Molecular Characterization, Sequence Analysis, and Taxonomic Borderlines of Different Dermatophyte Species Using Calmodulin Gene

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Objectives: Since the calmodulin gene has not systematically been used in dermatophyte taxonomy, our aim was the sequencing and analysis of calmodulin gene as a new genetic marker in different dermatophyte species, as well as the assessment of its application in identification, phylogenetic and taxonomy studies according to intraspecies and interspecies variation.
Methods: Several reference and clinical strains including 202 strains of 29 species were used in sequence analysis of partial calmodulin gene. DNA was extracted and purified from fungal colonies. For identification of clinical isolates and approval of standard strains, PCR by the universal primers ITS1/ITS4 and subsequently RFLP by *Mva*I enzyme were done. PCR of calmodulin gene was performed by designing a set of primers, and the target was sequenced. Consensus sequences were used for multiple alignment and phylogenetic tree analysis and the levels of intra- and interspecific variation were assessed.
Results: Sequence diversity among 29 dermatophyte species ranged from 0 to 200 nt. Intra-species differences were found within strains of *T. interdigitale, A. simii, T. rubrum* and *A. vanbreuseghemii*. Closely related species in different groups formed well-supported clades in the calmodulin tree, such as *T. interdigitale*, *T. tonsurans*, and *T. equinum* (bootstrap value, 99%); *A. simii*, *T. mentagrophytes*, and *T. schoenleinii* (bootstrap value of 100%); *T. rubrum* and *T. violaceum* (bootstrap value, 100%); members of the *A. benhamiae* complex (bootstrap value, of 93%); *M. racemosum*, *M. cookie* and *E. floccosum* (bootstrap value 100%); and *T. ajelloi* and *T. eboreum* (bootstrap value 99%).
Conclusion: Characterization of calmodulin gene was done in broad range of the various dermatophyte species and the number of reliable calmodulin sequences of dermatophytes was increased in Genbank. Calmodulin gene tree topologies were almost identical in ITS, BT2 and TEF1α regions.

Relationship between Genetic Diversity of *Candida glabrata* Yeasts Isolated from Patients in Iran by MLVA Method

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Objectives: In recent years, *Candida* species have caused diseases in dramatic rates due to the increased risk factors in people with impaired immune systems. Although *C. albicans* is important as the most common cause of candidiasis, other species such as *glabrata*, because of their resistance to antifungal drugs, have become more important. Azole group, once self- medicated by the patients, may cause the development of resistance to fluconazole. As a result of lack of response to treatment, patients who are not cured decide to refer to other doctors. On the other hand, *C.glabrata* has shown resistance to azole group, and due to lack of studies on the genetic diversity of *C. glabrata* in Iran, we were prompted to investigate the epidemiological study of this species by using microsatellite markers for the first time in Iran.
Methods: 83 clinical samples of *C. glabrata* were collected from different cities of Iran such as Tehran, Kashan, Isfahan, and Shiraz in a two-year period. Conventional, PCR- RFLP and sequencing methods were used for identification of the species. To perform MLVA technique, 6 series of specific primers were selected. After amplification, to determine the number of desired alleles and repetitive sequences analyses, the samples were sequenced. Phylogenetic tree was drawn for comparing the relationship between the strains and the other factors.
Result: Based on MLVA Techniques, for primer 4, five alleles, for primer 5, four alleles, for primers5, 6, 10 and 11, three alleles were identified. By this method, 72 different genotypes were reported. 22 isolates had the same genotypes in pairs.
Conclusion: In general, among the cities under investigation, Tehran showed the greatest genetic variation. The findings of this study will be helpful in identifying the pathogenesis of this yeast, so it is hoped that our finding can contribute to determining the genetic map for future health plans and selecting suitable antifungal drugs.

Molecular Characterization and In Vitro Antifungal Susceptibility of 316 Clinical Isolates of Dermatophytes in Iran

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Objectives: Dermatophytosis is a common mycotic infection of the skin, nail and hair, associated with major public health concerns worldwide. Various species of dermatophytes show significant differences in susceptibility to antifungals. Here, we present the antifungal susceptibility of a large collection of molecularly identified dermatophyte isolates obtained from tropical region of South of Iran (Shiraz and Ahvaz).
Methods: A total of 9485 patients clinically suspected to have cutaneous fungal infections were examined. Dermatophytosis was confirmed in 1502 cases (15.8%) by direct microscopy and culture. Three hundred and sixteen isolates recovered in culture were identified to species level using PCR-sequencing of ITS region and PCR-RFLP.
Results: Tinea corporis was the most prevalent type of clinical manifestation (35.2 %), followed by tinea cruris (17 %), tinea capitis (12.8 %), tinea pedis (11.3 %), tinea manuum (11 %), tinea unguium (6.9 %) and tinea barbae (5.8 %).
Trichophyton interdigitale was the most common isolate (49, 36%), followed by T. *rubrum* (18.98%), *Epidermophyton floccosum* (13.29%), *Microsporum canis* (9.17%), *Arthroderma benhamiae* (*T. anamorph of A. benhamiae*) (5.38%) and *T. tonsurans* (3.79%). Overall, irrespective of the geographical region, terbinafine was the most potent antifungal against all isolates, with an MIC range of 0.002 to 0.25 µg/mL, followed by itraconazole (0.004 to 0.5 µg/mL), griseofulvin (0.125 to 8 µg/mL) and fluconazole (4 to 128 µg/mL).
Conclusion: Analysis of our data revealed a significant increase in the frequency of *A.benhamiae*, which definitely warrants further investigation to explore source of this infection in South of Iran. Moreover, terbinafine was the most effective antifungal against all isolates, in vitro.

Assessment of Risk Factors Associated to Neonatal Thrush and Oral *Candida* Colonization

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Objectives: Oral thrush occurs frequently in neonates. Numerous predisposing factors for this infection in neonates have been proposed. Gestational ages, blood group, gender, type of birth, diet, using pacifier or bottle in neonates as well as vaginal colonization, residential area, and level of education in mothers might have a role in this colonization. In this study we identified risk factors associated with progression towards thrush in neonates and genetically defined the respective *Candida* isolates associated with disease and colonization.
Methods: The studied population consisted of 705 neonatesreferred to the Screening Center of Paramedic School of Shiraz University of Medical Sciences. Samples were taken from the oral mucosa of all the neonates using sterile swab within the first and the second 15 days after birth thorough examination of the oral cavity. All samples were cultured on Chromagar *Candida*. Morphologic analyses were used to identify potential yeast-positive cultures which were then further characterized using PCR-RFLP analysis.
Results: In the first and the second half-months, only 2.1% (15) and 15.2% (107) of the infants showed manifestations of oral thrush. The most commonly isolated species was *Candida albicans* (56%) followed by *C. parapsilosis* (18.3%), *C. tropicalis* (6.3%), *C. krusei* (6.3%), *C. glabrata* (3.7%), *C. kefyr* (3.1%), *C. rugosa*(1.6%), *C. famata* (1.0%), *C. dubliniensis* (0.5%) and *C. guilliermondii* (0.5%). Birth type, use of pacifier, prematurity were the only factors with a statistically significant association with Candida colonization (i.e. isolation of ≥ 100 CFU) ($p < 0.05$) . Moreover, the presence of vaginal infection in mothers during the two weeks before birth and their occupation was significantly associated with this colonization (≥ 100 colonies) ($p < 0.05$). Logestic regression test showed that the presence of oral thrush in the infants of mothers with symptoms on their breasts, was significantly more than other infants (OR = 3.67). Also the prevalence of oral thrush in neonates who were fed using baby bottle was significantly higher than the breast-fed neonates (OR = 2.72). Moreover, the risk of oral thrush in neonates with AB-blood group is four times higher than those of O-blood group (OR = 4.20). Based on Chi-square test, natural birth (39.67%) was associated with the incidence of oral thrush in the neonates compared to the Caesarian birth (13.11%). Further analysis with Cochran–Mantel–Haenszel statistics showed this significance to be mainly due to parameters like gender, weight, diet, and prematurity.
Conclusion: Asoral thrush usually cause annoyance and discomfort in neonate and in some cases might lead to malnutrition and low weight, this infection should be considered more seriously. Many factors might have a role in this colonization and could be prevented before child birth or during infancy (such as type of child birth, feeding, using pacifier, mothers' vaginal Candidiasis, etc). In this study, the prevalence of oral thrush in neonates and factors associated with this colonization were fully discussed.

Biochemical and Molecular Studies on the Effect of Histone Deacetylases Inhibitors on *Aspergillus flavus* the Cause of Pistachio Infection

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Objectives: *Aspergillus flavus* is a saprophytic soil fungus that infects and contaminates pre-harvest and pos-tharvest seed crops with carcinogenic secondary metabolite aflatoxin. Histone deacetylases (HDACs) play important roles in the regulation of gene expression through histone modification, modifies core histone, and participates in large regulatory complexes that suppress and enhance transcription. HDACs can play an important role in secondary metabolites production and normal vegetative growth.
Methods: In this study, Suberoylanilide Hydroxamic Acid (SAHA), a classical HDAC inhibitor (HDACi) was applied to investigate its effect on the development and biosynthesis aflatoxin genes of *A. flavus*.
Results: Real time RT-PCR analysis indicated that SAHA affected the gene expression of genes that are required for the production of aflatoxin. In aflatoxine genes, SAHA reduced the expression of *AflR* and increased *Ver-1* and *Nor-1.* The results also indicated that SAHA decreased the production of conidiophores, aflatoxin B1, and pathogenesis. Further studies revealed that SAHA reduced conidial chain elongation and, hence, the vegetative growth.
Conclusion: These results suggested that HDACs are important for fungal cell functions like aflatoxin biosynthesis, growth and pathogenesis. This approach could contribute to developing new therapies for fungal infection and lead to the identification of new compounds with fungal cell selectivity. If so, valuable antifungal drugs can be produced.

Study of The Effects of Ozonated Olive Oil In The Treatment of VulvovaginalCandidiasis

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Objectives: Vulvovaginal candidiasis is the most common infection of the vulvovagina which manifests with itching, burning sensation and leucorrhea. Conventional treatments are azoles to which tolerance has been reported, especially in immunosuppressed patients. New studies suggest antifungal effects of ozone, the allotropic form of oxygen. This study compared the effects of ozonated olive oil and clotrimazole for the treatment of vulvovaginal candidiasis.
Methods: One hundred patients with confirmed vulvovaginal candidiasis were randomly classified into two groups and treated by ozonated olive oil or clotrimazole for 7 days. The study outcomes were changes in itching, burning, leucorrhea and culture before and after the treatment, which were evaluated by an interview and paraclinical examination. Statistical analysis was done by SPSS software, version 17. The significance level stood at 0.05.
Results: Both Ozone and clotrimazole reduced the symptoms significantly and led to negative specimen cultures (p0.05). However, ozone decreased burning sensation significantly better than clotrimazole ($p<0.05$)
Conclusion: Considering the potential efficacy of ozonated olive oil for the improvement of clinical and paraclinical aspects of patients with vulvovaginal candidiasis, it could be suggested as an effective alternative topical treatment for the treatment of these patients.

Development of a High Resolution Melting Analysis Assay for Rapid and High Throughput Identification of Clinically Important Dermatophyte Species

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Objectives: Dermatophyte infections have a universal distribution and are considered as public health problems in underdeveloped and developing countries. Accurate identification of dermatophyte species is important for epidemiological studies and implementing antifungal treatment strategies. Although nucleic acid amplification-based assays have several advantages over conventional mycological methods, their major disadvantage is the high cost. Additionally, some of these assays are time-consuming and low through-put. In the present study, a rapid and accurate Real-time PCR based high-resolution melting (HRM) assay was developed for detection and differentiation of the most common dermatophyte fungi.
Methods: Thirteen reference strains of dermatophyte species and

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)
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250 clinical isolates from patients with dermatophytosis were used in this study. Using designed HRM primers for highly conserved regions of the dermatophyte`s ribosomal DNA, Real-time PCR and HRM analysis were performed for differentiation of common dermatophyte species. To evaluate the performance characteristics of the method, all clinical isolates were tested in comparison with the long established PCR-RFLP method, and the discordant results were reassessed using DNA sequencing as the reference standard method.
Results: This assay was able to differentiate 13 common dermatophytes species including *T. rubrum*, *T. tonsurans*, *T. violaceum*, *T. verrucosum*, *T. erinacei*, *A. benhamia, T. interdigitale*, *T. schoenleinii*, *M. ferrugineum, E. floccosum*, *M. gypseum, M. canis* and *M. audouinii* using either normalized melting peak or difference plots analysis methods. All clinical isolates had identical typing results by HRM and PCR-RFLP methods. The results were confirmed by sequencing, as well.
Conclusion: The results showed that, in comparison to PCR-RFLP, the developed HRM assay was able to differentiate 13 common dermatophytes species with a higher speed and accuracy. These results indicated that the described HRM assay will be a useful, sensitive, high through-put and cost-effective method for differentiating the most common dermatophyte species.

Design, Synthesis, Biological Evaluation, and Molecular Modeling Study of Novel Indolizine-1-Carbonitriles Derivatives as Potential Anti-Microbial Agents

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Objectives: Invasive bacterial and fungal infections are well recognized as diseases of immuno-compromised patients. Over the last twenty years, there have been significant increases in the number of recorded instances of resistant bacterial and fungal infections. It has been prospected that in the year 2050, the rate of mortality due to microbial resistance will surpass mortality due to cancer. Thus, the discovery of novel antimicrobial agents is crucial in coming years and it is required to guarantee positive therapeutic outcomes in patients. In this study, a novel one-pot two- step tandem reaction for the synthesis of indolizine-1-carbonitrile derivatives **(6a-6i)** was identified.
Methods: The route comprised 1,3-dipolar cycloaddition reaction of 2,4'-dibromoacetophenone, 2-chloropyridine, aromatic aldehyde derivatives **(2a-i)** and malonitrile under ultrasound irradiation. The product compounds were tested in vitro and in silico against bacteria and fungi.
Results: It was revealed that compound **6b** had the most antifungal activity (range MICs = 8–32 µg/mL) and compound **6g** had the most antibacterial activity (range MICs = 16–256 µg/mL). Molecular docking of compounds **(6a-6i)** into fungal 14α-demethylase and bacterial protein tyrosine phosphatase active sites were also performed and probable binding mode of compounds **6b** and **6g** were determined.
Conclusion: The present study described the docking, synthesis, structure elucidations, in vitro antibacterial and antifungal activity assay of indolizine derivatives. We synthesized and evaluated a series of indolizine-1-carbonitrile compounds in good yields. All the synthesized compounds were evaluated for in vitro antibacterial activity against a number of Gram-positive and negative bacteria. The compounds were also evaluated for in vitro antifungal activity against a number of filamentous fungi and yeasts. Good results were achieved, and they might be helpful for the design and synthesis of compounds with stronger activities.

Interaction of the Novel Azole Derivatives (10h&11h) with Fluconazole against *Candida* Species in Vitro

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Objectives: Candidiasis is a serious life-threatening infection that has caused significant mortality and morbidity in the last several decades, especially in immunocompromised patients. Azoles are a widely applied class of antifungal agents, and fluconazole has been shown to be as effective, high solubility, wide tissue distribution with low toxicity. However, the efficacy of fluconazole and other antifungal agents would be improved by using combination therapy. Therefore, in the current study, we evaluated the interaction between two antifungal compounds (10h and 11h) with fluconazole against *Candida* species in vitro.
Methods: The interaction of novel azole derivatives with fluconazole (10h plus fluconazole and 11h plus fluconazole) was tested against 39 *Candida* species recovered from candidemia, endocarditis, cystic fibrosis, vulvovaginitis and onychomycosis by Checkerboard microdilution assay. Data obtained by visual reading were further analyzed using the fractional inhibitory concentration index (FICI).
Result: The results revealed that MICs range for 10 and 11hs were 0.016-32 and 0.016–64 mg/L, respectively, while for fluconazole was 0.016–64 mg/L. Checkerboard analysis showed 10 and 11hs as novel azoles displaying synergistic activity against 67.5% and 45% of testes strains, respectively. In contrast, for *C. glabrata*, *C. parapsilosis and C. krusei* strains, the effect was different (FICI >0.5 and ≤4.0). No antagonistic effects were observed.
Conclusion: When used alone, novel fluconazole derivatives showed potent activity against all *Candida* species. Checkerboard results indicated that a synergistic effect occurred against most isolates. In conclusion, the combination of the new azole with fluconazole showed a synergistic effect against most *Candida* isolates. However, in the future, an animal experiment of candidiasis might be used to test whether the general anti-Candida and synergistic effects are retained.

Studying the Distribution of Genotypes of *C. albicans* in Patients Suffering from Recurrence with Various Clinical Signs Using the Method of Molecular Sequencing of PCR-SSCP in Zahedan

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Objectives: *Candida albicans* is considered the most common and the most important pathogen in patients with recurrent vaginal candidiasis. In terms of epidemiology, evaluation and classification of strains of *Candida albicans* in patients with recurrence are important to prevent the spread of infection. There are a number of CAI loci in the genome of *Candida albicans* that appear in the non-coded and significantly determine the polymorphic strains. Therefore, short repetitive sequences or microsatellites are widely used for genetic typing of microorganisms. The aim of this study was to investigate the distribution of genotypes of *C. albicans* in patients in Zahedan suffering from recurrence of various clinical signs by using the method of molecular sequencing of PCR-SSCP.
Methods: Vaginal samples obtained from patients with suspected recurrence of the disease were inoculated and incubated in the SDA and corn meal agar. PCR-RFLP reaction was conducted on the part ITS1- 5.8s - ITS2 with *MspI* enzyme for identification of *Candida albicans* strains, and the enzyme *MboI* was used to confirm the types of *albicans* and *dubliniensis* species*.* For conducting PCR-SSCP reaction, DNA was prepared and microsatellite CAI locus was distributed.
Results: With respect to candidates, 165 out of 350 samples (47.14%) were positive. Species identified via PCR-RFLP method included *Candida albicans*, 100 isolates (60.6%), *Candida dubliniensis*, 6 isolates (3.6%), *Candida glabrata*, 38 isolates (23%), *Candida krusei*, 19 isolates (11.5%), and *Candida tropicalis,* 2 isolates (1.2%). Among the total 165 patients, 118

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patients (71.5%) had recurrent candidiasis type and 47 patients (28.5%) had acute type. In the acute phase of infection and recurrence of *C. albicans* and non-*Candida albicans*, species showed no significant difference (P = 0.6). CAI fragments were distributed based on different patterns, and through comparing bands with standard strains, 26 different genotypes were identified on the basis of conformation and the spatial form. Genotypes A, K, Q, and I were the most abundant and considered as dominant. Genotype I in the acute form and genotype A in the chronic form of the disease were observed. The phylogenetic tree, using 5.8S sequence, showed that the genetic distance created by PCR-SSCP method corresponded with the results of the sequences within the scope of genus and species.
Conclusion: Molecular analysis of DNA sequencing fragments containing 5.8S and PCR-SSCP marker CAI showed that there is diversity in the two markers. The study could collect a comprehensive panel of phenotypic and genotypic markers in unique strains. This method can be used in large scale epidemiological studies to determine the strains of *Candida albicans* and small evolutionary changes in microsatellites.

Biocontrol Activity of the Entomopathogenic Fungus *Aspergillus niger* against *Anopheles stephensi*, Vector of Malaria

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Objectives: Malaria disease is one of the most important diseases caused by parasites all over the world. “Mosquito control” is the control of mosquito-borne diseases through the interruption of disease transmission by killing or preventing mosquitoes from biting humans. The aim of the present study was the- assessment of biocontrol activity of the *Aspergillus niger* against larvae and adult stages of *Anopheles stephensi*.
Methods: The spores of *A.niger* were released in rearing water at three dosages of 5×10^5, 10×10^5 and 15×10^5. The application methods including topical application of spores on sucrose solution, freely exposure to infected culture media and combination of them were assessed. The fungi invasion in both larval and adult *stages* were detected using three dimensional microscope and taking high resolution photos of them as well as preparation of 10% KOH wet mount from dead bodies followed by the preparation of tissue sections and staining with hematoxylin and eosin (H&E).
Results: Three dosages of 5×10^5, 10×10^5 and 15×10^5 spores yielded 16.0%, 24.0% and 24.0% mortalities, respectively compared to 3% mortality in the control group, which showed significant differences ($p<0.05$). Adult emergence was found in 82.0%, 65.0% and 22.0% respectively in the tested dosage compare to 97.0% of the control group ($p>0.05$). The survival rate of treated blood-fed mosquitoes was 10.0% in comparison to66.0% in control group ($p>0.05$).
Conclusion: Based on significant larval mortality or reduction of adult longevity, it is highly recommended to isolate the metabolites from local strain of *A.niger* for this purpose. The efficacy of such fungal metabolites might be further determined against malaria vectors at laboratory and field conditions.

Multilocus Enzyme Electrophoresis (MLEE) for Differentiation of Six Medically Important *Candida* Species.

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Objectives: *Candida* species are opportunistic yeasts that cause infections ranging from simple dermatosis to potentially life -threatening fungemia. These yeasts are capable of causing infections varying from simple dermatosis to widespread candidemia with relatively high mortality rate. In the present study, Multilocus Enzyme Electrophoresis (MLEE) was used for the discrimination of six medically important *Candida* species.
Methods: Six enzymatic systems including malate dehydrogenase (MDH), phosphoglucomutase (PGM), glucose-phosphate isomerase (GPI), Glucose-6-phosphate dehydrogenase (G6PD), 6Phosphogluconate dehydrogenase (6PGD), and Malic enzyme (ME) were used for the differentiation of the species.
Results: Of the examined systems, only ME showed no activity. MDH provided the best species-specific pattern for the discrimination of the species, and both MDH and G6PD provided a discriminatory pattern for the differentiation of *C. dubliniensis* from *C. albicans*.
Conclusion: Concerning species-specific patterns found in some of the above mentioned systems, MLEE might be used for the identification of the species in particular *C. dubliniensis* due to its good reproducibility and discriminatory power.

Identification of Asymptomatic Carriers of *Pneumocystis jirovecii* among HIV Positive People

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Objectives: HIV positive people with decreasing CD4+ Tcell count are at risk of *Pneumocystis* pneumonia (PCP).Although anti *Pneumocystis jirovecii* prophylactic drugs are administrated for these people, the success of this prophylactic regimen is unknown. The aim of this study was to identify asymptomatic carriers of *P. jirovecii* among HIV positive people and to evaluate the efficacy of using prophylactic drugs.
Methods: 151 Oropharyngeal wash samples were collected from HIV positive individuals resident in four different provinces of Iran. They did not have any clinical signs of PCP at the time of sampling or during the last six mounts prior to the study. *P. jirovecii* DNA was detected in the samples using two nested-PCR methods targeting different genes of the organism. CD4+ T cells count and history of taking anti *P. jirovecii* drugs by study population were taken from their health consultation reports. The correlation between the frequency of asymptomatic carriers and the use of prophylactic drugs was statistically evaluated.
Results: 103 (68.2%) and 48 (31.7%) of participants were male and female, respectively. The mean age of them was 37 years. The mean CD4+ T Cells count was 331(50 to 964) Cells/µl. *P. jirovecii* DNA was detected in 6 (4%) out of 151 OPW samples by both nested-PCR methods. All PCR positive people and 94%of study population did not take prophylactic drugs regularly.
Conclusion: Although the rate of asymptomatic carriers of *P. jirovecii* among the Iranian HIV positive people is not too high, they are at risk for developing PCP if they are not screened for the presence of the organism or if they are not followed up for their prophylactic programs.

Genotyping of Clinical and Environmental *Aspergillus flavus* Isolates from Iran Using Microsatellites

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Objectives: *Aspergillus flavus* has been described as the second most important *Aspergillus* species causing human infections in the tropical countries. Despite the growing challenge due to *A. flavus* in Iran, the molecular epidemiology strains isolated from patients and their environment has not been well studied. Herein, by use of microsatellites for genotyping of *A. flavus* isolates from Iran we evaluated the genetic relationships between clinical and environmental isolates.
Methods: A panel of nine microsatellite markers was used to analyze the genetic relatedness between 208 clinical and environmental isolates of *A. flavus*. The discriminatory power of

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the microsatellite markers was calculated using Simpson's index of diversity.
Results: 208 strains were analyzed, of which 67 strains proved to be a mixture of two or more different *A. flavus* isolates. The STR typing of 143 (n=119 clinical and n=24 environmental) pure isolates revealed that 118 different genotypes could be recognized. The discriminatory power (D) for the individual markers ranged from 0.4812 to 0.9457 and the panel of all nine markers combined yielded a diversity index of 0.9948.
Conclusion: We have seen a large genotypic diversity in *A. flavus* isolates from Iran. High resolution typing method such as microsatellite analysis in the present study yielded better understanding of the molecular epidemiology of *A. flavus* complex. Therefore, microsatellites is a powerful typing tools for discriminating between *A. flavus* isolates from different sources.

Diagnosis of fungal rhinosinusitis in paraffin-embedded tissue by Semi Nested PCR

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Objectives: *Fungal rhinosinusitis* (FRS) is a disease whose history dates back to several decades ago. This disease is commonly divided into two categories: Invasive and non-invasive. Histopathology is the "gold standard" method to diagnose this disease. Histopathological diagnosis of fungi in tissue samples requires great experience in this field, and this method is not able to determine the genus and species of the causative microorganism. In addition, the culture of half of the histopathologically positive samples yielded no growth. This study was an attempt to evaluate the diagnostic power of PCR method for the diagnosis of fungal sinusitis in tissue samples.
Methods: In this study, 110 paraffin-embedded biopsy specimens from sinus tissues were subjected to DNA extraction. The amplification of a β-globin gene by PCR- based method was used for confirming the quality of extracted DNA. Semi Nested- PCR using ITS primers were performed for the detection of Fungal DNA.
Results: Of 110 biopsy samples suspected of sinusitis, 60 were positive by histopathologic methods. 53 out of the 60 histologically proven positive were positive in PCR, and 7 samples were negative. Out of 50 negative samples by histopathological methods, 45 samples were negative in PCR while 5 were positive.
Conclusion: Direct identification of fungal DNA in tissues by PCR method make it possible to confirm the diagnosis of fungal sinusitis; in addition, by sequencing PCR products, the etiologic agents can be determined with no need to culture the specimen.

Genes Expression Profiling of Polyene-Resistant *Candida parapsilosis* Strains

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Objectives: The rate of candidiasis among patients has increased mostly in recent years. One of the five common yeasts is *Candida parapsilosis* that is involved in invasive candidiasis.
Amphotericin B (AMB) has activity against a number of pathogenic fungi. Resistance to polyenes is less common than azoles, but recently it is increasingly reported in filamentous fungi and pathogenic yeasts. Using gene expression pattern can be realized responses mechanisms of action these genes and resistance to this antifungal drug. In the present study, three resistant strains obtained to following *in vitro* susceptibility testing of *C. parapsilosis* clinical isolates. Our aim was changes in gene expression (*ERG3, ERG6 ,ERG11*) were then determined using a Real-time PCR.
Methods: 120 clinical samples of *C. parapsilosis* were tested against Amphotericin B (AMB) (Sigma-Aldrich, USA). By molecular methods, these isolates had previously been identified as C. *parapsilosis*. Reference antifungal susceptibility testing of isolates was performed by the broth microdilution method described in Clinical and Laboratory Standards Institute (CLSI) guidelines, document M27-S3. *C. parapsilosis* ATCC 22019 type strain was used for quality control of antifungal susceptibility testing. To quantify possible changes in the ERG3, ERG6, ERG11 genes expression in *C. parapsilosis* was measured by quantitative real-time RT-PCR.
Genes expression was normalized to the housekeeping gene ACT1 and analyzed by using REST (2008 V2.0.7) software.
Result: Evaluation of the antifungal susceptibility profile showed that only two (1.66 %) *C. parapsilosis* strains were resistant to AMB. ERG3, ERG6, ERG11 and ACT1 (housekeeping gene) mRNA levels were examined in two resistant strains (AMB_{R1}, AMB_{R2}). ERG11 up regulation was observed in AMB_{R2}, a significant difference in ERG11 regulation wasn't observed in AMB_{R1}. Down regulation in ERG3 and ERG6 was observed in (AMB_{R1}, AMB_{R2}).

Conclusion: The investigation of antifungal resistance profiles in clinical isolates of *C. parapsilosis* may provide important information for the control of antifungal resistance as well as distribution and susceptibility profiles in populations. Although the development of resistance is rare, this suggests that there is a multitude of factors responsible for resistance to polyenes beside target alteration.

Seeking for Amino acid Substitutions in erg11p of Fluconazole-Resistant Clinical Isolates of *Candida glabrata:* Effective Substitutions and Homology Modeling

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Objective: Understanding of the mechanisms responsible for resistance to fluconazole (flu) in *C. glabrata* is not only crucial for the development of new antifungals, but also important in choosing the appropriate antifungals for the patients at the earliest stages. Up regulation of the ABC transporter genes is the major cause of drug resistance. Nevertheless, the essential role of erg11p, encoded by the *ERG11* gene, should not be neglected. The aim of the present study was screening the erg11p amino acid substitutions in flu-resistant clinical isolates of *C. glabrata*.
Methods: A total number of 60 clinical isolates of *C. glabrata* were investigated. Antifungal susceptibility to fluconazole was determined for each isolate using the broth microdilution reference method (CLSI document M27-A2). The *ERG11* gene was amplified by PCR and sequenced afterwards. A flu-susceptible isolate was also subjected for sequencing. The sequencing results for each part were multi aligned by MEGA6 software. A homology model of the *C. glabrata ERG11* gene was created by SWISS-MODEL software using the crystal structure of *S. cerevisiea* Erg11p as a template and the predicted binding sites to fluconazole were investigated.
Results: Four isolates demonstrated high MIC values (≥64µg/ml). The flu-resistant strains were isolated from BAL (n=1), Vagina (n=1), sputum (n=1), and nail (n=1). We found 26, 16, 24, 11 mutations of which 17, 6, 9, 4 were silent mutations without amino acid changes. According to homology modeling results, Y126, F134, I139, Y140, F236, G310, V311, G314, G315, T318, L380 and M509 are responsible for binding to flu in S. cerevisiae. The amino acid substitution G236V was at the binding site and mutations H146Q and D234 were near to the binding site of triazoles.
Conclusion: There is little documented data about the azole-resistant related mutations in the yeast *C. glabrata*. Here, we reported mutations which have not been documented before. Our study was the first one using homology modeling to predict the hot

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point mutations in flu-resistant *C. glabrata* isolates.

Development a Multiplex Real-Time PCR for Differential Detection of Causative Agents of Onychomycosis

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Objectives: Onychomycosis is a common nail disease that is responsible for up to 50% of nail disorders. The conventional methods used to diagnose onychomycosis are microscopy and fungal culture. Microscopy, although fast and economical, has a sensitivity dependent upon many factors, including the skill of the operator and the quality and quantity of nail samples obtained. Recently, PCR assays have been developed for the direct detection of fungi in nail samples. Multiplex PCR was reported as a reliable alternative. This study aim to detect non- dermatophyte molds, as well as *Candida* and dermatophyte species, in the nail samples suspicious for onychomycosis using a multiplex real-time PCR culture-independent method.
Methods: Four hundred fifty gene sequences from the NCBI database were analyzed to design three sets of primers and taqman probes from 5.8s, β-tubulin and D1D2 regions that target the non-dermatophyte molds, dermatophyte and *Candida* species, respectively. We investigated the specificity of these primer sets and probes using fungal genomic DNA prepared from different fungal species. To assess the clinical applicability of the primers and probes, 147 nail specimens were analyzed in parallel by multiplex real-time PCR and direct examination. DNA was extracted using a conical bullet and commercial kit after putting one mg of samples in -70°C for 30 minutes.
Results: The primers and probes specifically detected fungal DNA. The positive rates of direct examination specimens were 8.8%, 23.1% and 13.6% for *Candida*, dermatophyte and Non dermatophyte, respectively. In contrast, PCR analysis yielded a positive rate of 17.6% for each of the three groups of pathogen fungi. Multiplex real-time PCR showed positive results for more than one agent in 15.6% of samples. Among 80 samples that were negative by direct examination, 34 were positive by real-time PCR. Thirty four samples were negative by both direct and real-time PCR.
Conclusions: The unique advantage of the Multiplex PCR was that more than one target sequence could be amplified, which is clinically useful as an efficient and fast procedure for the detection of pathogens. The number of specimens that were positive by real-time PCR was more than the numbers that were positive by direct examination alone. It could be due to the high sensitivity of designed primers and ,therefore, detection of fungi which are rarely true pathogens but may occur as contaminants or normal flora will not be diagnosed by direct examination.

Occurrence and Species Distribution of Pathogenic Mucorales from Unselected Soil Samples in France

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Objectives: Mucormycosis is a life-threatening invasive fungal disease that affects a variety of human patient and animals. Although Mucorales are mostly opportunistic pathogens, originating from soil or decaying vegetations.There is currently little data on the prevalence of this kind 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 France.
Methods: Soil samples were collected in different regions. Two grams of soil were homogenized in 8mL of sterile saline containing 0.05% Tween 80. Suspensions were placed on Sabouraud dextrose agar (SDA) and RPMI agar supplemented with itraconazole (4 mg/L) or voriconazole (1mg/L). 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. Each strain was identified phenotypically, and molecular identification was performed by ITS sequencing.Antifungal susceptibility testing was done in accordance with EUCAST guidelines.
Results: A total of 173 soil samples were analyzed. Forty-five(26.1%) samples were positive for Mucorales. Among these isolates, there were 30*Rhizopusoryzae,*11 *Mucorcircinelloides,* two *Lichtheimia corymbifera,* one *Rhizopus microspores* and one *Cunninghamella bertholletiae*. Direct sequencing confirmed the phenotypic identification in all cases. Positive soil samples came from cultivated fields (including fields of maize, sunflower, and rapeseed) but also from other types of soil such as flower beds. Mucorales were retrieved from samples obtained in different geographical regions of France. It was found out that growth of Mucorales was more frequent on mediums containing voriconazole. The resulting geometric mean MIC for *Rhizopus oryzae* and *Mucor circinelloides* isolates were 0.95 µg/mL and3.11 µg/mL for posaconazoleand0.55 µg/mL and 0.14 µg/mL for amphotericin B, respectively.
Conclusion: The present study showed that pathogenic Mucorales are frequently recovered from soil samples in France. Species diversity should be further analyzed on a larger number of soil samples from different geographic areas in France and in other countries.

High prevalence of clinical and environmental triazole resistant *Aspergillus fumigatus* in Iran: is a big challenge?

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Objectives: Recently, triazole resistance in *Aspergillus fumigatus* is an emerging health problem in medical cares due to long term use of azole drugs. In addition, prolonged exposure of *A. fumigatus* to fungicides in agriculture could develop resistance in environment.
Methods: during the three years of study from 2013 to 2015, a total of 513 clinical (n= 213) and environmental (n= 300) isolates which had been gathered across ten provinces of Iran were processed and examined for growth of itraconazole and voriconazole resistant *A. fumigatus* by cultured on Sabouraud's dextrose agar plate comprising 4 and 1 mg/liter itraconazole and voriconazole, respectively at 45°C for 72 hours in dark**.** Identification based on sequencing of the partial beta-tubulin gene using the specific primers was performed. Minimum inhibitory concentrations (MICs) and minimum effective concentration (MEC) of antifungal agent were determined according to the Clinical and Laboratory Standards Institute M38-A2 document**.** To find possible mutations in isolates that reduced susceptibility to itraconazole and voriconazole (MIC of >2 µg/ml) according to CLSI breakpoints the whole region of the *cyp51A* gene was amplified using three pairs of primers.
Result: out of the whole isolates, 150 *A. fumigatus* isolates (n=71; clinical, n=79; environmental) were confirmed. A total of 10 (6.6%) (Clinical; 4.2% and environmental; 7.6%) strains showed MIC values above the clinical breakpoints for at least one of the antifungal agents tested by in vitro antifungal susceptibility testing. Among the *cyp51A* mutations found in the set of isolates with itraconazole-resistant, the TR34/L98H variant was the most prominent, but no other polymorphisms were identified by using this method.
Conclusion: In recent years, triazole-resistant clinical and environmental isolates of *A. fumigatus* have emerged in Europe, and in other continent have also been described. Resistant isolates with TR34/L98H mutation can always be observed in invasive aspergillosis and azole compounds should be used carefully for prophylactic and treatment purposes.

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Identification of *Candida* Species Isolated from Vulvovaginitis in Mashhad, Iran by the Use of the MALDI-TOF MS

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Objectives: Vulvovaginal candidiasis (VVC) is a common problem in women**.** The purpose of this study was to identification of *Candida* species isolated from vulvovaginitis women referred to Ghaem Hospital by MALDI TOF mass spectrometry techniques.
Methods: The experiment was performed on 65 clinical samples Vulvovaginitis women were collected in Ghaem Hospital. First of all specimens using phenotyping techniques such as microscopy and culture on Sabouraud dextrose agar and corn meal agar medium, *Candida* species were detected and were evaluated with MALDI TOF mass spectrometry.
Results: Of the 65 isolates analyzed, 93.8% were correctly identified by MALDI-TOF mass spectrometry. In this study, the most frequently isolated species were *Candida albicans* (58.5%), followed by *Candida tropicalis*(16.9%), *Candida glabrata*(7.7%), *Candida parapsilosis*(7.7%), *Candida guillermondii*(3.1%) and species not in database (6.1%).
Conclusion: Our results demonstrate that the MALDI TOF mass spectrometry is a fast and reliable technique, and has the potential to replace conventional phenotypic identification of Candida species and yeast strains routinely isolated in clinical microbiology laboratories

A Comparative Analysis on ITS, RPB-2 and EF1α Fragments, Aiming Species Delimitation in *Fusarium Solani* Species Complex (FSSC)

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Objectives: The genus *Fusarium*, one of the most prominent pathogens of plant and animal species, harbors multiple species and species complexes. *Fusarium solani* species complex (FSSC), defined mainly based on morphology, is indeed a diverse complex of many phylogenetic and/or biological species. These fusaria may be soil-borne although they cause a considerable range of fungal infections, like keratitis, in human. However, In spite of the fact that fusaria are among the most important fungal genera, binomial nomenclature may not be offered for a great deal of them and this lack of binomials seem to be problematic in FSSC. Besides, species delimitation may seem tedious in this species complex. Thus, a combined set of sequence loci; ITS, RPB-2 and EF-1α, which are used in species level identification in many fusaria, are here analyzed.
Methods: Using various sequence analysis approaches in BlastN, Geneious, MEGA5 and mrbayes programs, three genomic loci which are used for species delimitation in the genus *Fusarium* were studied. Thus, EF-1 α, ITS and RPB2 fragments of a collection of 109 isolates of FSSC, gathered from nucleotide database of GenBank in NCBI, and culture collections; IBRC, CBS and NBRC, were aligned using the default parameters of Clastal W multiple sequence alignment program in MEGA5. Thereafter, the sequence identity and the average pairwise identity values were calculated for multiple sequence alignments, singularly. Also, the same procedure was performed on the combined multi-loci sets; EF-1/ITS, EF-1/RPB-2, ITS/RPB-2, and ITS/EF-1/RPB-2. In addition, Bayesian analyses using Mrbayes package, and MP and ML analyses using MEGA5 package were conducted through the same sequence alignments to illustrate the robustness of each tree.
Results: Sequence analyses performed on EF-1α, RPB2, and ITS fragments of FSSC members showed that the sequence identity in EF-1α fragment cannot be more than 58% (average pairwise identity ~95%), while this value was around 65% for ITS fragment (average pairwise identity ~96.5%) and 70% for RPB2 fragment (average pairwise identity ~97.5%).
Conclusion: Evolutionary speaking, ITS fragment seems to be similar to EF-1α in FSSC, although the latter has been shown to possess a higher resolution. Additionally, although EF-1α fragment, as the most mutable fragment in FSSC, is recommended as the first choice for species delimitation in FSSC, the complementary effects of ITS and RPB-2 fragments should not be neglected. Evidently, they cause a higher robustness which can be inferred from the bootstrap and posterior probability values.

Epidemiological Status of Dermatophytosis in Khuzestan, Southwestern Iran, an Update

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Objectives: Dermatophytosis is amongst the most common superficial fungal infetions in Iran. In this study we aimed to update the clinical and microbial aspects of human dermatophytosis in Khuzestan, Southwestern Iran.
Methods: In the framework of a one-year survey a total of 4120 skin, hair and nail samples obtained from the outpatients with symptoms suggestive of tinea were analyzed by using direct microscopy, culture and molecular identification methods. Strains isolated from culture were subjected to amplification of the nuclear rDNA ITS regions in a PCR assay followed by RFLP analysis. For confirmation of species identification, 100 isolates as representatives of all presumable species were subjected to ITS-sequencing.
Results: Infection was confirmed in 1123 cases (27.25%) by direct microscopy and/or culture including 603 male vs. 520 female. Infection frequencies were the highest and the lowest in age groups of 21−30 and 11−20 years old, respectively. Tinea corporis was the most prevalent clinical manifestation followed by tinea cruris, tinea capitis, tinea manuum, tinea pedis, tinea unguium, tinea faciei and tinea barbae. *Trichophyton interdigitale* (58.7%) was the most dominant isolate followed by *Epidermophyton floccosum* (35.4%), *Microsporum canis* (3%), *T. rubrum* (1.5%), *T.* species of *Arthroderma benhamiae* (0.5%), *T. tonsurans* (0.3%), and *T. violaceum* (0.3%). Other species included *M. gypseum*, *M. fulvum* and *T. verrucosum* (each one 0.1%). The prevalence of dermatophytosis caused by zoophilic species increased, while infections due to anthropophilic species decreased.
Conclusion: To clarify the epidemiological trends of dermatophytosis and dermatophytes application of DNA-based

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methods is an important aid.

Distribution and Identification of Aspergillus Species in Bronco Alveolar Lavage in High Risk Patient Prone to Aspergillosis Referred to Massih Daneshvari Hospital, Tehran, Iran

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Objective: Aspergillus species are ubiquitous, saprophytic fungi. Among the human pathogenic species of aspergillus, *A. fumigatus* is the primary causative agent of human infections, followed by *A. flavus*, *A. terreus*, *A.niger.* Invasive Aspergillosis (IA) is perhaps the most devastating of aspergillus related diseases targeting severely immunocompromised patients. Those most at risk for this life-threatening disease are individuals with hematological malignancies ,lung transplant patients , patients on prolonged corticosteroid therapy , diabetic patients , COPD patients , and patients with cancers .Therefore, diagnosing aspergillosis in these patients is very important for survival. The aim of this study was the investigation of prevalence of aspergillus speciese in BAL sample in patients reffered to Massih Daneshvari hospital in 93-94.
Methods: The BAL sample of susceptive patients was collected in steril condition and transferred immediately to mycology laboratory. After centrifuging and procceding with pankratin, the perception was used to direct smear with KOH 3% and then cultured on Chapkes agar and Saboroud dextrose agar medium for 7 days in 25 ˚ c.
After this time, the growth of aspergillus was cheked, then the species were identified by using conventional phenotyping method .Galactomannan assay were done for positive patients.
Results: Among 480 ball samples of susceptive patients, the aspergillus species were identified in 20 species by direct smear and slide culture overall including 7 case *A. fumigatus* , 6 cases *A. flavus* , 4 case *A. terreus* , 3 cases *A .niger*. Also these patients were positive for galactomannan antigen in ELISA test.
According to the history, the patients had one primary disease such as lung transplant, cancer, COPD, old TB, corticosteroid therapy, luckemia.
Conclusion: Our finding showed, that these patients are susceptive and high risk to aspergillosis and we know that species can cause IA (specially *A.fumigatus* and *A.flavus*) in these patients. [Exploration and diagnose aspergillus speciese is very important and lead us to use antifungal drugs for curing these patients for more survival.] In conclusion, antifungal therapy must be applied for high risk individual.

Identify Species of Dermatophytes Using Random Primers OPAA17 by AP-PCR Method

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Objectives: Dermatophytes are closely related to keratinophilic fungi that invade the keratinzed tissues in humans causing dermatophytosis. Identification of dermatophytes with conventional methods is time- consuming and sometimes problematic because of similarities and variabilities of species. Genetic amplification has made rapid and perfect identification of dermatophytes possible. AP-PCR method is used for typing, but the aim of this research was evaluation of AP-PCR method for identification of dermatophytes and to find a suitable approach for a rapid distinguishing of dermatophytes.
Method: Fifty-two isolates from 10 species of dermatophytes including: *T. mentagrophytes* (10), *T. verrucosum* (9), *T. rubrum* (5), *T. tonsurans* (3), *T, violaceum* (2), *T. schoenleinii* (1), *M. gypseum* (8), *M. canis* (4), *M. ferrugineum* (2), *E.floccosum* (8) were collected and confirmed with microscopic, macroscopic and biochemical tests. After DNA extraction, Molecular identification was done using AP-PCR (arbitrarily primed PCR) with random primer OPAA17. These primers amplified bands of different sizes in species of dermatophytes DNA.
Results: All species of dermatophytes were recognized with a distinct DNA band patterns on gel agarose. The range of obtained bands was between 200 to 1400 bp for all dermatophyte except *trichophytons choenleinii in which* no band was seen.
Conclusion: In laboratory, distinguishing *T. mentagrophytes* from *T. rubrum* has always been problematic, but using this method, recognizing the two species of fungus can be easily done. AP-PCR is such a sensitive method that the slightest change in the concentration of DNA, primer, MgCl2 may yield different results. Therefore, using this approach as a diagnostic procedure is not recommended.

Long Non-Coding Rnas as New Potential Biomarkers in Mycotoxins: Evaluation of Lincrna-ROR in Aflatoxin-Induced Hepatocellular Carcinoma

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Objectives: Aflatoxins are carcinogenic, mutagenic, immunosuppressive and teratogenic mycotoxins contaminating food and feed produced by Aspergillus spp as their by-products. Aflatoxins interact synergistically with HBV/HCV infection which increases the risk of hepatocellular carcinoma, an important cause of death all over the world. Clearly, the theory that carcinogenetic processes are initiated and sustained by cancer stem cells is current in the mycotoxin aflatoxin-induced hepatocellular carcinoma. Non-coding RNAs, such as microRNAs and long non-coding RNAs may be important in the maintenance of fungal infections and mycotoxin mechanisms such as aflatoxin-induced hepatocellular carcinoma. lincRNA-ROR is a newly-discovered lncRNA which is involved in stem cell properties. According to the recent reports, molecular mechanisms of potential effect of aflatoxin and lincRNA-ROR have some common points in the cell. The objective of this study was to evaluate the expression changes of reprogramming-related long non-coding RNA (lincRNA-ROR) in aflatoxin-treatment hepatocellular carcinoma.
Methods: In this study, human cell line hepatocellular carcinoma, HepG-2, (NCBI Code: C158) were purchased from the National Cell Bank of Pasteur Institute (Tehran, Iran) and cultured in Eagle's Minimum Essential Medium in fetal bovine serum to a final concentration of 10%. Cells were treated with specific concentrations of aflatoxin which MMT tests showed they were less toxic for the cells. RNA isolations, cDNA synthesis and quantitative Real-Time PCR were carried out for lincRNA-ROR and p53 in specific times. Data analysis was down in raw data. Results: Preliminary data showed some changes on lincRNA-ROR in cells in comparison to non-treatment cells.
Conclusion: Relationship between aflatoxin exposure, development of human primary hepatocellular carcinoma and mutations in the p53 tumor suppressor gene has been proved before. LincRNA-RoR is up-regulated in most tumor cells. Since lincRNA-ROR and p53 have a feedback auto-regulatory system, p53 may be a common point for these two molecules by which they act.

Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of *C. albicans* and *C. dubliniensis*

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Objectives: Candidiasis is the main opportunistic fungal infection which has increased over the past 30 years with high mortality ranging from 40% to 60%. *C. albicans* is a commensal and a constituent in mucosa of healthy individuals. It's also the most common opportunistic human fungal pathogen. In addition, *C. dubliniensis* is closely related to *C. albicans* and shares many phenotypic properties traits with *C. albicans* such as the ability to form chlamydospores and germ tubes. This strain exhibits increased

Official publication of Iranian Society of Medical Mycology (ISMM) and Invasive Fungi Research Center (IFRC)
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adherence to epithelial cells and may have higher propensity to develop azole antifungal drug resistance than *C. albicans*. The present study was performed in order to evaluate MALDI-TOF MS and PCR assay by using hyphal wall protein1 (hwp1*)* gene to discriminate between *C. albicans and C. dubliniensis*.

Methods: Initial identification was performed by phenotypic methods and PCR-amplification of ITS rDNA region followed by RFLP analysis with the *MspI* enzyme. For discrimination between *C. albicans*, *C. dubliniensis* and *C. africana*, a PCR assay was done by using *hwp1* gene as well as a MALDI-TOF MS identification were then performed with the ethanol/formic acid extraction protocol according to Bruker Daltonics.

Results: A total of 121 *Candida* isolates were obtained from sputum and BAL samples of patients suspected to respiratory infection. 83 of these strains which were previously identified as *C. albicans* by *MspI* enzyme restriction analysis, 89.15%, 9.63%, 1.2% were identified as *C. albicans*, *C. dubliniensis* and *C. africana* respectively by *hwp1* gene. However, 90.35% and 9.63% were identified as *C. albicans* and *C. dubliniensis* respectively by performing MALDI-TOF MS method.

Conclusion: *C. dubliniensis* is merging as a pathogen and has frequently been misidentified as *C. albicans* by clinical laboratories. *C. dubliniensis* exhibits increased adherence to epithelial cells and may have higher propensity to develop azole antifungal drug resistance than *C. albicans*. Although the MALDI-TOF is a highly useful method for the routine identification of yeasts, we identified C. *africana* as C. *albicans* by this method, PCR assay could identified *C. africana* from *C. albicans* complex by *hwp1* gene. Species distribution of *Candida* species may play an important role in achieving successful therapeutic treatment due to variation of virulence, acquired and intrinsic antifungal susceptibility of *candida* species

The Effect of Aloe Vera Gel Compared with Clotrimazole Cream in the Treatment of Vaginal Candidiasis

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Objectives: vaginal candidiasis is the second most common infection of the vagina. Statistics show that about 75 percent of women of child-bearing age of 20 to 40 years have problems with the vaginal candidiasis. Native people in southern Iran use aloe vera gel to treat this disease. IOn the other hand, clotrimazole is a broad-spectrum antifungal that is used in the treatment of vaginal candidiasis caused by C. albicans and other Candida species .The aim of this study was to determine the effect of aloe vera gel, compared with clotrimazole cream, in the treatment of vaginal candidiasis.

Methods 80 women with vaginal candidiasis aged 20-40 were examined. Half of them used aloe vera gel and the other half used clotrimazole cream for a week. Data were analyzed by SPSS (ver13),T- test, chi-square and fisher exact test.

Results: After 1 week, reduction in the severity of symptoms in those consuming clotrimazole was 75 % while it was 20% among those using aloe vera gel. This difference was statistically significant ($p<0.05$).

Conclusion: Compared with aloe vera gel, A significant reduction of symptoms and complaints was seen when using clotrimazole cream. So taking clotrimazole cream seems more effective in the treatment of vaginal candidiasis. Further studies on aloe vera gel as an alternative treatment for synthetic drugs for vaginal candidiasis is recommended.

Study the Effects of Some Non-Steroidal Anti –Inflammatory Drugs on Morphogenesis and Pathogenesis of *Candida Albicans*

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Objectives: The polymorphic fungus, *Candida albicans*, is known as the most common fungal pathogen in human causing both mucosal and systemic infections. The yeasts of genus *Candida* are the fourth most common cause of nosocomial bloodstream infections. It has been also suggested that biofilms formed by Candida species play an important role in its pathogenicity. Nowadays, anti-inflammatory drugs are among the most popular medications to control pain and inflammation especially in hospitalized patients and transplant recipients. This study was done to evaluate the effect of NSAIDs (non-steroidal anti-inflammatory drugs) including Diclofenac and Piroxicam on biofilm formation of *C. albicans* and the expression of genes related to its morphogenesis and pathogenesis.

Methods: The growth of biofilm of *C.albicans* (standard strain) was investigated using XTT reduction assay after exposing fungal cells to different concentration of drugs compared with untreated cells. Expression of adhesion-related genes (ALS1,3), hypha specific genes(HWP1,EAP1),hypha specific secreted aspartyl proteinase(SAP4,6), and regulatory gene(EFG1) were analyzed by RT- PCR in the treated cells with different concentration of the mentioned drugs.

Results: Diclofenac at the concentration up to 1.56 mg/ml inhibited biofilm formation of *C.albicans*. Also, Piroxicam had a similar effect on the inhibition of biofilm formation at the concentration up to 2.5 mg/ml. Furthermore, these drugs increased the expression of ALS1 and SAP6 significantly, in dose dependent manner. However, HWP1 and SAP4 genes were down regulated in the treated cells compared to untreated controls.

Conclusion: These data provided new insight into the effect of anti-inflammatory drugs on *C.albicans* growth and transition. According to these results, broad-spectrum use of these medications might affect the pathogenesis of Candida, as human microflora, and should be considered in their prescription.

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