suggested that glabridin may induce apoptosis through the caspase-independent way and might be considered as an anti-*Candida* agent.

Keywords: Glabridin, apoptosis, *AIF* gene, *Candida albicans*

P-140

Determination of changes in the expression of *miR-212* and *EGFR* genes in clinical samples from areas infected with

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***Trichophyton rubrum* compared with non-infected areas**

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Introduction: Skin infections with dermatophytes are common human infections called dermatophytosis. *Trichophyton rubrum* is the most common cause of dermatophytosis. Antimicrobial peptides (AMPs), which have potential antimicrobial effects, are affected by epidermal growth factor receptor (*EGFR*) gene and increasing expression of this gene in skin cells activates AMPs and prevents cloning of dermatophytes in keratinocytes. However, mRNA of *EGFR* gene is muted with increasing expression of microRNAs (miRNAs) and in particular *miR-212* in this study. Therefore, *EGFR* inhibition may have a negative effect on AMP localization in dermatophyte-infected keratinocytes.

Objectives: This study aimed to determine the changes in the expression of *miR-212* and *EGFR* genes in cutaneous tissue affected with *T. rubrum* as compared to adjacent spots.

Materials and Methods: The number of samples in this study was estimated to be 72. The fungus was cultivated on Sabouraud dextrose agar medium. Isolation and optimization of total RNA as well as synthesis and optimization of cDNA for *EGFR* and *miR-212* genes were performed. Amplification of these target genes was

performed on Real-Time PCR. In data aggregation and analysis, changes in expression of target genes were calculated with $2^{-\Delta\Delta Ct}$ ratio. P.value was considered <0.05 .

Results: In samples infected with *T. rubrum*, *miR-212* significantly reduces the expression of *EGFR* gene, and in these samples, the expression of *miR-212* gene is 8 times higher compared to the expression of *EGFR* gene. In control sample, the expression of *miR-212* is much lower than that of *EGFR* gene.

Conclusion: By increasing expression of *miR-212* in this study, the function of *EGFR* gene mRNA has been turned off, which leads to the reduction of AMPs that results in the colonization of *T. rubrum* and progression of dermatophytosis on the skin.

Keywords: Dermatophytosis, *Trichophyton rubrum*, AMP, *EGFR*, *miR-212*

P-141

Antifungal activity evaluation of new methyl and methoxy acetophenonic isoxazolin compounds

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Introduction: Fungal infections have become one of the major causes of morbidity and mortality in recent decades. The emergence of drug-resistant fungi poses a major threat to human health. Discovering new drugs promising new therapies. We designed and fabricated three *Acetophenonic isoxazolin* derivatives to evaluate antifungal activity against *Candida albicans*.

Materials and Methods: After design and synthesis a new series of *Acetophenonic isoxazolin* derivatives, their antifungal effect was evaluated against *Candida albicans* using microdilution method according to CLSI guideline.

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Results: All synthesized compounds were found to have considerable antifungal activity. The Minimum Inhibitory concentrations (MICs) ranged 64-250 $\mu\text{g/mL}$ against *Candida albicans*.

Conclusion: The favorable antifungal activities of the synthetic derivatives against *Candida albicans* may have a considerable potential for therapeutic application.

Keywords: Acetophenonic isoxazolin, Antifungal, *Candida albicans*

P-142

Evaluation of biogenic selenium nanoparticles effects on *ERG11* and *CDR1* genes expression in fluconazole resistant *Candida albicans* strains

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Introduction: *Candida albicans* remains as the most opportunistic yeast isolated from fungal infections. Azoles resistance in *Candida* species have been considerably raised in the last decades. Because of the toxicity of antimicrobial drugs, resistance to antifungal agents and drug interactions, need for new antifungal agents seems essential. In this study we assessed the effects of selenium biogenic nanoparticles on *C. albicans* by antifungal susceptibility test and also determined the effect of selenium nanoparticle on expression of *ERG11* and *CDR1* genes.

Materials and Methods: Selenium nanoparticles were synthesized with *Bacillus* sp. Msh-1. The ultrastructure of selenium nanoparticles was evaluated with transmission electron microscope. The antifungal susceptibility test for fluconazole resistant *C. albicans* (ATCC76615) and wild

type *C. albicans* (ATCC 10231) isolates was performed according to the CLSI M27-A3 standard protocol. The RNA extraction of *Candida* species was performed with the manufacturer's instruction kit. Synthesis of cDNA was conducted with the 1621K kit (Life Science kit) and according to the manufacturer's instruction. The changes in expression levels of the *CDR1* and *ERG11* genes were analyzed with a quantitative real-time PCR assay.

Results: The azole resistance *C. albicans* and wild type *C. albicans* strains were inhibited to with 100 µg/ml and 70 µg/ml of selenium nanoparticles concentration, respectively. The expression of *CDR1* and *ERG11* genes was significantly down regulation in this selenium nanoparticles concentrations.

Conclusion: The present study exhibited that selenium nanoparticles have an appropriate antifungal activity against fluconazole resistant and susceptible *Candida albicans* strains. Also Se NPs reduced expression of *CDR1* and *ERG11* genes that associated with azoles resistance. Further studies are needed on the synergistic effects of selenium nanoparticles with other antifungal drugs.

Key words: *Candida albicans*, Selenium nanoparticles, Azole-resistance

P-143

Evaluation of antifungal effects of nanoliposomal fluconazole against fluconazole susceptible and resistant *Candida* species isolated from patients *in vitro* and comparison with common fluconazole

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Introduction: The aim of this study was to produce fluconazole loaded liposomal nanoparticles, to analyze their physicochemical properties and to compare their antifungal effects with the free fluconazole drug *in vitro* against the fluconazole susceptible and resistant *Candida* species isolated from patients.

Materials and Methods: Six common candida species including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. guilliermondii* were tested. The Liposomal nanoparticles were prepared using thin layer hydration method and soybean lecithin, cholesterol, and fluconazole at a ratio of 10: 1: 1. The nanoparticles were analyzed in terms of size, poly dispersity index, zeta potential, morphology, entrapment efficiency of drug and the amount of drug released. To investigate the antifungal effects of liposomal nanoparticles and compare them with the free form of fluconazole, we used Broth Microdilution as described in CLSI M27-A3.

Results: The results were analyzed using Student's T-test and indicated the greater antifungal effects of the liposomal nanoparticles containing fluconazole than the normal form of the drug. It was shown that MIC of fluconazole was put in the range of sensitive species after exposure with the fluconazole nanoliposomal in most fluconazole resistant *Candida* species except for the *krusei* species.

Conclusion: Therefore, it is likely that we can use the new system for drug delivery to prevent drug release from the cell. In addition, this is the first research using fluconazole lipid nanoparticles against *C. krusei*.

Key words: Nanoliposome, Fluconazole, Antifungal activity, *Candida*

P-144

Concentration and type of bioaerosols before and after conventional disinfection and sterilization procedures inside hospital operating rooms

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Introduction: Operating rooms (ORs) in hospitals are sensitive wards because patients can get infections. This work aimed to characterize the type and concentration of bioaerosols in nine ORs of an educational hospital before and after sterilization and disinfection.

Materials and methods: During 2017, fungal samples were incubated at 25–28 °C for 3–7 days and bacterial samples at 37 °C for 24–48 hr.

Results: The study results showed that the concentrations of fungi before cleaning procedures (for both of disinfection and sterilization) were limited from 4.83 to 18.40CFU/m³ and after cleaning procedures ranged from 1.90 to 8.90CFU/m³. In addition, the concentrations of bacteria before cleaning procedures were limited 14.65–167.40CFU/m³ and after cleaning procedures ranged from 9.50 to 38.40CFU/m³. The difference between the mean concentrations of airborne bioaerosols before and after sterilization was significantly different than the suggested value of 30CFU/m³ ($p \leq 0.05$). The bacterial concentration was higher than the recommended value (30CFU/m³) in 41% of the ORs. The main fungal species identified in the indoor air of ORs (before vs. after sterilization) were *Aspergillus fumigatus* (25.6 vs. 18.3%), *A. niger* (11.6 vs. 5.8%), *Penicillium* spp. (5.5 vs. 3.3%), *Alternaria* spp. (2.8 vs. 0.7%), *Fusarium* spp. (9.7 vs. 3.7%), *Mucor* spp. (15 vs. 12.7%), *Cephalotrichum* spp. (1.7 vs. 0.8%), *A. flavus* (24.6 vs. 18.5%), *Cladosporium* spp. (2.6 vs. 0.8%), and *Trichoderma* spp. (0 vs. 0.9%).

Conclusion: The growth of biological species even after sterilization and disinfection likely resulted from factors including poor ventilation, sweeping of OR floors, inadequate HVAC filtration, high humidity, and also lack of optimum management of infectious waste after surgery. Designing well-constructed ventilation and air-conditioning systems, replacing HEPA filters, implementing more stringent, frequent, and comprehensive disinfection procedures, and controlling temperature and humidity can help decrease bioaerosols in ORs.

Keywords: Bioaerosol, Operating room, Sterilization, Indoor air, Shiraz

P-145

Identification of *Aspergilli* isolated from outdoor air using PCR-RFLP

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Introduction: *Aspergillus* species are the most common and widely distributed genera of fungi associated with diverse group of human disease such as allergic bronchopulmonary, mycotic keratitis, otomycosis, nasal sinusitis as well as invasive infection. In this study we attempted to identification of *Aspergillus* isolated from outdoor air in Tehran by using *EcoRI* and *TaqI* restriction enzymes and the PCR-RFLP molecular method.

Materials and methods: In the present study, airborne *Aspergilli* were identified in 22 regions of Tehran city by using a combination of morphological criteria, sequencing of ITS1/ITS4 regions and

restriction fragment length polymorphism (RFLP) techniques. PCR fragments, produced using ITS1/ITS4 primers, were digested using *EcoRI* and *TaqI* restriction enzymes.

Results: The results showed that by using *EcoRI* enzyme, all species followed a similar pattern and produced a fragment of 300 bp. *A. niger*, *A. flavus*, *A. terreus*, *A. amstelodami*, *A. tubinjesis*, *A. oryzae* and *A. versicolor* produced fragments with 60, 160 and 210 bp when they exposed to *TaqI*. Other species produced very different fragments. The result of digestion by *TaqI* in *A. flavipes* yielded four fragments with 60,90,150 and 190 bp, in *A. japonicas*, three fragments with 60,120 and 220 bp, in *A. ochraceus* two fragments with 60 and 180 bp, in *A. nidulans* three fragments with 60, 180 and 200 bp and in *A. fumigatus* two fragments with 80 and 180 bp.

Conclusion: The results demonstrated that the ability of *TaqI* enzyme in the production of 6 different patterns provided the differentiation of 5 species of *A. flavipes*, *A. japonicas*, *A. ochraceus*, *A. nidulans* and *A. fumigatus* from each other and from other species.

Keywords: *Aspergillus* spp., Molecular identification, PCR-RFLP technique

P-146

Structural study of the *Annexin* evolution in Fungi

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Introduction: The annexins are a large family of proteins that bind to Ca²⁺ and cell membrane phospholipids. These proteins are composed of two domains: N-terminal domain that interacts with membrane phospholipids and C-terminal domains that interacts with other annexins and proteins. Annexins implicated in variety functions such as exocytosis and endocytosis, signal transduction, organization of the extracellular matrix, resistance to reactive oxygen species, subcellular transport, membrane repair, and DNA replication. Moreover it has been suggested that they have a critical role on the tip growth of fungal hypha. The aim of this study is using bioinformatics methods to study of sequence diversity and structural evolution of fungal annexins.

Materials and methods: In this study, amino acid sequence of fungal annexins was retrieved from NCBI and UNIPROT databases. Then multiple sequence alignment and phylogentic investigated by Clustal and Omega software. The positive selection performed by means of Data monkey web server. Then protein structure prediction and molecular dynamic were performed using MODELLER9v12 and Gromacs, respectively.

Results: The obtained results of the study on 48 fungal annexins showed these proteins in fungi have four repeats in C-terminal domain that each repeat composed of approximately 60 amino acids. Results of selection mapped on the phylogenetic tree showed strength of selection in some fungi such as *Paracoccidioides* and *Emericella* that are pathogen in human or *Ustilago maydis* that causes smut on maize and teosinte was high.

Conclusion: The results indicated fungal annexin can be correlated with pathogenicity of some fungal pathogens.

Keywords: Fungal annexin, Diversity and Evolution, Structural study

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Differentiation of the most common causes of human dermatophytosis (*Trichophyton rubrum* and *Trichophyton interdigital*) by Duplex PCR

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Introduction: Dermatophytes create the most common contagious fungal disease in humans, called dermatophytosis (tinea). The two species of *Trichophyton rubrum* and *Trichophyton interdigital* are responsible for over 80% of all types of dermatophytosis in iran and in many countries of the world. So far, several morphological and physiological methods have been used to distinguish these very similar species, but the methods are generally time-consuming and have a low specificity. The purpose of this study is to introduce a simple and rapid Duplex PCR reaction to differentiate these two species from each other.

Materials and methods: The nucleotide sequences of the 4 regions of *ITS*, *beta-tubulin*, *elongation factor 1 alpha* and *calmodulin* in the two considered species of fungi were conducted bioinformatics analysis and differences and similarities of nucleotides between two species in each of these genes were studied for selecting the primer. The specificity of selected primers in the laboratory was tested for PCR reaction against isolated sequences of common *dermatophyte* species.

Results: According to the total data the primers selected for the *elongation factor 1 alpha* gene have better conditions and was selected as specific primers. In fact, these primers produced a product of 173 and 384 bp, in *Trichophyton rubrum* and *Trichophyton interdigital*, respectively. The primer pairs designed, faced with various *dermatophytes* species have a high specificity.

Conclusion: The method set up in this study is a specific diagnostic and differential

method which is more accurate and quicker than routine culture and can be useful in the field of laboratory diagnosis, epidemiological studies and therapeutic choices.

Key words: Dermatophyte, *Trichophyton rubrum*, *Trichophyton interdigital*, *Elongation factor 1 alpha*, Duplex PCR

P-148

Arlequin: An integrated software package for population genetics data analysis
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Introduction: Arlequin is the French translation of "Arlecchino", a famous character of the Italian "Commedia dell'Arte". As a character he has many aspects, but he has the ability to switch among them very easily according to its needs and to necessities. This polymorphic ability is symbolized by his colorful costume, from which the Arlequin icon was designed.

Methods: Arlequin, like the computation of standard genetic diversity indices, the estimation of allele and haplotype frequencies, tests of departure from linkage equilibrium, departure from selective neutrality and demographic equilibrium, estimation or parameters from past population expansions, and thorough analyses of population subdivision under the AMOVA framework. Arlequin can handle several types of data either in *haplotypic* or *genotypic* form. The basic data types are:

- DNA sequences
- RFLP data
- Microsatellite data
- Standard data
- Allele frequency data

Results: Arlequin is a software package integrating several basic and advanced methods for population genetics data analysis.

Keywords: Arlequin, Population genetics, Genetic data analysis, AMOVA

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Antifungal activity of silver nano-particles and evaluation of *Aspf1* gene expression as

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a major allergen of *Aspergillus fumigatus*
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Introduction: *Aspergillus fumigatus* as the
most common etiologic agent of aspergillosis
play a significant role in allergic and invasive
aspergillosis. This study was done to
determine the antifungal activity of silver
nano-particles (nano-Ag) on *A. fumigatus*.

Materials and methods: Antifungal
susceptibility test was performed on *A.*
fumigatus that isolated from bronchoalveolar
lavage and standard strain by using micro-
titer broth dilution method according to CLSI
(clinical laboratory standards institute) M38-
A2 document. The expression of *Asp1* gene
before and after exposure to silver
nanoparticles were measured using real-time
PCR.

Results: From total of 60 samples, *A. flavus*
was isolated in 21 cases, followed by *A. niger*
in 3 cases and *A. fumigates* in one case. The
minimum inhibitory concentration (MIC₉₀)
and minimum fungicidal concentration
(MFC) of nano-silver on standard and
clinical isolates were 0.25, 0.5 and 0.5, 1
µg.ml-1, respectively. The expression ratio of
Asp1 gene in comparison to β -*tubulin*
internal control in standard and clinical
isolates after exposure to silver nano-
particles was 0.007 and 0.5, respectively (*P*-
value < 0.05). It can be concluded that silver
nano-particles have high potency of
antifungal activity against *Aspergillus*
isolates.

Conclusion: It can be concluded that nano-
particles of silver have high potency of
antifungal activity against *Aspergillus*
fungus. Their higher activity may be
correlated to the small size of them.
However, this matter that the nano-particles
can be used as alternative antifungal agent for
therapy of pathogenic fungi, more other

studies are required in comprehensive level
in the future.

Keywords: *Aspergillus fumigatus*, *Asp1*,
Silver nano-particles

P-150

**Evolution effect of antibiofilm activity of
acetic extract from *Mazouj* gall against
*Candida albicans***

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Introduction: Candidiasis as one of the most
common infection in human is usually caused
by *Candida albicans* and hard to treat. The
majority of *C. albicans* infections are
associated with its ability to form biofilms.
Biofilm formation is a risk factor for
mortality in patients with Multidrug-resistant
Candida albicans infection. The difficulty in
treatment of candidiasis by antifungal agents
to penetrate is due to the biofilm matrix. New
alternative antifungal agents are urgently
needed to prevent the emergence of fungal
resistance in treatment of candidiasis. *Mazouj*
gall have been shown to have many
medicinal properties. This gall is caused by
gall-wasp species, that is, *Cynips tinctoria*.
The objective of this project was to
investigate the effects of antibiofilm activity
of Acetic extract from *Mazouj* gall against
Candida albicans.

Material and methods: The MIC and MFC
of *Mazouj* (identified by the Herbarium of
Research Institute of Agriculture Jihad of
Lorestan, Iran) acetic extract against *C.*
albicans PTCC 5027 were determined using

the broth microdilution method. Antibiofilm activity was evaluated using a 3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium-bromide (MTT) assay.

Results: The (Minimum inhibitory concentration) MIC and (Minimum fungicide concentration) MFC acetonic extract against *C. albicans* PTCC 5027 were 0.5 mg/ml and 1 mg/ml respectively. This activity depended on the time and concentrations of the extract. Mazouj extract acts as a potent antibiofilm agent with reduced biofilm biomass of *C. albicans* in the concentrations higher than 0.6 mg/ml.

Conclusion: The present results demonstrate that the *Mazouj gall* is potentially useful as antifungall and anti-biofilm agents in preventing Candidiasis.

Keywords: *Candida albicans*, Biofilm, *Mazouj gall*

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Evaluation of antibacterial activity of methanolic extract of the polypore fungus

Fomes

fomentarius (Polyporaceae) against

Staphylococcus aureus

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Introduction: Since ancient times medicinal mushrooms have been used in traditional medicine. *Basidiomycetes*, especially polypores, have been exploited for pharmaceutical and medicinal applications. The tinder polypore, *Fomes fomentarius*, has been widely used for the treatment of various diseases, e.g. dysmenorrhea, hemorrhoids, bladder disorders, oesophagus gastric and uterine carcinoma. Due to the expansion of

antibiotic resistance among bacteria, further researches are needed on the discovery and development of new antibacterial agents. Since *Staphylococcus aureus* is a major reason of hospital-acquired infection (HAI), and raised *antibiotic resistant* isolates, the aim of this study was to assess the antibacterial effect of *F. fomentarius* Methanolic extract against this *bacterial* species.

Material and methods: *Fomes fomentarius* was collected in December 2017 from Abbasabad forests, Mazandaran province, Iran. The *macro- and micro-morphological* of the isolates were identified. Then the antibacterial activities of the Methanolic was extract evaluated by broth microdilution method against *S. aureus* ATCC25923.

Results: The (Minimum inhibitory concentration) MIC and (Minimum bactericide concentration) MBC of Methanolic extract of *F. fomentarius* against *S. aureus* ATCC25923 were 0.7 mg/ml and 12.5 mg/ml respectively.

Conclusion: The results of this study showed that *F. fomentarius* has inhibitory activity against *S. aureus* which deserves more studies about antimicrobial properties of this fungus.

Keywords: *Fomes fomentarius*, *Staphylococcus aureus*, Polyporaceae, antibacterial

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Evaluation of on anti - Candida effect of essential oil of Rose and compound mouthwash (Rose essential oil, grape vinegar) on Candida strains (albicans and non-albicans)

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Introduction: In this experimental study, an experiment was carried out using an *in vitro* method to determine the antifungal effects of plant compounds. Serial dilution method is used to determine the minimum inhibitory concentration of fungal growth (MIC). Essential oil of Rose with distilled water and Rose Stoke solution with vinegar are mixed according to 8 parts versus 2 parts .

Standard strains studied: *C.albicans* ATCC10231- *C.dubliniensis* ATCCCD60- *C. glabrata* ATCC90030- *C. parapsilosis* ATCC22019. Nistatin chemical was used as a positive control.

Results: Based on the results obtained in this study, (Minimum inhibitory concentration) MIC of *Candida albicans* is 6µg/ml and 8µg/ml for Rose essential oil and compound mouthwash, respectively. The MIC of *Candida dubliniensis* for Rose essential oil is 31µg/ml and for compound mouthwash is 12µg/ml. Also, the MIC of *Candida parapsilosis* strain for Rose essential oil is 62µg/ml, and for compound mouthwash is 25µg/ml, and MIC of *Candida glabrata* for essential oil of Rose is 31µg/ml and for compound mouthwash is 12µg/ml. According to the results of the Punched-Whole test the average diameter of the non-growth halo of Rose oil and vinegar for *Candida albicans* is 17 and 6mm, respectively. The results of the Punched-Whole test for essential oil of Rose for all *Candida dubliniensis*, *Candida parapsilosis* and *Candida glabrata* is 15mm while the results of the test for Mouthwash for mentioned *Candidas* is 0mm.

Conclusion: According to the results, essential oils of Rose and Mouthwash on standard strains of *Candida albicans* and non- *albicans* have good anti-fungal effects. Also, *Candida glabrata* is more resistant than *Candida albicans*. Due to this research, the essential oil of Rose and Grape vinegar can be used to make oral herbal mouthwash for patients with removable prosthodontics who have oral Candidiasis.

Keywords: Mouthwash, *Candida*, Grape vinegar, Rose essential oil

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Antifungal activity of Iranian desert truffle extract against *Malassezia* species obtained of Dandruff samples

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Introduction: Dandruff is a mild clinical state of seborrheic dermatitis, caused by *Malassezia* species. There are many medications to treat this disease, but the recurrence rate of this disease is still high. Truffle is believed to have potential antifungal activity. Regarding this, the present study conducted to investigate the anti-*Malassezia* activity of Iranian Desert Truffle Extract (IDTE).

Material and methods: In this study, 60 skin samples were collected from people with Dandruff. The samples were examined under a microscope using 10% potassium hydroxide solution and cultured in modified Dixon agar for 5 days. The *Malassezia* species were further confirmed by PCR-sequencing method. Then anti Dandruff activity of IDTE was studied.

Results: Of 60 samples of Dandruff, 43 (71.6%) samples were positive for *Malassezia* yeast as compared to healthy individuals (22%). The prevalence of *Malassezia* species was as: *M. globosa* (39.5%), *M. resteri* (25.5%), *M. furfur* (32.5%) and *M. sympodialis* (2.5%) respectively. Accordingly, Metanolic extract showed effective anti-fungal activity against the isolated *Malassezia* fungus. Especially IDTE had the highest MIC value against *M. furfur*.

Conclusion: The all of the isolated yeast of Dandruff samples were susceptible to IDTE. Therefore, this extract can be effectively used in formulation of natural Anti-Dandruff shampoo.

Keywords: Dandruff, *Malassezia* yeasts, Iranian desert Truffle extract (IDTE)

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Determination of antifungal and antibiofilm activities of vitamin D3 against *Candida* species and expression of genes related to morphogenesis and pathogenesis.

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Introduction: Vitamin D3 is a material which the body needs for hemostasis of calcium and healthy bones. Deficiency of vitamin D3 has been related to many diseases and microbial infections. Also, *Candida* species is known as opportunistic fungal pathogens that could cause candida infections in people. It has been suggested that biofilm formation by *C.albicans* play an important role in its pathogenicity. Therefore, we determine to investigate the effect of vitaminD3 on growth of *Candida* species and, biofilm formation and the expression of genes related to morphogenesis and pathogenesis of *Candida albicans*.

Material and methods: Fungi static and fungicidal activities of *Candida* species was evaluated by micro-broth dilution method based on CLSI protocol. Biofilm formation by *Candida albicans* was measured using XTT reduction assay after exposing yeast cells to different concentrations of vitamin D3 in comparison with untreated cells. Also, Expression of adhesion-related gene (ALS1), hyphal cell wall protein gene (HWP1), secreted aspartyl proteinase (SAP6), and morphogenesis pathway regulatory gene (EFG1) were analyzed by RT-PCR in the

treated yeast cells with different concentrations of vitamin.

Results: Vitamin D3 indicated fungi static and fungicidal activity on *Candida* species in different concentration. Furthermore, inhibited biofilm formation in a dose dependent manner. RT-PCR analysis of RNA extracted from *C. albicans* indicated that different concentrations of vitamin D3 could change the levels of expression of genes, in a different manner. These changes has been shown as increasing in expression of *ALS1*, *SAP6* and *EFG1* genes while had no considerable effect on *HWP1* expression.

Conclusion: Obtained data provided new insight into the effect of vitamin D3 on growth, transition, biofilm formation and pathogenicity of *C.albicans* .According to prevalence of drug resistance in *Candida* species and unfavorable side effects of conventional antifungal drugs, vitamin D3 could be useful as a new antifungal agent which because of its liposolubility might change the integrity of the cell membrane.

Key word: Vitamin D3, *Candida*, Morphogenesis, Pathogenesis and Real Time PCR.

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RNA interference-based silencing of the multidrug resistance protein 1 (*MDR1*) gene in voriconazole- resistant *Aspergillus flavus* isolates

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Introduction: RNA interference (RNAi) is a post-transcriptional gene silencing (PTGS) phenomenon by which RNA molecules knock down essential genes responsible for vital as well as virulence factors. Lately, Asian patients with Invasive aspergillosis (IA) develop resistance to Azole as a result of

a long term exposure to this drug as a treatment. On the basis of recent studies, roughly 40% of Voriconazole-resistant *Aspergillus flavus* demonstrates a wide range of *MDR1* overexpression compared with the wild-type strain. Thus, this study is an endeavor to delve into the silencing potentials of siRNA on *MDR1* in Voriconazole-resistant *Aspergillus flavus* strains as the target gene.

Material and methods: In this study, four Voriconazole-resistant *Aspergillus flavus* strains and four Voriconazole-susceptible strains were used (These isolates were identified previously and were stored in the culture collection of the Medical Mycology Laboratory, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran). We designed three *MDR1*-specific siRNAs and after the co-transfection of siRNA into *Aspergillus flavus* strains, using lipofectamine, we investigated the effect of different siRNA concentrations (5, 15, 25, 50nM) on *MDR1* expression by qRT-PCR. Finally, the Minimum Inhibitory Concentrations (MICs) of Voriconazole for isolates were determined by the broth dilution method.

Results: *MDR1* siRNA induced 2, 4, 8, 17-fold reductions in *MDR1* mRNA expression in Voriconazole-resistant strains following the treatment of the cells with concentrations of 5, 15, 25, 50nM siRNA, respectively. The results demonstrated the MIC values of Voriconazole were significantly reduced in the treated groups with *MDR1*-specific siRNA, both at concentrations of 25nM (1, 2, 4, 4µg per ml) and 50nM (0.5, 2, 4, 4µg per ml), when compared to the untreated groups (4, 8, 16, 16µg per ml).

Conclusion: In this study, we suggested that siRNA-mediated specific inhibition of *MDR1* gene can play role in Voriconazole-resistant *Aspergillus flavus* strains and this could be one of the target genes for inactivation. However, we recommend evaluating various multidrug resistance efflux pumps (MDR-EPs) and performing experiments on the effect of siRNA on human cells in the future studies. The current study promises a bright prospect for the 5th Iranian Congress in Medical Mycology, Tehran, Iran, All rights reserved. For permissions, please e-mail: cmm.mazu.com

treatment of invasive aspergillosis through the effective deployment of RNAi and gene therapy.

Keywords: *MDR1* gene, RNA silencing, Voriconazole, *Aspergillus flavus*.

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Somatic protein profile of six various yeasts isolated from patients with Psoriasis

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Introduction: Psoriasis is an immune-mediated skin disorder that seems to be increasing in prevalence throughout the world. This disease characterized by erythematous lesions covered with silvery scales and infiltration of leukocytes into both the dermis and the epidermis. Although the exact etiology of psoriasis is still unknown, it is known that yeasts can play a significant role in exacerbation of psoriasis via stimulation of immune responses by their protein antigens. Among the yeast *Candida* spp, and *Saccharomyces cerevisiae*, that are found in human and environment, are more important in stimulating the immune system. Somatic proteins are the most important antigens and allergens of yeasts that can provoke and exacerbate psoriasis by stimulating the inflammatory response of the immune system. Most studies have examined only somatic proteins of *Candida albicans* and not other species. The genus *Candida* comprised more than 150 species but only five species are regarded as frequent colonizer of skin and mucosal surfaces of patients with psoriasis. So this study aimed to assess somatic proteins of five various species of *Candida* genus and as well as *Saccharomyces cerevisiae* isolated from patients with psoriasis.

Material and methods: Two isolates of *Candida albicans*, two isolates of *Candida parapsilosis*, two isolates of *Candida guilliermondii*, one strain of *Candida lipolytica*, one strain of *Candida tropicalis*, and as well as one strain of *Saccharomyces*

cerevisiae were isolated from patients with psoriasis. These yeasts fungi were identified by using the sequence of the D1/D2 domain of the 26S rRNA gene. Somatic proteins of isolated yeasts were analysed using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis.

Results: Isolated yeasts had 56 different somatic protein bands, which ranged from 11 to >180kDa. *Candida albicans*, *Candida parapsilosis*, *Candida guilliermondii* and *Candida lipolytica* had 12 protein bands, and *Candida tropicalis* and *Saccharomyces cerevisiae* had 7 protein bands and 8 protein bands, respectively. There was no significant difference in the number of somatic protein bands between *Candida albicans* and other yeasts (P=0.391).

Conclusion: The results of this study showed that different strains of the *Candida* genus have the same electrophoretic pattern of somatic proteins.

Keywords: *Candida albicans*, *Saccharomyces cerevisiae*, Somatic protein, Yeast

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Comparison of the inhibitory effects of ethanolic and aqueous extract of *Vitexagnus-castus* with fluconazole against *Candida albicans*

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Introduction: Vulvovaginal candidiasis is one of the most common infections in female genital organs, which is caused by *Candida* species. *Candida albicans* is the causative agent of more than 80% of infections, and the role of non-*Candida* strains in the disease etiology is less prominent. The expansion of Azoles resistance among *C. albicans* strains is considered an important medical problem. According to previous studies, *Vitex agnus-castus* (vitex) has some antimicrobial effects. We aimed to evaluate the anti-fungal effects of aqueous and alcoholic extracts of vitex against clinical vaginal isolates of *C. albicans* in comparison with Fluconazole.

Material and methods: Gas chromatography-mass spectrometry analysis was performed on vitex to identify its possible bioactive components. Forty *C. albicans* clinical isolates were identified by using germ tube, chlamyospore production, culture on CHROM agar, and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Finally, after the extraction of vitex, drug susceptibility test was carried out according to the clinical laboratory standards institute (CLSI) M27-S4 document guidelines.

Results: The major chemical components of vitex leaf as determined by gas chromatography included α -Pinene, isoterpinolene, caryophyllene, and azulene. The minimum inhibitory concentrations (MICs) of aqueous and alcoholic extracts of vitex, as well as Fluconazole were within the ranges of 15.62–62.5, 7.81–15.62, and 0.25–8 μ g/mL, respectively.

Conclusion: Our findings showed that the alcoholic and aqueous extracts of vitex had antifungal activity against clinical isolates of *C. albicans*. Moreover, the alcoholic extract of vitex and Fluconazole were more effective against clinical vaginal isolates of *C. albicans* compared to the aqueous extract of vitex.

Keywords: Antifungal activity, *Candida albicans*, *Vitex agnus-castus*.

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Antifungal effects of two endemic aqueous extracts on oral yeasts in diabetic patients

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Introduction: Diabetic patients are particularly susceptible to fungal infections due to modifications that occur in their immunological system. Herbal extracts with inhibitory activity have been useful as oral antifungal drugs for the control of yeasts in oral cavity in diabetic patients. The major aim of this study was to determine the effects of *Chenopodium Album* (36HYU2385) and *Apium Nodiflorum* (36HYU2362) aqueous extracts on *Candida albicans* isolated from oral cavity in diabetic patients. These two herbs have been traditionally used as therapeutic agents for infections. They grow around the Zagros Mountains in Iran. *Apium nodiflorum* decreases the oxidative damage in liver and kidney, also it has anticancer activities. *Chenopodium album* has been used in the treatment of cardiovascular disorders, abdominal pain, eye disease and throat troubles. To our knowledge, this is the first report of the effects of these extracts on the growth of *Candida albicans* in oral cavity.

Material and methods: In this report, fifty clinical isolates of *C. albicans* were isolated from diabetic patients. The antifungal susceptibility testing of aqueous herbal

extracts were performed according to CLSI document M27-S3.

Results: Results indicated that minimal inhibitory concentration (MIC) in the extracts of *C. album* and *A. nodiflorum* against *C.albicans* growth were 31.25mg/ml and 15.62mg/ml respectively.

Conclusion: According to the results, it can be suggested that these herbal extracts may have antifungal potential to be used in diabetic patients as yeast controller.

Keywords: Diabetic patients, *Candida albicans*, Herbal extracts

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Determination of antifungal and antibiofilm activities of vitamin D3 against *Candida* species and expression of genes related to morphogenesis and pathogenesis.

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Introduction: Vitamin D3 is a material which the body needs for hemostasis of calcium and healthy bones. Deficiency of vitamin D3 has been related to many diseases and microbial infections. Also, *Candida* species is known as opportunistic fungal pathogens that could cause candida infections in people. It has been suggested that biofilm formation by *C. albicans* play an important role in its pathogenicity. Therefore, we determine to investigate the effect of vitaminD3 on growth of *Candida* species and, biofilm formation and the expression of genes related to morphogenesis and pathogenesis of *Candida albicans*.

Material and methods: Fungi static and fungicidal activities of *Candida* species was evaluated by micro-broth dilution method

based on CLSI protocol. Biofilm formation by *Candida albicans* was measured using XTT reduction assay after exposing yeast cells to different concentrations of vitamin D3 in comparison with untreated cells. Also, Expression of adhesion-related gene (*ALSI*), hyphal cell wall protein gene (*HWPI*), secreted aspartyl proteinase (*SAP6*), and morphogenesis pathway regulatory gene (*EFG1*) were analyzed by RT-PCR in the treated yeast cells with different concentrations of vitamin.

Results: Vitamin D3 indicated fungi static and fungicidal activity on *Candida* species in different concentration. Furthermore, inhibited biofilm formation in a dose dependent manner. RT-PCR analysis of RNA extracted from *C. albicans* indicated that different concentrations of vitamin D3 could change the levels of expression of genes, in a different manner. These changes has been shown as increasing in expression of *ALSI*, *SAP6* and *EFG1* genes while had no considerable effect on *HWPI* expression.

Conclusion: Obtained data provided new insight into the effect of vitamin D3 on growth, transition, biofilm formation and pathogenicity of *C. albicans*. According to prevalence of drug resistance in *Candida* species and unfavorable side effects of conventional antifungal drugs, vitamin D3 could be useful as a new antifungal agent which because of its liposolubility might change the integrity of the cell membrane.

Key word: Vitamin D3, *Candida*, Morphogenesis, Pathogenesis and Real Time PCR.

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The possible effect of vitamin D3 on *Candida* growth and pathogenicity

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Introduction: Vitamin D3 is a substance which the body needs for hemostasis of calcium and healthy bones. Deficiency of this vitamin has been related to many diseases and microbial infections. Recently, researchers have found a positive relationship between vitamin D and *Candida* infection. As biofilm formation by *C. albicans* play an important role in its pathogenicity, we investigated the effect of vitamin D3 on the growth, biofilm formation and expression of genes related to its morphogenesis and pathogenesis.

Material and methods: Antifungal activities of vitamin D3 against *Candida* species was evaluated by micro-broth dilution method based on CLSI protocol. Inhibition of *Candida albicans* biofilm formation by different concentration of vitamin D3 was measured using XTT reduction assay. Moreover, expression of adhesion-related gene (*ALSI*), hyphal cell wall protein gene (*HWPI*), secreted aspartyl proteinase (*SAP6*), and morphogenesis pathway regulatory gene (*EFG1*) were analyzed by RT-PCR in the treated yeast cells with different concentrations of vitamin D3.

Results: Vitamin D3 exhibited fungistatic activity on *Candida* species with minimum inhibitory concentration at concentration of 1 to 128µg/ml. Furthermore, vitamin D3 inhibited the formation of *C. albicans* biofilm in a dose dependent manner. RT-PCR analysis of RNA extracted from *C. albicans* indicated that different concentrations of vitamin D3 could change the of expression levels of genes, in a different manner.

Conclusion: Based on our results and those of previous studies that found a high prevalence of vitamin D deficiency in patients with candidiasis, vitamin D3 might be used in treating *Candidiasis* in addition to antifungal therapy.

Keywords: Vitamin D3, *Candida*, Antifungal

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Determination of chemical composition and anti-fungal activities of aromatic water of *Zataria multiflora* Boiss

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Introduction: *Candida* species, as a part of normal flora of mucocutaneous surfaces, may cause wide range of clinical symptom from superficial infection to mucocutaneous or visceral candidiasis. These yeasts may also cause upper gastrointestinal especially among those with imbalance normal flora or compromised immune system. Regarding universal emergence of antifungal-resistant *Candida*, there is growing tendency in finding novel antifungal agents especially from natural resources. Among which, aromatic waters (AW) distilled from medicinal plant containing essential oils with known antimicrobial properties might be a good candidate. The aim of this study was to determined *in vitro* and *in vivo* antifungal activities of *Zataria multiflora* Boiss AW against *Candida* species.

Material and methods: The chemical composition of the essential oil from AW *Zataria multiflora* analyzed by gas chromatography-mass spectrometry (GC-MS). The antimicrobial activity of the essential oil against *Candida* species was evaluated by broth micro-dilution as per the Clinical and Laboratory Standards Institute (CLSI) methods. Moreover, biofilm formation inhibition and antioxidant activity of the AW was measured by using a XTT reduction and DPPH methods, respectively. Experimental activity of the AW in the prevention or treatment of GI candidiasis was also evaluated in animal model by both culture and histopathological methods.

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Results: GC-MS analysis revealed that the major constituents of the essential oil of AW were thymol (40.67%) and carvacrol (46.56%). The *Zataria multiflora* AW exhibited antimicrobial activity against all tested yeasts with MICs in the range of 0.25–0.5V/V. In addition, the EO inhibited the biofilm formation of *Candida albicans* at concentration up to 0.5V/V (70%). The AW significantly decreased the CFUs in mice receiving AW in comparison with those of control group. Similarly, histopathological analyses showed that *Candida* colonization decline in mice following administration of AW of *Zataria multiflora* in therapeutic trial.

Conclusion: The considerable antifungal activity of the AW against the examined *Candida* species might be related to high concentration of phenolic monoterpenes in the EO distilled from AW. In addition to considerable antimicrobial effects of the AW, antioxidant activity of the AW attributed to inducing the healing process of tissue necrosis found in mice treated with AW in comparison to the controls. Considering wide range of antifungal activities of the examined AW, it might have potential to be used in the management of alimentary candidiasis or as mouthwash or other pharmaceutical products.

Keywords: *Candida albicans*, *Zataria multiflora* Boiss, Aromatic water, Antifungal activity

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Antifungal activities of the essential oils from Iranian medicinal plants against common causes of vaginal candidiasis

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Introduction: Vaginal candidiasis is one of the most prevalent fungal infections and 70-75% of healthy, grown-up women get infected by this disease at least once during their reproductive age. Over the past two decades, emergence of resistance to routine antifungal drugs in particular Azoles has been accelerated dramatically. To overcome resistance, there is a great tendency in using herbal products originating from natural resources such as essential oils (EOs). Seven aromatic plants used in this study are among popular traditional Iranian medicinal plants with potential application in modern medicine as anti-oral infectious diseases. This study was conducted to determine the chemical composition and antimicrobial activities of essential oils from seven medicinal plants against *Candida spp.*

Material and methods: The chemical compositions of EOs distilled from seven plants were analyzed by gas chromatography/mass spectrometry (GC/MS). These plants included *Satureja khuzestanica*, *Satureja bachtiarica*, *Ocimum sanctum*, *Artemisia sieberi*, *Zataria multiflora*, *Carum copticum* and *Oliveria decumbens*. The antimicrobial activities of the essential oils were evaluated by broth micro-dilution in 96 well plates as recommended by the Clinical and Laboratory Standards Institute methods.

Results: The tested EOs inhibited the growth of the examined *Candida* at concentrations of 0.015-2 μ L/mL. All the *Candida spp.* were killed by the EOs at about the same or twice the concentration of their corresponding MICs. Of the examined EOs, *Satureja khuzestanica*, and *Zataria multiflora* respectively showed the highest antifungal activities, while *Artemisia sieberi* exhibited the lowest antimicrobial properties.

Conclusion: Based on these results, the EOs of the above mentioned plants might be used as an antifungal agent in treatment and control of the vaginal candidiasis.

Keywords: Essential oil, *Candida*, Antifungal activity

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The evaluation of the methanol extract of Azadiractin leaves on fungi growth and aflatoxin production by aflatoxin producing strain

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Introduction: Mycotoxin is the second metabolite and their effect on separate from infection on their lifestyle. One of the most important of them is aflatoxin which produces by species *Aspergillus*. As regards Azadiractin from past up to now is attractive because of antimicrobial properties and usability as the pharmaceutical plant, so the aim of this research the evaluation of the methanol extract of Azadiractin leaves on fungi growth and aflatoxin production was designed.

Material and methods: The methods were designed to gain the aim include of produce the methanol extract of Azadiractin leaves, check effect of antifungal activity on four species of *Aspergillus* (*Aspergillus flavus* 24, *Aspergillus flavus* 39, *Aspergillus parasiticus* and *Aspergillus niger*) by spreading on agar and then minimum fungicide concentration (MFC) and minimum inhibitory concentration (MIC) method, in next step evaluation inhibit of produce aflatoxin by three species(*Aspergillus flavus* 24, *Aspergillus flavus* 39, *Aspergillus parasiticus* NRRL 2999) by methanoli extract of Azadiractin by high-performance liquid chromatography (HPLC).

Results: Result of agar diffusion showed that the Azadiractin extract did not inhibit four species of *Aspergillus* and this problem after growing fungi on broth culture except negative control, this step proved by MIC and MFC methods. In addition by growing mycelium beside of different density of

Azadiractin extract and weight them in three times, showed that by gradually increasing the concentration of Azadiractin extract and weighing them in three times, showed that by gradually increasing the concentration of Azadiractin extract, mycelium growth decreased, especially in 20 ml of density of extract, *Aspergillus flavus* 39 and in 15 ml and 20 ml density of extract, *Aspergillus flavus* 24 and *Aspergillus parasiticus* did not had any mycelium growing. At the end by HPLC technique its prove that this concentration of extract (10, 15 and 20 ml) can inhibit toxins which produced by *Aspergillus flavus* 39, also *Aspergillus parasiticus* toxin can inhibit beside of 2,55,10 and 15 ml of Azadiractin extract. Moreover, by increasing in the concentration of Azadiractin extract the produced toxin by *Aspergillus flavus* 24 decreased.

Conclusion: The results indicated that the Azadiractin leaves extract power on the produce reduction or amount of aflatoxin toxin.

Keywords: Mycotoxin, Aflatoxin, *Aspergillus*, Azadiractin

P-164

Investigation alcoholic and aqueous extract feature of *Artemisia sieberi* plant with fluconazole drug on the *Candida* strains

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Introduction: In recent years, fungal systematic infections which have been created by making disease fermentative especially for those patients who have stayed in the hospital, the common anti-fungal therapies are not effective by common drugs. The studies showed that different forms of anti-fungal drugs have been investigated,

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they showed good synergistic effects in front of some fungal disease. The present study has been done to investigate alcoholic, aqueous extract feature of *Artemisia sieberi* plant with fluconazole drug on the four *Candida* strains to introduce one effective and safe product.

Material and methods: In this method, a fresh culture of *Candida* strains on the Sabouraud dextrose agar has been used, the fresh fungal suspension has been provided. Fluconazole drug stock, aqueous and alcoholic *A. Sieberi* plant stock and combination of aqueous stock and drug, alcoholic stock and drug were provided. The values of minimum inhibitory concentration (MIC) and minimum fungicide concentration (MFC) were calculated by microdilution method for each compound.

Results: It has been observed that *A. Sieberi* aqueous extract was ineffective alonely and alcohol extract was effective on *C. albicans* and *C. krusei*. Synergism effect of *A. Sieberi* aqueous extract and fluconazole on *C. albicans* and *C. tropicalis* were observed the most amount and alcohol extract synergism effect of *A. sieberi* and fluconazole on *C. tropicalis* and *C. albicans* was equally effective.

Conclusion: With respect to the obtained results of this study, Fluconazole drug alonely highly effective on *C. albicans* and *C. tropicalis*. On the *C. glabrata*, aqueous extract *A. sieberi* and fluconazole synergism had most effective. *A.sieberi* alcoholic extract and fluconazole drug synergism had the highest effect on *C. krusei*.

Keywords: *Artemisia sieberi*, Fluconazol, *Candida* spp

P-165

Pseudohyphae formation in *Candida glabrata* due to exposure to CO₂

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Introduction: Pseudohyphae formation considered as a virulence factor in *Candida* species. Generally, *Candida glabrata* grow as budding yeast cells; however, reports display that *C. glabrata* could form pseudohyphae cells in response to some stimuli. Carbon dioxide (CO₂) is an important gaseous molecule which could act as a stimuli and induced filamentation in some yeast cells. In this study, we evaluated the ability of *C. glabrata* in forming pseudohyphal cells under different concentration of carbon dioxide.

Material and methods: *Candida glabrata* reference strain (ATCC 90030) was used in this study. Yeast sample were cultured on sabouraud dextrose broth (SDB) medium and incubated under 3%, 5%, and 10% CO₂ concentrations for 24, 48 and 72 hours. Control cultures also maintained without CO₂ pressure for 3 days. The possibility of pseudohyphae and mycelium formation in *C. glabrata* were evaluated.

Results: The results of this study revealed that the most branching filament-like cells were obtained in high CO₂ concentration (10%) after 72 hours. In low CO₂ concentration (3%), after 3 days only yeast and budding cells were observed without any pseudohyphae formation.

Conclusion: Obtained results in this study showed the positive effect of high CO₂ pressure on morphological changes which induced pseudohyphae formation in *C. glabrata* yeast cells.

Keywords: *Candida glabrata*, pseudohyphae, CO₂ pressure

P-166

Investigation of Antifungal activity of *Teucrium polium* L. against *Candida albicans*

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Introduction: Medicinal plants such as *Teucrium polium* L. are believed to be an important source of new chemical substances

with potential therapeutic effects. The main goal of this study is to evaluate the mechanism of antifungal potency of *T. polium* L. compared to amphotericin B against *Candida albicans*.

Materials and Methods: *T. polium* L. was collected from Isfahan, dried and powdered. Then methanol extract was prepared. *C. albicans* was added to extract, and the presence of sodium, potassium, glucose and amino acids were determined by flame photometer, autoanalyzer and HPLC.

Results: Results showed that Sodium, potassium, glucose and amino acids were released from *C. albicans*. So ethanolic extract of *T. polium* has inhibitory effect on the growth of *C. albicans*. Based on the HPLC results, 2181.232, 330.935, and 296.374 mg/ml of glutamine, glycine, and glutamic acid were in extract respectively.

Conclusion: The results suggest that antifungal mechanism of *T. polium* against *C. albicans* is similar to amphotericin B.

Keywords: *Teucrium polium*, Amphotericin B, Antifungal, HPLC.

P-167

Effect of *Morgana* extract on *Candida albicans* and *Trichomonas vaginalis* growth *in vitro*

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Introduction: Vaginitis is the most common gynecologic diseases of women throughout the world. The most common causes of vaginitis in symptomatic women are vaginal candidiasis (20-25%), Trichomoniasis (15-20%) and bacterial vaginosis (40-45%). *Candida* species are the most important infectious agents, Types resistant to common drugs are on the rise. *Morgana peregrina*

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grows in tropical and subtropical regions of the world and in Iran, it grows in Sistan and Baluchestan province. The seeds and oil of this plant have medicinal properties including antimicrobial, Anti-tumor and anti-inflammatory. The aim of this study was to find an alternative medicine to treat *Candida albicans* and *Trichomonas vaginitis*. The aim of this study was to investigate the anti-trichomonas and anti-candida effects of acetone extracts of *Moringa peregrina* in vitro.

Material and Methods: *C. albicans* exposure to various concentration (2, 1, ... and 0/003 mg/ml) of acetone extracts of *Moringa* and the rate of proliferation yeast cell was examined by Minimum Inhibitory Concentration (MIC) of the extract for *C. albicans* (ATCC 10231). We used acetone extracts of *Moringa peregrina* at concentrations of 375, 750, ... 3000 and 4000 µg/ml for the treatment of trichomoniasis. We evaluated the effect of the extracts after 24 and 48 hours. The final number of viable parasites were determined by trypan blue staining and neobar lamella, and IC50 (50% Inhibitory Concentration) value was calculated. The cytotoxic effect of the extract on the mice macrophage cells was investigated.

Results: The funding showed that yeast growth in 2 mg/ml of acetone extracts reduced approximately to (> 1%) and MIC value was 2 mg/ml for *C. albicans*. Comparison between treatment and control groups revealed a significant decrease in the viability of parasites in the treatment group at all concentrations after both 24 and 48 hours (P <0.05). After 24 hours the IC50 and SI values were calculated as 682 and 4.1 for parasite respectively.

Conclusion: in this study, investigated of different concentration of acetone extracts of *Moringa peregrina* on inhibiting the growth of *C. albicans* and *T. vaginalis*. The observed result compared to previous studies showed the inhibitory effect of extracts *Moringa* extracts on the growth of *Candida* and *Trichomonas*. It can be the identification and isolation of active ingredients of the plant, may lead to use of this extract for the

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treatment of both infections in the future studies.

Keyword: *Candida albicans*, *Trichomonas vaginalis*, *Moringa* extract, vaginitis, MIC

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In vitro Synergistic Interaction of clotrimazole/amphotericin B/terbinafine on Fluconazole-Resistant *Candida* Species Hashem Ahmadi^{1,2}, Fahima Alizada²

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Introduction: The number of opportunistic infections caused by *Candida* species has increased significantly in recent years. The antifungal drugs for the treatment of serious fungal infections remain limited. One of the biggest threats to clinical practice is the emergence of resistance for most of antifungal drugs. Combination therapy is the most widely used technique for the treatment of diseases. In this study, we were aimed to evaluate the combined antifungal effects of clotrimazole/amphotericin B/terbinafine on fluconazole-resistant *Candida* species.

Material and Methods: Eighteen *Candida* species were isolated from immunocompromised patients and identified by the morphological and biochemical methods. Antifungal susceptibilities were performed using the CLSI standard reference method (M27-A3 and M27-S4). Eventually, time-kill studies were performed on antifungal agents with each isolate.

Results: Indicated that the combination of clotrimazole/amphotericin B/terbinafine exerted synergistic effects with fractional inhibitory concentration index ranged from of fractional inhibitory concentration (FIC₅₀) = 0/3-2 and a range of FIC₉₀ = 0/18-1/5. In fluconazole-resistant *Candida* species. Time-kill studies showed reducing the number of yeast cells (p<0/05).

Conclusion: These results suggest that the combination of clotrimazole/amphotericin B/terbinafine would be worth exploring in the treatment of candidiasis. Additional

studies are nevertheless required to dissect the mechanisms.

Keywords: *Candida* Species, synergistic interaction, fluconazole

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Isolation and molecular identification of soil antifungal bacteria against *Aspergillus fumigatus*

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Introduction: Aspergillosis is an opportunistic infection that has the highest number of mortality among fungal disease in the world. The occurrence of azole-resistant aspergillosis strains, limiting and toxicity, and numerous side effects have created several problems for the treatment of aspergillosis. The aim of current study is characterization and identification of inhibitory soil bacteria with emphasis on antifungal effect against *Aspergillus fumigatus*.

Material and methods: A total number of 30 soil samples were collected from the northern forests of Gilan, Mazandaran and Golestan areas so that from different parts in these areas. The suspension prepared from each soil sample and tested for antifungal activity against *A. fumigatus* by agar plate bioassay. Active bacteria isolated and purified from soil samples with antifungal activity against *A. fumigatus* and investigated their antifungal activity. Genomic DNA of active bacteria extracted and 16S ribosomal sequences from DNA were amplified by using the universal primers 27F and 1492R in a thermal cyclor.

Results: A total of 162 actinomycete isolates were obtained from 30 soil samples based on their morphological characteristics (opaque, rough, granular, velvety and granular) on agar plates and gram staining. 4 isolates with cods H-57, I-65, I-71 and I-92 showed high potential on inhibiting *A. fumigatus* growth. 40 isolates had moderate inhibition, 33

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isolates had low inhibitory powers and 85 isolates did not have the power to inhibit *Aspergillus spp* growth. Based on the 16s rDNA sequence analyzer 4 strong inhibitory bacteria were identified *Streptomyces libani*, *Streptomyces platensis*, *Bacillus subtilis*, *Sphingopepyxis sp.* isolates.

Conclusion: Identified bacteria was shown as finding rich sources of useful antifungal metabolites for designing new antifungal drugs discovery.

Keywords: Soil bacteria, *Aspergillus fumigatus*, Molecular identification

P-170

Inhibitory effect of garlic extracts on growth of *Candida albicans* and *Geotrichum candidum*

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Introduction: Garlic is one of the most important plants used in foods for its flavoring, prophylactic and therapeutic properties. It has revealed that garlic has activity against bacteria and fungi. The aim of this study was to evaluate the antifungal activity of *Allium sativum* aqueous, methanolic and ethanolic extracts against different strains of *Candida albicans* and *Geotrichum candidum*.

Material and methods: Disc and well diffusion methods were applied to measure inhibitory effects of the extracts against all targeted strains tested in the experiment. Minimal inhibitory concentration (MIC) of the extracts were determined for each strain.

Results: The highest antifungal activity was observed at a concentration of 300 µg/ml of aqueous extract. The MIC was determined to

be 350 µg/ml *Geotrichum candidum* and 300 µg/ml for *Candida albicans*. The MIC of methanolic and ethanolic extracts were higher than corresponding figures for aqueous extracts.

Conclusion: The results demonstrated that garlic has notable antifungal activity against *Candida albicans* and *Geotrichum candidum*.

Keywords: *Allium sativum*, antifungal effect, *Candida albicans*, *Geotrichum candidum*

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Workers exposure to aflatoxin B1 in urban wet waste sorting centers

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Introduction: Inhalation of contaminated dust particles, such as bio-aerosols containing toxins, including aflatoxins, is one of the biggest threats to workers in the waste industry, especially urban and domestic waste. Aflatoxin B1 is the most common aflatoxin, which is a genotoxic and cytotoxic agent. Monitoring of aflatoxin B1 (AFB1) levels in the settled dust of wet waste sorting occupational environments were the aim of this study.

Material and methods: In four different sites of sorting municipal recyclable wet waste centers, 200 male workers (the Mean ± SD and range of age was 29.65 ± 7.1 and 18 - 41 years, respectively) were involved in the processing of recyclable wet waste in semi-enclosed silos, in Halgheh-Darreh Recycling Center area in Karaj city of Iran. In this study,

40 settled dust samples in sorting centers and control surface samples were obtained in the spring and fall seasons of the year. Each of randomly selected surface sample was 10 × 10 cm². The settled dust of each selected surface, was sampled using of personal air sampling pump on fiberglass filter with 0.7 µm pore size and 2.5 cm diameter.

AFB1 concentration of settled dust samples was measured using HPLC with Fluorescence detector and post column electrochemically generated bromine cell, after extraction with Methanol-H₂O (80/20) and clean up with use of AflaTest immune affinity column. The mobile phase consisted of a deionized water-acetonitrile-methanol mixture (50:20:30, v/v) with 1 mM KBr at 1 ml/min isocratic flow rate and 185 bars pressure. Fluorescence detection was achieved at 362 nm of excitation and 435 nm of the emission wavelength.

Results: The mean ± SD and range of AFB1 in settled dust samples in wet waste processing sites were 0.09 ± 0.0619, 0.013 - 0.21 and 0.1082 ± 0.0608, 0.011 - 0.206 ng/100cm² in the spring and fall seasons respectively. The AFB1 in settled dust of control samples was not detected.

Conclusion: The results of this study showed AFB1 was present in all of the deposited dust surface samples in wet waste processing center as compared with control samples (p<0.001). Based on our results there were no differences between the amount of AFB1 in spring and fall (p>0.05). The detection of AFB1 in these environment provides evidence that workers are exposed to AFB1 and the dangers of this toxin in this industry and this pose great levels of risk on human life on creating of especially hepatocellular carcinoma.

Keywords: settled dust, aflatoxin B1, wet waste processing, Municipal waste sorting

P-172

Activity of ethanol extract of *Rumex alveolatus* on *Candida glabrata*

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Introduction: The *candida* is undoubtedly one of the most important and most opportunistic fungal diseases in human. *Candida glabrata* is important for hospital infections. Due to its resistant form, this fungus is rapidly resistant to many azole compounds, especially fluconazole. *Rumex alveolatus* is traditionally used for the treatment of pain and inflammation. The aim of this research was the study of the ethanol extract mechanism of *R. alveolatus* on *C. glabrata*.

Materials and Methods: In the present study, the mechanism of anticandidal activity of *R. alveolatus* toward Amphotericin B was investigated by flame-photometry, autoanalyzer and HPLC. The type of damage to *C. glabrata* was investigated by SEM.

Results: In this study, results showed the ethanolic extract of *R. alveolatus* causes the release of Na⁺, K⁺, glucose and amino acids from *C. glabrata* similar to Amphotericin B. Scanning electron microscope showed the damage in *C. glabrata*.

Conclusion: According to the results of this study, the effect of *R. alveolatus* on the fungal cell membrane and cause single ion leakage capacity, as well as amino acids, are the width of the membrane. In other words, the destruction of cell walls results in leakage of cellular out of the cell, resulting in cell death.

Keywords: *Rumex alveolatus*, *Candida glabrata*, HPLC, ESM. Flame photometry

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In vitro* antifungal susceptibility testing of six azole agents against *Candida glabrata

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Introduction: *Candida glabrata* is the most common non-*albicans Candida* (NAC) cause of candidiasis, particularly vaginal infections. Furthermore, during the last decades, a decreasing in susceptibility to some antifungal agents was observed among them. The aim of this study was to compare the *in vitro* antifungal activities of luliconazole with five available azoles including; itraconazole, voriconazole, posaconazole, fluconazole and clotrimazole against *C. glabrata* isolates from patients with *Candida* vaginitis.

Material and methods: Sixty one *C. glabrata* isolates were identified by DNA sequencing and antifungal susceptibility assays were carried out using a modified resazurin broth microdilution method according to the EUCAST definitive document EDef 7.3.

Results: The sequence analysis of the isolate confirmed as *C. glabrata* and recorded on NCBI GenBank (Accession numbers, LC389224-84). The resistance or nonwild-type rates of the isolates to clotrimazole, itraconazole, posaconazole, fluconazole and voriconazole were 65.6%, 13.1%, 3.3%, 1.6% and 0% respectively. Importantly, none of the isolates of *C. glabrata* were susceptible to fluconazole. Luliconazole showed the best activity with the lowest geometric mean as 0.1 µg/mL, compared to 5.5 µg/mL, 1.7 µg/mL, 1, 0.3 µg/mL, 0.14 µg/mL respectively for fluconazole, itraconazole, clotrimazole, posaconazole and voriconazole agents.

Conclusion: Luliconazole was highly active against *C. glabrata* isolates, and according to no available luliconazole cream vaginal for treatment so we recommend further investigations of in the field of therapeutic effect luliconazole on vulvovaginal candidiasis with the cause of *C. glabrata*.

Keywords: *Candida glabrata*, Vulvovaginal candidiasis, luliconazole, itraconazole, clotrimazole, posaconazole, voriconazole

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Anti-dermatophyte effect of *Olea europaea* leaf extract on *Trichophyton*

mentagrophytes and *Microsporum gypseum*

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Introduction: Dermatophytosis is a common fungal disease that caused by dermatophytes. Dermatophytes are of keratin friendly fungi that involve keratinized tissues such as hair, nail and skin. In recent years, increased antifungal drug resistance was reported. In addition, antifungal drugs have limited effects and have many side effects. For example Griseofulvin has been available antifungal agent for the treatment of dermatophytosis for about 40 years, but it has side effects such as nausea, diarrhea, headache, rash, and sometimes allergy, ketoconazole causes the inhibition of liver enzymes and an increase in the concentration of other drugs, and inhibition of synthesis of steroid hormones. Recently scientists interested in treatment infection diseases by herbs. Herbals are effective alternatives for chemical treatment methods. In this research, the anti-dermatophyte effect of *Olea europaea* leaf extract on *Trichophyton mentagrophytes* and *Microsporum gypseum* was studied.

Materials and Methods: We collected olive leaves and dried in shade then put them in powder form and extracts of *Olea europaea* leaf were extracted using Soxhlet. *T. mentagrophytes* (PTCC 50541) and *M. gypseum* (PTCC 5070) were used in this study were prepared from fungal colocation of Tehran University then cultured in SCC and incubated in 28°C for 7-14 day. Antifungal effect of them was measured by disc diffusion method, minimum inhibitory concentration (MIC₈₀) and minimum fungicidal concentration (MFC).

Results: The results showed that the diameter of the inhibition zone of the *Olea europaea* leaf extract was 24±0/5 mm for *T. mentagrophytes* and it was 21±0/5 mm for

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M.gypseum Which are not significantly different from that of griseofulvin (p≤0.05). The concentration of extract obtained as MIC₈₀ has been 0/78 ±1 mg/ml for *T. mentagrophytes* and it has been 3/125±1 mg/ml for *M. gypseum*. The concentration of extract obtained as MFC has been 0/39±1/2 mg/ml for *T. mentagrophytes* and it has been 1/56±1 mg/ml for *M. gypseum* which are not significantly different from that of griseofulvin, nystatin and terbinafine (p≤0.05).

Conclusion: According to the results, the *Olea europaea* leaf extract has a high antifungal effect potential on *T. mentagrophytes* and *M. gypseum*. As a result, the extract of plants is a very suitable and safe substitute for the treatment of fungal diseases such as dermatophytosis.

Keywords: Dermatophytes, Dermatophytosis, *Olea europaea*, *Trichophyton mentagrophytes*, *Microsporum gypseum*.

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Evaluation of antifungal activity of Sumac (*Rhus coriaria*) extract and its effect on expression of secreted aspartyl protease 9 in *Candida albicans*

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Introduction: *Candida albicans* is the opportunistic fungal pathogen in human. Factors such as long-term use of antibiotics and steroids, diabetes, weakened immune system and so on can make a person susceptible to candidiasis. Among the factors in the pathogenesis of *C. albicans*, secreted aspartyl proteinase (*SAP*) is a potential virulence factor. *SAP9* has the greatest impact on virulence and adhesion of this yeast. Due to the high cost and side effects of drugs and drug resistance leading to unsuccessful treatment, special attention has been paid to the use of medicinal plants. Sumac has a long history in treating disease. Hence, we decided to evaluate the effect of Sumac extract on a standard strain of *C. albicans* ATCC 10231 and its impact on *SAP9* expression in this study.

Material and methods: Yeast cell suspension was prepared from a 24-hour culture of standard *C. albicans* ATCC10231. Different concentrations of the hydro-alcoholic extract of Sumac and medium culture RPMI1640 according to CLSI protocol were added to the wells of a microplate and incubated at 35 °C for 48 hours. Then Real Time PCR was performed to evaluate the *SAP9* expression at concentrations 500 and 1500 mg/ml and positive control.

Results: Minimum inhibitory concentration (MIC) against 1500 mg/ml was achieved and *SAP9* expression at both concentrations (500 and 1500 mg/ml) was reduced.

Conclusion: This study showed that Sumac extract had the significant antifungal effect. Thus, this extract has a role in reducing *C. albicans* virulence by lowering *SAP9* expression and inhibiting the growth.

Keywords: *Candida albicans*, Sumac extract, *SAP*, Real Time PCR

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Effect of CO₂ concentration on drug sensitivity pattern of clinical isolates

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Introduction: Candidiasis is a common opportunistic fungal infection caused by yeasts that belong to the genus *Candida*. There are over 20 species of *Candida* yeasts that can cause infection in humans and animals, the most common of which is *Candida albicans*. In recent years drug resistance is an important object in medical because resistance to some of the drugs in patients especially immune compromised affects many factors like environmental factors such as CO₂ concentration. Because in the atmosphere with airborne contamination the percentage of CO₂ increases from the normal range and it affects many virulence factors of fungi like as drug resistance. The aim of this study was the evaluation of the drug sensitivity pattern of *C. albicans* isolates.

Material and methods: In this study, *C. albicans* isolates obtained from different sources of patients in Shariati hospital and identified by restriction fragment length polymorphisms (RFLP-PCR) method (2017-2018) and we evaluated the pattern of drug sensitivity by minimum inhibitory concentration (MIC) test with the M27-A4 protocol of CLSI. All samples of *C. albicans* was exposed to %5 CO₂ at 37 degrees for 4 weeks.

Results: Our finding indicated about 90% of the samples were resistance to Fluconazole and %5 CO₂ can change drug sensitivity in *C. albicans* isolated from patients.

Conclusion: These results can help us to introduce the better health protocol to patients.

Keywords: Drug resistance, *Candida albicans*, CO₂, Fluconazole

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Antifungal susceptibility of oral *Candida albicans* isolated from diabetic individuals

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Introduction: *Candida albicans* is the most fungal yeast in oral candidiasis. It is known that diabetic individual is predisposed to candidiasis. The prolonged diabetic condition can cause *C. albicans* infections and enhance mucosal changes. Thus, effective treatment can prevent of development of infection. The aim of this study was to determine the drug susceptibility pattern from *C. albicans* isolates were obtained diabetic patients.

Material and Methods: Samples were collected during a period of 12 months in Tehran diabetic center. *Candida* species were isolated and identified by using conventional germ tube and *CHROMagar* tests and molecular diagnostic method restriction fragment length polymorphisms (PCR-RFLP). Antifungal susceptibility profiles for fluconazole and itraconazole were performed based on CLSI M27-A4 method.

Results: *C. albicans* identified and included in this study 23% of *C. albicans* isolates were resisted to Fluconazole and 32% were susceptible to itraconazole.

Conclusion: The results of this study show that *C. albicans* are the main *Candida* species that causing oral candidiasis in Tehran diabetic center. It was concluded that these isolates had resistance against routine antifungal drug (fluconazole) whereas some of them were sensitive to itraconazole. These data can be helpful to the better decision to treat.

Keywords: *Candida albicans*, itraconazole, fluconazole, diabetic

P-178

***In vitro* antifungal effect of *Aureobasidin A* against *Candida albicans* growth**

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Introduction: In recent years, the incidence of deaths caused by important fungal infections (such as *candida albicans*) has been increased due to the inherent or acquired resistance to common antifungal drugs. It has attracted researchers' attention in finding safe antifungal compounds with a high impact as an alternative low toxicity drug in the treatment of fungal infections which can also be appropriate candidates to use in the drug design. *Aureobasidin A* (AbA) is a cyclic depsipeptide antibiotic, isolated from the filamentous fungus *Aureobasidium pullulans* R106, which is toxic to yeast such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans* and some of the *Aspergillus spp.* at low concentrations.

Material and method: In this study, antifungal activity of AbA determined according to a standardized broth microdilution method (Clinical and Laboratory Standards Institute (CLSI) document M27-A2), against fluconazole resistance *C. albicans* ATCC 10231, in comparing with fluconazole.

Result: The minimum inhibitory concentration (MIC₅₀) and MIC₉₀ of AbA and fluconazole were assessed in range 2, 0.25 (µg /ml) and 1024, 512 (µg /ml) against *C. albicans* ATCC 10231, respectively.

Conclusion: This study demonstrates that AbA has good inhibitory effect on the growth of fluconazole resistance *C. albicans*, which provides a basis for further researches to find more effective combinations regarding other natural products or drugs.

Keywords: *Candida albicans*, *Aureobasidin A*, Antifungal, Minimum Inhibitory Concentration (MIC)

P-179

Antidermatophyte effect of copper nanoparticle on *Trichophyton rubrum* (PTCC 5143)

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Introduction: *Trichophyton rubrum* is one of the most common dermatophytes that cause dermatophytosis in the world and Iran, it can cause infection in the skin, hair and nails. According to epidemiological reports, fungal diseases are on the rise and on the other hand, drug resistance has also been observed, and the fungal drugs that are used today have high side effects, so researchers are looking for a new drug for the treatment and control of dermatophytosis infections. Metal nanoparticles are one of the best alternatives. Nanoparticles are particles with the dimensions between 1 and 100 nm. Among them, copper nanoparticles, have been suitable chemical and physical characteristics and numerous applications in medicine and pharmacology. In this study, we used copper nanoparticles against *T. rubrum* (PTCC 5143).

Materials and Methods: *T. rubrum* (PTCC5143) that used in this study was prepared from fungal colocation of Tehran University then cultured in SCC and incubated in 28 °C for 7-14 day. Copper nanoparticles were prepared from Nano Nasb Pars Company, the size of them was 20 nm. Antifungal effect of them was measured by disc diffusion method, minimum inhibitory concentration (MIC₈₀) by macrodilution and microdilution method and minimum fungicidal concentration (MFC).

Results: The results showed that the diameter of the inhibition zone of the copper nanoparticles were 24.43±1 mm which is not significantly different from that of griseofulvin ($p \leq 0.05$). The concentration of essential oil obtained as an MIC₈₀ has been 15.6 ±0.5 µg/ml and the concentration of copper nanoparticles obtained as an MFC has been 31.25±0.85 µg/ml which is not significantly different from that of griseofulvin, nystatin and terbinafine ($p \leq 0.05$).

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Conclusion: According to the results, the copper nanoparticles have a highly antifungal effect potential on *Trichophyton rubrum*. As a result, the copper nanoparticles are a very suitable and safe substitute for the treatment of fungal diseases such as dermatophytosis.

Keywords: *Trichophyton rubrum*, copper nanoparticles, dermatophyte

P-180

Emergence of azole resistance in

Aspergillus fumigatus: a global problem

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Introduction: *Aspergillus fumigatus*, a ubiquitously distributed opportunistic pathogen, is the global leading cause of aspergillosis and causes one of the highest numbers of deaths among patients with fungal infections. Infections caused by *A. fumigatus* are a significant clinical issue and represent the second most-common form of fungal infection. Infections associated with azole-resistant *A. fumigatus* have a significantly-increased mortality in recent years.

Material and methods: Data were collected by performing searches using a specified set of Medical Subject Heading (MeSH) terms like *A. fumigatus*, Azole, Resistance, TR34/L98H, TR46/Y121F in the following databases and search engines: MEDLINE, ISI Web of Science, Ebsco, Science Direct, Scopus, and Google Scholar.

Results: The first azole resistance isolate was detected in 1997 in the U.S and azole resistance has been reported from many other countries too.

A. fumigatus resistance to Azole drugs has been raised as a global problem. For this reason, in late 2000, the medical community was forced to consider these reports, it was also found that most cases of azole resistance disease are due to the environmental

resistance of *A. fumigatus*. Azole Resistance in *A. fumigatus* can be Appearance in two ways: a Resistant that is generated during long clinical treatments and another Resistant caused by the environment due to the extensive use of demethylation inhibitors in agriculture (Multiple studies have now demonstrated TR34/L98H triazole resistance strains of *A. fumigatus* from soil. TR34/L98H is the predominant resistance mechanism of environmental origin in *A. fumigatus*).

Since the first report of the *A. fumigatus* azole resistance strain, several studies have been published investigating the underlying molecular mechanisms. In *A. fumigatus*, the main targets of the azoles are Cyp51 proteins, encoded by two different, *cyp51A* and *cyp51B*.

Tandem repeat sequence insertions at the *cyp51A* promoter consisted of overexpression and substitution. The integration of a 34-bp tandem repeat (TR34) with a substitution of leucine 98 to histidine (TR34/L98H) and a 46-bp tandem repeat insertion in the promoter region and substitutions of tyrosine 121 to phenylalanine and threonine 289 to alanine (TR46/Y121F/T289A) leading to an overexpression of *cyp51A* along which is related to VRC resistance also overexpression of the ATP-binding cassette (ABC) proteins transporter Cdr1B has been detected in recent years.

Recent epidemiological data show that this mechanism is an expanding problem, with reports from China, Iran, and India. Triazole resistance rates of *A. fumigatus* isolates with integration in the *cyp51A* promoter by Belgium 5.7 %, Denmark 4.5 %, France 0.85 %, Germany 3.2 %, Portugal 0 %, Spain 0.6 %, UK 6.6 %, Turkey 10.2 %, China 5.5 %, India 1.94%, Iran 3.3%, Japan 11.2 %, Kuwait 12.5%, USA 0.55 %, Australia 2.1% has been reported.

Conclusion: Due to the increased resistance of this fungus to modern drugs, we need to use new drugs with new technologies, such as nano, that have better therapeutic effects. Using the new methods of incidence and prevalence *A. fumigatus* resistant to Azole in the future is expected.

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Keywords: Azole Resistance, *Aspergillus fumigatus*, Emergence, TR34/L98H, TR46/Y121

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Study of aqueous and alcoholic extract of the Coriander effect on *Candida albicans* Yazdanfar Hasan¹, Yazdanfar¹ P, Soedi Z¹

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Introduction: Fungal infections, especially *Candida* species, are the most common opportunistic fungal infections and the treatments with chemical drugs have many side effects Coriander antimicrobial effects that have caused it to be considered in many microbial treatments. In this study, it has been tried to compare this herbal efficacy on *Candida albicans*.

Material and Methods: Aqueous and ethanol extracts of Coriander was obtained by drench method. The diameter of non-growing zone of *C. albicans* was estimated by the microdilution method.

Results: The mean diameter of the non-growing zone related to the extracts on *C. albicans* was 17.83 mm, 20.34mm, 23, 14mm (versing 5.75 mm, 8.45mm, 10, 15mm) of aqueous extracts .in25% and 50% and 75% concentration of Coriander seed extract , ethanol was more effective than distilled water.

Conclusion: The research made an experimental and used the coriander seed extract with 3 different concentrations of 25%, 50% and 75% with 2 different solvents (ethanol and distilled water) on *C. albicans*. The result showed the antifungal effect of the Coriander seed with ethanol was more than distilled water and the cause might be damage of cytoplasmic membrane and subsequent leakage of intercellular compounds such as DNA.

Keywords: Coriander. *Candida albicans*, infection, candidiasis, mouth infection

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The plant extract roles on the pathogenesis of *Cryptococcus neoformans*

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Introduction: *Cryptococcus neoformans* is encapsulated yeast that causes cryptococcosis. Cryptococcosis has changed from a relatively obscure fungal pathogen that infects immunocompetent patients to a leading cause of central nervous system infection in the world's enlarging immunocompromised populations. Two species in particular, *C. neoformans* (three serotypes: A, D, and AD) and *C. gattii* (two serotypes: B and C), are considered dangerous to humans. Two varieties of *C. neoformans* have been distinguished: *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii*. Cryptococcosis is a worldwide infection especially in North America and sub-Saharan Africa. Cryptococcal infections have been infrequently reported from Iran. The first published data is related to study from 1970. Most of the antifungal agents are classified in the azole antifungal drugs, especially fluconazole and echinocandins drugs. Due to the increased resistance to azoles and echinocandins, the application of medicinal plants, with or without current drug strategies, in order to reduce the side effects has been gained more importance. Recent clinical trials showed that antifungal agents which extracted from natural sources (herbs) showed limited side effects with high significance. Various plant extracts such as Carvacrol, *Thymus vulgaris*, *Coreopsis tinctoria*, *Laurus nobilis*, Eugenol, and *Mentha savolence* have been shown to inhibit the growth levels and degradation of plasma membranes of *C. neoformans*.

Conclusion: Cryptococcosis is the major fungal disease which has high mortality rate with vast spectrum of clinical forms. This infection is known as the third death cause among the AIDS patients. Due to the raising of different spectrum of side effects following the use of antibiotics (chief among them azoles), and also due to the expensive

treatment costs that cause the unavailability to these drugs, special attention should be paid to this field. Studies showed that the use of plants and their components play effective roles in the growth and fine-tuning of fungal gene expression, furthermore, on the capsules biosynthesis and pathogenicity *C. neoformans*. Application of studied plant extracts can be replaced by current strategies in order to the therapeutic goals for treatment of cryptococcosis.

Keywords: *Cryptococcus neoformans*, plant extracts, pathogenesis

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In vitro combination of Crocin with fluconazole against *Candida* species

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Introduction: The incidence of invasive fungal infections has been increased in recent years. The increasing use of azole drugs both for prophylaxis and treatment results in the gradual emergence of azole-resistant species. Accordingly, introducing a new strategy to improve the management of *Candida* infections is an urgent need. The present study evaluated the antifungal activity of *Crocin* alone and in combination with fluconazole.

Material and methods: Fifty clinical isolates of *Candida* species were applied. The identity of the isolates was confirmed using internal transcribed spacer (ITS) identification system. The interactions of *Crocin* and *fluconazole* were investigated by using a microdilution checkerboard method based on the CLSI reference technique with 96-well microtiter plates. To assess the interaction of combinations of drugs, the fractional inhibitory concentration index (FICI) was calculated.

Results: The minimum inhibitory concentration (MIC) obtained against *Crocin* alone indicated relatively high concentrations (MIC₅₀, 1 µg/mL). Our results demonstrated indifferent interactions between *Crocin* and *fluconazole* with FICI range values between 0.5 and 4 against *Candida* strains.

Conclusion: The High MIC value for *Crocin* against *Candida* species indicated no appropriate antifungal activity and even *fluconazole* did not significantly reduce the MICs. Therefore, other mechanisms which are not related to the mechanism of azole drugs are involved at High concentration of *Crocin*.

Keywords: *Crocin*, *Fluconazole*, *Combination*, *Candida*

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Bio-monitoring of ochratoxin A in human breast milk based on the HPLC method

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Introduction: Ochratoxin A is a nephritic and carcinogenic toxin that contaminated a variety of food and feeds in worldwide. The adverse effects of the toxin are affected by various factors including; type, dose, and duration of consumption toxin, recipient age (younger people are more sensitive), environmental factors such as stress and lifestyle. In this survey, we determined the concentration of OTA in nursing breast milk samples and evaluated the potential risk for the newborn babies based on the mycotoxin taking.

Material and methods: All 177 milk prepared of Sari and Isfahan lactating women assessed to clean-up by HPLC analyses.

Results: In total samples, only three cases (1.7%) were contaminated with ochratoxin A at 45, 90 and 140 ng/l levels. No significant differences observed between parameters age, body mass index, job, dietary pattern, and personal habits with OTA breast milk levels.

Conclusion: Although the OTA incidence was low, many internal and external factors including analytical methods and seasonal differences can also influence this ratio. Thus, further investigations on mycotoxin contamination in food and biological fluids as well as protection strategies to decrease the risk asses in other parts of the world are recommended.

Keywords: Mycotoxin, Ochratoxin A, Human breast milk, Infant foods

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In vitro activity of new azoles luliconazole and lanoconazole compared with ten other antifungal drugs against clinical dermatophyte isolates

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Introduction: In spite of high prevalence of dermatophytosis worldwide, the antifungal susceptibility patterns for dermatophyte family have been poorly evaluated. Luliconazole and lanconazole are new generation of imidazole antifungal agents. We evaluated the *in vitro* activity of 12 antifungal agents including anidulafungin, caspofungin, econazole, butenafine, tolnaftate, fluconazole, itraconazole, miconazole, terbinafine, lanconazole, luliconazole and griseofulvin against clinical dermatophytes.

Material and methods: One hundred clinical isolates of dermatophyte belonging to five species including *T. rubrum* (n=29), *T. interdigitale* (n = 52), *E. floccosum* (n = 4), *T. tonsurans* (n = 13), *M. canis* (n=2) were obtained from the collection of Invasive Fungi Research Center, Mazandaran University of Medical Sciences, Sari, Iran. *In vitro* antifungal susceptibility tests were determined according to recommendations stated in the Clinical and Laboratory Standards Institute (CLSI) M38-A2 documents.

Results: Overall, luliconazole, caspofungin and anidulafungin were more active against the dermatophyte isolates than the other used agents. MIC₅₀ for these agents were 0.016, 0.016 and 0.008 µg/ml respectively. However, MIC₅₀ of caspofungin for two species, *T. tonsurans* and *E. floccosum* was 0.008 µg/ml. MIC results of all dermatophyte isolates showed they were susceptible to antifungal agents, except for fluconazole.

Conclusion: Three agents including luliconazole, caspofungin and anidulafungin had the best activity against the dermatophyte isolates. However, terbinafine and tolnaftate also showed almost close effects. The current study demonstrated that all of the used

antifungal agents displayed excellent activity, although the majority of dermatophyte isolates were resistant to miconazole and fluconazole and showed very low susceptibility to griseofulvin.

Keywords: Dermatophytes, luliconazole, lanconazole, antifungal susceptibility

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Inhibitory effect of garlic extracts on growth of *Candida albicans* and *Geotrichum candidum*

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Introduction: Garlic is one of the most important plants used in foods for its flavoring, prophylactic and therapeutic properties. It has revealed that garlic has activity against bacteria and fungi. The aim of this study was to evaluate the antifungal activity of *Allium sativum* aqueous, methanolic and ethanolic extracts against different strains of *Candida albicans* and *Geotrichum candidum*.

Material and methods: Disc and well diffusion methods were applied to measure inhibitory effects of the extracts against all targeted strains tested in the experiment. Minimal inhibitory concentration (MIC) of the extracts were determined for each strain.

Results: The highest antifungal activity was observed at a concentration of 300 µg/ml of aqueous extract. The MIC was determined to be 350 µg/ml *Geotrichum candidum* and 300 µg/ml for *Candida albicans*. The MIC of methanolic and ethanolic extracts were higher than corresponding figures for aqueous extracts.

Conclusion: The results demonstrated that garlic has notable antifungal activity against *Candida albicans* and *Geotrichum candidum*.

Keywords: *Allium sativum*, antifungal effect, *Candida albicans*, *Geotrichum candidum*

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Effect of *Aspergillus*, *Mucor* and *Candida* supernatants' on the viability of *Leishmania (L) major* promastigotes an in vitro study

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Introduction: Leishmaniasis is an intracellular protozoan- parasitic disease, in which the sand fly is the common vector of transmission. Both zoonotic and Anthroponotic Cutaneous Leishmaniasis are endemic in different foci. With regard to the cutaneous form, 1.0–1.5 million cases are reported annually with 90% of the cases occurring in 8 countries. Although antimony-containing compounds that are the main drugs used to treat Leishmaniasis has been recommended for Cutaneous Leishmaniasis treatment by World Health Organization, but there are some restrictions in this case including high expense, side effects, frequent injections need, and incomplete efficacy.

Materials and methods: *Aspergillus*, *Mucor* and *Candida* were cultured for preparing supernatant, then *Leishmania (L) major* strain [MRHO/IR/75/ER] promastigotes cultured in NNN and RPMI 1640 media. The cell proliferation of enzyme-linked immunosorbent assay (ELISA), Badu (Chemiluminescent) was performed as described by Roche Diagnostics.

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Results: Mean of Viability Promastigote of *Leishmania (L) major* strain [MRHO/IR/75/ER] in culture according to *Aspergillus*, *Mucor* and *Candida* supernatant, Glocantime concentrations and control group by the ANOVA test was run shows statistically there was a significant difference (P<0.05). *Aspergillus*, *Mucor* and *Candida* supernatant inhibits growth of *Leishmania (L) major* strain [MRHO/IR/75/ER] Promastigotes.

Conclusion: Interestingly, *Aspergillus*, *Mucor* and *Candida* supernatants appear to be potent anti-parasitic of the three isomers against type *L major* promastigotes and amastigotes. These exciting results suggest that *Aspergillus*, *Mucor* and *Candida* supernatants have significant therapeutic potential as a novel anti-Leishmania.

Keywords: Cutaneous Leishmania, *Aspergillus*, *Mucor*, *Candida*, *Leishmania major*.

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Antifungal activity of aqueous fraction of *salvia rhytidea Benth* extract compared with nystatin against *Candida albicans* and *Candida glabrata*

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Introduction: *Salvia rhytidea Benth (S. rhytidea)* has been used since ancient times in medicine and subjected to extensive pharmacognostic researches. It has significant biological activities in medicine including antifungal activity. *Candida* infections are increasing at an alarming rate, and this is especially true for

immunocompromised individuals, such as AIDS patients, transplant patients, and neonates. The present study was aimed to evaluate antifungal activity of aqueous fraction of *S. rhytidea Benth* extract compared with Nystatin against *C. albicans* and *C. glabrata*.

Material and methods: Minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) aqueous fraction of *S. rhytidea Benth* extract compared with Nystatin against *C. albicans* and *C. glabrata* was measured.

Results: MIC and MFC of aqueous fraction of *S. rhytidea Benth* extract was 100µg/ml and $\geq 200\mu\text{g/ml}$ for *C. albicans* and 50µg/ml and $\geq 200\mu\text{g/ml}$ for *C. glabrata*. While, MIC and MFC of Nystatin against *C. albicans* were 16 and 32 and for 64 and 128µg/ml for *C. glabrata*.

Conclusion: Aqueous fraction of *S. rhytidea Benth* extract shows anti candida effect. This could be considered as new antifungal compounds to treat *Candida* infections.

Keywords: Aqueous fraction of *S. rhytidea Benth* extract, *C. albicans*, *C. glabrata*, Nystatin

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Genoprotective effects of *Zataria multiflora* and *Myrtus communis* with ketoconazole in the COMET assay on human B-lymphocytes

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Introduction: In the traditional systems of medicine, medicinal plants are important sources of safe chemical substances with potential therapeutic effects. In the recent years, tendency to herbal medicine has been increased and people have recognized and used of many cultivated or wild plants and its products have less toxic effects than synthetic drugs and are a good source for novel therapeutic agents. *Zataria multiflora* and *Myrtus communis* are known as two Iranian herbal medicines, which widely used for remedy of different disorders. In this study, we investigated the protective effect of hydro-alcoholic extracts of these plants against genotoxic induced by Ketoconazole using Comet assay.

Materials and Methods: Human B lymphocytes were treated with 1% of *Zataria multiflora*, 0.5% of *Myrtus communis* hydro-alcoholic extracts and ketoconazole simultaneously for assessment of DNA damages of incubated B lymphocytes by using Comet assay.

Results: Our results showed that these hydro-alcoholic extracts significantly prevented from DNA damage within 3 and 24 hours after incubation of B lymphocytes compare to ketoconazole control group after 24 hours. We observed a statistically significant in DNA damages in treated B lymphocytes with herbal extracts to compare with ketoconazole after 24 hours ($P < 0.05$).

Conclusion: Results of this study has shown that hydro-alcoholic extracts of *Zataria multiflora*, of *Myrtus communis* were not genotoxic agents but exhibited significant protective activity during use in leukocytes. However, further *In vitro* and *in vivo* research is necessary for evaluation of safety of these extracts.

Key words: Genotoxicity, *Zataria*, *Myrtus*, Ketoconazole, Comet assay

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Evaluation of new antifungal activity of three acetophenonic isoxazolin derivatives on *Candida albicans*

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Introduction: Invasive fungal infections have emerged in immunocompromised patients during recent decades. Although several new antifungal drugs have been licensed increasingly. Isoxasoline derivatives have recently interested as candidates for drugs. We designed and fabricated color, flour and hydrogen Acetophenonic Isoxazolin derivatives against *Candida albicans*.

Materials and Methods: After synthesis a new series of Acetophenonic Isoxazolin derivatives, their antifungal effect was evaluated against *Candida albicans* using microdilution method according to CLSI guideline.

Results: All synthesized compounds were found to have considerable antifungal activity. The Minimum Inhibitory concentrations (MICs) ranged 32-250 µg/mL against *Candida albicans*.

Conclusion: The favorable antifungal activities of the synthetic derivatives against *Candida albicans* may have a considerable potential for therapeutic application.

Keywords: Acetophenonic Isoxazolin, flour, Color, Antifungal, *Candida albicans*

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Major Mycotoxins in Food; Hazards, Formation and Prevention Methods

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Introduction: Mycotoxins are a group of toxic metabolites produced by some fungal species, most of which include *Aspergillus*,

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Penicillium and *Fusarium spp.* Mycotoxins cause serious health risks both for human and livestock, including different types of cancers, neurological disorders, liver disorders, teratogenic effects, and immune system insufficiency. Environmental conditions and moisture content are the major factors affecting the formation of mycotoxins. Formation of these compounds must be prevented, because of their potential risks, as well as the widespread occurrence in food.

Materials and Methods: A comprehensive literature review in different databases and sources such as recent published articles and reference books was conducted with the related keywords.

Results: The findings of our searches indicate that these compounds can be present in various foods such as milk, nuts, bread, cereals, rice, dried fruits, juices, meat products, etc. In addition to poisoning through contaminated food, poisoning through breathing and contact with the skin is possible too.

The prevalent mycotoxins include a variety of aflatoxins, ergot alkaloid, fumonisin, ochratoxin, patulin, trichothecene and zearalenone. Among mycotoxins, aflatoxins excreted from *Aspergillus* species are more important for carcinogenicity than other types. They are divided into four types B1, B2, G1 and G2 in which B1 is more toxic and causes liver cancer. Furthermore, M1 and M2 are the secondary types formed in milk and dairy products.

Production of mycotoxins is common in foods with high moisture content and high water activity, thus to control their formation, the moisture content of the food should be restricted to certain critical levels. Another affecting factor is temperature. The mycotoxin production is usually significant at 25 to 30°C while, at 8 to 10°C it is lower and takes longer to produce. The oxygen concentration, pH, nutritional value and microbial interactions are also factors influencing the formation of these toxins.

Conclusion: By precise control of the factors discussed above, during harvesting, preparation and storage of food products, it is

possible to largely prevent the formation of these mycotoxins.

Keywords: Mycotoxin, food, *Aspergillus*, aflatoxin

P-192

Biofilm-producing ability of clinical isolates of *Candida parapsilosis* species complex: comparison of two methods

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Introduction: *Candida parapsilosis* is one of the major pathogens of the nosocomial infections. Mainly due to their ability to form biofilms on surfaces of indwelling medical devices could lead to invasive fungal infection (IFI) which is associated with high morbidity and mortality rates. The aim of the study is to examine biofilm formation ability of clinical isolates of *C. parapsilosis* species complex measured by quantification of total biomass and assessment of cell activity.

Material and methods: Crystal violet (CV) staining and 2-(4, 5-dimethyl-2-thiazolyl)-3, 5-diphenyl-2H-tetrazolium bromide (MTT) reduction assays for visualization and measurement of biofilm-producing ability have been used at different time intervals. Scanning electron microscopy (SEM) was used to visualize the ultrastructural characteristics and comparing the efficiency of the MTT and CV methods.

Results: 60 clinical samples of *C. parapsilosis* complex have been tested for biofilm-producing ability. The isolates included 47 strains of *C. parapsilosis sensu stricto* (78.3%), 11 *C. orthopsilosis* (18.3%) and 2 *C. metapsilosis* (3.3%). According to cut-offs by CV assay, all isolates were able to form biofilms and no significant difference was found in the biomass production at

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different time intervals (24, 48, 72, 96h) ($p > 0.05$). *C. parapsilosis sensu stricto* was considered a high biofilm producer at four tested time points independently, whilst *C. orthopsilosis* was considered mostly a moderate biofilm producer (with the exception at 72h time point). Regarding to results of MTT reduction assay, no statistically significant difference was observed, only *C. orthopsilosis* demonstrated higher metabolic activity at 24h time point ($p < 0.05$). A significant difference was observed in the ability of CV and MTT assays to quantify biofilm production ($p < 0.05$). SEM analysis demonstrated minor structural differences in morphology between *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *C. metapsilosis* isolates, also a quantitative correlation was found between the extent of biomass assessed by CV method and that observed microscopically through assessment of surface coverage by SEM.

Conclusion: All *C. parapsilosis* clinical strains had biofilm-producing ability. Similar surface topography with slight differences was observed. The significant variation was found between the results of the CV and MTT assays. Mainly the microscopic results showed more compatibility with the results of the CV method.

Keywords: *Candida parapsilosis* complex, biofilm production, crystal violet staining, scanning electron microscopy, cell activity.

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Prevention and detoxification of mycotoxins in the food chain: post-harvest and pre-harvest strategies

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Introduction: Mycotoxins are secondary metabolites present worldwide in agricultural commodities and produced by molds that cause carcinogenic effects and also economic losses. Mold growth and mycotoxin formation are mainly dependent on environmental factors (temperature and water availability), agricultural practices and storage conditions.

The most common approach to detoxify mycotoxin-contaminated food and feed is the sorbent materials for removal of toxins by adsorption during passage through the gastrointestinal tract. Some microorganisms are capable to detoxify by enzymatic transformation and Biodegradation of mycotoxins. This review is concentrated on strategies to inhibit toxigenic fungal growth, reduce mycotoxins production and finally maximize consumer safety.

Materials and Methods: Thank to the importance of food safety aspects of the human diet and with regards to toxicity hazards of mycotoxins for human and animal, we carried out a comprehensive review of various strategies including pre-harvest prevention strategies and post-harvest detoxification procedures e.g physical, chemical and biological methods and other developing innovative strategies by research in recently published reports.

Results: Strategies for mycotoxin prevention and control will most likely result not from a single treatment but from a combination of appropriate environmental factors, good agricultural and manufacturing practices, suitable storage condition, proper quality assurance Programs and bio-safe postharvest detoxifying methods throughout the production process.

Conclusion: The most effective methods are those carried out before the fungal infestation and mycotoxin production on the plant. Determination of the main critical control points during harvesting, drying and storage stages especially in the cereal production chain is essential to control and prevention of mycotoxin formation.

Key Words: mycotoxin, fungi, Detoxification, food safety

P-194

Mycotoxins in food and feed and their impact on human and animal health: a review

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Introduction: Nowadays, the worldwide contamination of foods and feeds with mycotoxins is a significant food safety problem. Mycotoxins are secondary metabolites produced by fungi that have adverse effects on human and animal upon ingestion, inhalation, or skin contact that result in illnesses and also economic losses. The disease caused by exposure to mycotoxins are known as mycotoxicosis that does not need to the presence of the toxin-forming fungi. The metabolism of ingested mycotoxins could result in mycotoxin accumulation in different tissues or organs, entering into the food chain through milk, meat, or eggs. A number of fungi that are capable of producing mycotoxins include *Aspergillus*, *Penicillium*, *Fusarium*,

Claviceps, and *Alternaria* species. The most important mycotoxins found in food are aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, and ergot alkaloids. In the present review study, we discussed about various mycotoxins and their carcinogenic rate and the other negative effects on human and animal health.

Materials and Methods: Due to the importance of food safety aspects of the human diet and with regards to toxicity hazards of mycotoxins for human and animal, we carried out a comprehensive review on toxicological effects of mycotoxins and their negative impacts on health by research in recently published reports.

Results: Mycotoxins have various acute and chronic effects on humans and animals depending on mycotoxin species and susceptibility of human or animal, such as hepatotoxic, genotoxic, immunosuppressive, nephrotoxic, teratogenic, or carcinogenic effect.

Conclusion: Factors influencing the presence of mycotoxins in foods or feeds such as environmental conditions (temperature and moisture content) should be controlled. They should be completely eliminated during food processing operations and should not be allowed to contaminate finished processed food products.

Key words: Mycotoxin, Fungi, Health, Food Safety

P-195

Comparative *in vitro* activity of old and novel antifungal agent against a national collection of dermatophyte species caused tinea pedis

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Introduction: Dermatophytes are a group of keratinophilic fungi that invade and infect the keratinized tissues and cause dermatophytosis. Dermatophytosis has different clinical manifestation. Tinea pedis is a dermatophytic infection of the feet that also called as athlete foot. We investigated novel Triazole luliconazole and laniconazole, compared with old antifungal agent against dermatophyte species associated with tinea pedis.

Material and methods: A total of 59 dermatophytes isolates comprising of *Trichophyton mentagrophytes* (n=47), *T. rubrum* (n=10), *T. tonsurans* (n=1) and *E. floccosum* (n=1), recovered from infected patients with tinea pedis in Tehran, Iran. Identification to the species level of all isolates was confirmed by DNA sequencing of the ITS1-5.8S rDNA-ITS2 rDNA region. *In vitro* antifungal susceptibility testing was adjusted in microdilution plates for old and novel antifungal drugs according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 documents.

Results: Novel triazoles had potent activity against the dermatophyte isolates. The geometric mean MICs were the lowest for luliconazole (0.0008 and µg/mL), followed by laniconazole (0.003 µg/ml), terbinafine (0.019 µg/ml), itraconazole (0.085 µg/ml), ketoconazole (0.089 µg/ml), econazole (0.097 µg/ml), griseofulvin (0.351 µg/ml), voriconazole (0.583 µg/ml) and fluconazole (11.58 µg/mL).

Conclusion: These results suggest that the novel triazole is promising candidates for the treatment of dermatophyte species compared to the old antifungal agent.

Key words: Luliconazole, Laniconazole, Dermatophyte, Novel Triazole

P-196

In Vitro Interactions of echinocandins with triazoles against *Candida parapsilosis* complex isolates from clinical specimens

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Introduction: The *Candida parapsilosis* complex has been described as one of the most common yeast species isolated from patients with bloodstream infections worldwide. This complex consists of three species: *C.parapsilosis* sensu stricto, *C.orthopsilosis*, and *C.metapsilosis*. Although *C.parapsilosis* strains are usually susceptible to azoles, recent reports indicate the emergence of invasive infections due to fluconazole (FLC) resistant *C.parapsilosis* complex isolates and all species show elevated MICs for the echinocandin class drugs relative to other *Candida* species. We therefore investigated the efficacy of echinocandins with triazoles against clinical *candida parapsilosis* complex isolates.

Materials and Methods: *In vitro* susceptibility to triazoles and echinocandins of *C. parapsilosis* (n=80), *C. orthopsilosis* (n=20) and *C. metapsilosis* (n=3) was tested using CLSI broth microdilution M27-A3 methodology. The *in vitro* interactions between echinocandins (micafungin, anidulafungin) and azoles (fluconazole, itraconazole) determined against fifteen triazoles resistant and high MICs echinocandins *C. parapsilosis* complex strains by use of a microdilution checkerboard technique.

Results: The combined interaction by micafungin with itraconazole or fluconazole (FICI range: 0.2-0.5) and anidulafungin with itraconazole or fluconazole (FICI range: 0.2-0.5) provided synergic interaction. No antagonism and indifferent interactions was observed for any combination.

Conclusion: The combination of echinocandins with triazoles exhibited synergistic activity against clinical *Candida*

parapsilosis complex isolates suggesting an alternative approach to overcome antifungal drug resistance. The further studies in addition to determination of the underlying mechanism of this synergistic action will be need for using of this combination therapy in the *in vivo*.

Keywords: *Candida parapsilosis*, Azoles, Echinocandins

P-197

The effect of total extract and chlorophormic, watery and alcoholic fractions of *D. Persica* on some pathogenic fungal

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Introduction: Regarding to the increase using of anti-fungal drugs against human pathogenic fungal and side-effects of some of them, scientist are searching for salvage and alternative therapy such as using herbal drugs. This study objective is to evaluate the anti-fungi effect of total watery, methanol and chloroform extract of *Dicyclophora persica*.

Material and Methods: The total extract was prepared from flowering shoot of *D. persica*, after identifying and making powder using percolation method. In order to determine the anti-fungi effects, some concentrations of total watery, methanol, chloroform extract have been made and was evaluated on 10 fungal strains such as *Trichophyton rubrum*, *Epidermophyton floccosum*, *Aspergillus flavus*, *Microsporum canis*, *Mucor*, *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria*, *Cladosporium*, using Hole diffusion method. Making sure of the results, in addition to Hole diffusion method, mixing and MIC (minimum inhibitory concentration) determination methods was also used.

Results: According to the study results, there was no evidence of any anti-fungi effect of *D. persica* on *Candida albicans* and *Mucor*. The chloroform extract was only effective on dermatophytes in Hole diffusion method and concerning *Trichophyton floccosum* and *M*

icrosporium canis, it was not effective in both methods. The observed anti-fungal effect in both methods on *Cladosporium* was from *phaeohyphomycet* and regarding *Alternaria*, only watery and chloroform extracts in mixing method, had anti-fungal effects. The antifungal effect on 3 *Aspergillus* under study was not observed, although in hole diffusion method and MIC methods, it was observed only for *Aspergillus niger* and *Aspergillus flavus* in watery extract, and it was totally ineffective on *Aspergillus fumigatus*.

Conclusion: based on this study, *phaeohyphomycet* and *Tricophyton rubrum* showed susceptibility to antifungal effect of existing compounds in the extract of the *Dicyclophora Persica*.

Keywords: Pathogenic Fungal; Anti-fungal drugs, MIC.

P-198

Contamination of milk and dairy products with aflatoxin M₁ in Iran: A systematic review and meta-analysis

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Introduction: Aflatoxins are among of the most important mycotoxins with mutagenic, carcinogenic, teratogenic, hepatotoxic and immunosuppression properties, which have been classified by the International Agency for Research on Cancer in Group I carcinogenic compounds. Therefore, its monitoring in milk and dairy products is very important. The aim of this study was to determine the contamination of milk and dairy products with Aflatoxin M₁ in Iran.

Materials and methods: In this study, national and international databases such as:

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PubMed, Scopus, Science Direct and SID were searched without time limit and using keywords: Aflatoxin, Iran, milk, dairy products and Iranian food products. Data entry and analysis were performed with Stata software version 11, and by Q-test based on chi-square at the significance level of 0.1 and I² index.

Results: The findings of this study showed that the prevalence and overall mean of aflatoxin M₁ contamination with 95% confidence interval in milk types was 74%, 54.86 ng / l, and in dairy products, 73%, 96.91 ng / kg respectively. Also, aflatoxin M₁ contamination in milk and dairy products was 15, 39, 18 and 21 percent higher than Iran and the European Union.

Conclusion: The presence of aflatoxin in milk and dairy products is a major threat to health and public health. Therefore, in order to reduce aflatoxin in food, the attention of the government and people to food security through the consumption of healthy food should be drawn. This is also achieved by paying particular attention to the principles of Hazard Analysis and Critical Control Points (HACCP) in food production.

Key words: aflatoxin, Iran, milk, dairy products, Iranian food products

P-199

Antifungal activity of royal jelly and propolis on the growth of *Aspergillus parasiticus*

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Introduction: Due to the increased resistance of microorganisms to antibiotics, discovering new compounds with less side effects is more important than usual medications. The aim of this study was to evaluate the antifungal activity of royal jelly and propolis on the growth of *Aspergillus*

parasiticus as one of the most common pathogens of foodborne disease, which is the cause of mutagenic, teratogenic and carcinogenic effects.

Material and methods: Using the broth microdilution method based on the Clinical and Laboratory and Standards Institute M38-A2 guide, the antifungal activity of royal jelly and propolis at different concentrations against the standard *Aspergillus parasiticus* strain (ATCC 15517) was determined.

Results: Royal jelly and propolis could inhibit the growth of *Aspergillus parasiticus*. The minimum concentrations of royal jelly and propolis with an inhibitory effect on the growth of the fungus were 3200 µg/ml and 100 µg/ml, respectively.

Conclusion: In this study, royal jelly and propolis were found to have very good antifungal properties against standard *Aspergillus parasiticus* strain. Accordingly, they might be good alternatives to chemical preservatives for keeping food, however further studies are required.

Key words: Royal Jelly, Propolis, *Aspergillus parasiticus*

P-200

Evaluation of virulence factors between homozygote and heterozygote strains of *Candida albicans*

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Introduction: *Candida albicans* is the most common cause of candidiasis. Secretion of exoenzymes and ability to produce biofilm are considered as major factors in candida pathogenesis. There is less data about virulence factors in homozygous and heterozygous strains in *Candida albicans*. The aim of this study was to evaluate biofilm formation, phospholipase, and

proteinase and hemolysin activity between two genotypes of *Candida albicans*.

Materials and methods: A total of 30 homogenous and 30 heterogeneous strains of *Candida albicans* species isolated from vaginal candidiasis were enrolled in our study. Egg yolk agar, Sabouraud blood agar, BSA agar and visual method were used for evaluation of phospholipase, hemolysin, proteinase and biofilm activities, respectively. Fischer exact test was used for statistical analysis.

Results: Exoenzyme activity in homozygous strains was 83.3% for phospholipase, 100% for proteinase, 100% for hemolysin and 93.3% for biofilm formation. In heterozygous strains these rates were 96, 100, 100 and 96.6%, respectively. There was no significant relationship regarding virulence factors between two strains.

Conclusion: Virulence factors are considered as main cause in *Candida* pathogenesis. Both strains exhibited exoenzyme activity in different ranges.

Keyword: homozygous, heterozygous, *Candida albicans*, virulence

P-201

In-vitro and *in-vivo* regulation of *Candida albicans* morphogenesis and pathogenesis by probiotic bacterium – *Pediococcus acidilactici*.

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Introduction: *Candida* species is known as opportunistic yeast that could cause candidiasis in susceptible individuals. Oral candidiasis is one of most frequent form of this infection that caused by *Candida* species in particular, *Candida albicans* which normally reside on human mucosal surfaces. The transition of *C. albicans*, from budding yeast to filamentous hyphae allows for covalent attachment to oral epithelial cells, followed by biofilm formation, invasion and tissue damage. It has been suggested that biofilm formation by *C.albicans* play important role in its pathogenesis. Hence, the effect of *P. acidilactici* on the growth, biofilm formation and expression of genes related to morphogenesis and pathogenesis of *C. albicans* were investigated in vitro as well as in animal model.

Materials and methods: Inhibitory activity of *P. acidilactici* on *Candida* species was evaluated by broth microdilution method based on CLSI protocol. Moreover, inhibition of *C. albicans* biofilm formation by *P. acidilactici* was measured using XTT reduction assay. Also, expression of adhesion-related gene (*ALSI,3*), hyphal cell wall protein gene critical to biofilm formation (*HWP1*), secreted aspartyl proteinase (*SAP4,6*), morphogenesis pathway regulatory gene (*EFG1*) and *EAP1* were analyzed by RT-PCR in the treated yeast cells with different concentrations of *P. acidilactici*. The experimental activity of the probiotic bacterium in the prevention or treatment of oral candidiasis was also assessed in an animal model by both culture and histopathological methods.

Results: *P. acidilactici* inhibited the growth of different species of *candida* at concentration ranging from 8 to 256 µg/mL. Furthermore, this probiotic bacterium inhibited the formation of *C.albicans* biofilm in a dose dependent manner. RT-PCR analysis of *C. albicans* yeast treated with different concentration of probiotic bacterium showed reduction of *ALSI,3*, *SAP4,6*, *EAP1*, *EFG1* and *HWP1* genes. The *P.acidilactici* significantly decreased the CFUs in mice receiving this probiotic treatment compared to those of control group.

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Similarly, histopathological analyses showed that *Candida* colonization declined in the mice following administration of probiotic in a therapeutic trial.

Conclusion: Obtained data provided new insight into the effect of probiotic bacterium on growth, transition, biofilm formation and pathogenicity of *C. albicans*. Our novel results point to the down regulation of several *Candida* genes critical to the yeast–hyphae transition, biofilm formation, tissue invasion and cellular damage. According to recent emergence of drug resistance in *Candida* species and unfavorable side effects of conventional antifungal drugs, probiotic bacterium have the same activity like antifungal drugs. Considering the wide range of antifungal activities of the examined probiotic bacterium, it can be used in the management of alimentary candidiasis or as a mouth wash or other pharmaceutical products.

Keywords: *Pediococcus acidilactici*, *Candida albicans* genes, quantitative real-time PCR, animal model.

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Fungicidal activity of *Cinnamomum cayennense* and *Origanum majorana* var. *majoranoides* against fluconazole resistant *Candida* species

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Abstract

Introduction: Antifungal resistant is one of the causes of high mortality rates during invasive candidiasis. Since development of new antifungal agents is limited, researchers have focused on natural products including essential oils with antifungal properties. In immunocompromised patients fungicidal activity is of benefit. This study was designed

to evaluate the fungicidal/fungistatic activity of five Iranian essential oils against fluconazole resistant *Candida* species and to study the effect of their chemical composition on their antifungal properties.

Methods: Chemical composition of essential oils was determined using Gas chromatography-Mass spectroscopy (GC/MS). Fluconazole resistant *Candida* species were chosen and MIC values of studied essential oils were determined by broth microdilution method. MFC was determined as the lowest concentration with no fungal growth on solid media. Fungicidal activity was calculated by MFC/MIC ratio.

Results: The results showed that *C. albicans* and *C. tropicalis* isolates were susceptible to itraconazole and voriconazole while one species of *C. glabrata* and *C. krusei* each was resistant to itraconazole; and itraconazole resistant *C. glabrata* isolate was resistance to voriconazole as well. *Cinnamomum cayennense* and *Origanum majorana* var. *majoranoides* had high anti-*Candida* activity. All essential oils in this study had fungicidal activity.

Conclusion: In general, natural compounds tested are suitable to be used as anti-*Candida*. However more studies are needed on each chemical compound to evaluate their antifungal activity alone or in combination with other agents.

Keywords: Fungistatic activity; Fungicidal activity; *Candida* spp; Essentials oils, antifungal agents

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Antifungal activity of *Artemisia aromatica* A. Nelson against nosocomial *Candida* isolates in comparison to conventional azoles

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Abstract

Introduction: Incidence of invasive fungal infections (IFIs) has been increased in recent years. *Candida* species are the most frequent fungal pathogens isolated from IFIs. Development of new antifungals is challenging; because there are only few targets for antifungal actions and toxicity level of many antifungals is relatively high. In recent years, some researchers have focused on natural compounds including essential oils and herbal extracts derived from medicinal plants and other biomaterials with antifungal activities. This study was designed to evaluate the antifungal activity of *Artemisia aromatica* A. Nelson against nosocomial *Candida* isolates in comparison to voriconazole and itraconazole.

Methods: Chemical composition of *Artemisia aromatica* A. Nelson was determined using Gas chromatography-Mass spectroscopy (GC/MS). *Candida* species isolated from hospitalized patients were chosen and MIC values of *Artemisia aromatica* A. Nelson was determined using broth microdilution method according to CLSI M27-A3. MFC was determined as the lowest concentration with no fungal growth on solid media.

Results: The results showed that *C. albicans* and *C. tropicalis* isolates were susceptible to itraconazole and voriconazole while one species of *C. glabrata* and *C. krusei* each was resistant to itraconazole; and itraconazole resistant *C. glabrata* isolate was resistance to voriconazole as well. *Artemisia aromatica* A. Nelson had the highest MIC values against all *Candida* isolates.

Conclusion: The results showed that *Artemisia aromatica* A. Nelson could be nominated as a potential antifungal agent. However more studies are needed to determine the exact mechanism of this essential oil and its activity in combination with other commercial antifungal agents.

Keywords: *Artemisia aromatica* A. Nelson, nosocomial infections, *Candida albicans*, *Candida tropicalis*, *Candida glabrata*

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Anti-fungal effect of Horsetail hydroalcoholic extract against *Candida albicans* compared with nystatin: An *in vitro* study

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Background: Oral infections created by *Candida* species are widely increasing. One of the topical treatments for oral candidiasis is nystatin. Since the anti-fungal treatments normally have adverse effects and cause drug resistance, the use of appropriate strategies to replace synthetic drugs to eliminate these problems has been considered. The aim of this study was to compare the effects of Horsetail extract with nystatin on *Candida albicans*.

Methods: In this experimental study, hydroalcoholic extract of Horsetail was prepared at concentrations of 4, 2, 1, 0.5, 0.25 and 0.12 mg/ml. *C. albicans* ATCC 10231 was cultured in Sabouraud Dextrose Agar (SDA) medium, and in each culture plate, one disk containing each concentration of plant extract, one 100-unit nystatin disc as positive control and one distilled water disks as negative control were placed. After 24 hours, the mean diameter of the inhibition zone of different concentrations of Horsetail extract and nystatin was compared by one-way ANOVA test.

Results: The mean diameters of inhibition zone Horsetail extract at the concentrations of 4 and 2 mg/ml were 38 and 26 mm, respectively, while the diameter of inhibition zone of nystatin disc was 32 mm. The results showed that by increasing the concentration of Horsetail extract, the diameter of the inhibition zone increased. The inhibition zone diameter of the highest concentration of Horsetail extract was not significantly different with nystatin ($p > 0.05$)

Conclusion: The results of this study showed that Horsetail extract could be considered as a suitable candidate for the elimination of oral infections caused by *C. albicans*.

Keywords: *Candida albicans*, Nystatin, horsetail