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Genetic diversity of the emerging human fungal pathogen Pitella norvegensis
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Objective: Pitella norvegensis (i.e. Candida norvegensis) is increasingly isolated from hospital settings, especially from immunocompromised patients. Understanding the rare pathogen, including its emergence and distribution, is crucial for accurate diagnosis and infection prevention. We studied the genetic diversity of a large collection of clinical P. norvegensis isolates obtained from Dutch hospitals along with a set of non-Dutch clinical and environmental isolates.

Methods: Clinical (n = 234, 90.6%) and environmental (n = 24, 9.2%) P. norvegensis isolates were subjected to Amplified Fragment Length Polymorphism (AFLP) fingerprinting and a novel size-foc microsatellite typing panel. Data were analysed with BioNumerics and Structure. We applied a novel mating type assay to determine the MATa locus and pseudocalsequencer.

Results: AFLP fingerprinting separated the P. norvegensis isolates into three main clusters. Two clusters fully consist of clinical isolates, the third represented a mix of clinical and environmental isolates. By microsatellite typing, the overall genetic diversity was low (Simpon’s D = 0.969), due to a large number of Dutch clinical isolates with similar genotypes. Minimum spanning tree analysis showed that Dutch clinical isolates fell into two clusters. Environmental and non-Dutch isolates were more distant and related. STRUCTURE analysis showed the presence of four genotypes, with signs of genetic admixture between geographic locations and clinical/electronic isolates. Nearly all isolates harbor the MATa mating-type allele.

Conclusions: The P. norvegensis isolates obtained from Dutch hospitals appeared to be largely closed, independent of geographic origin and isolation date. The observed clonality is supported by the common number of AFLP isolates. Microsatellite typing indicated potential admixture between clinical and environmental isolates.

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First report of Mycosoma due to Madurella fajalii from India
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Objective: To describe the first case report of Madurella fajalii from India.

Methods: A 70-year-old non-diabetic man from Bhadrak district who worked as a soldier in past came to us with a history of pus discharge containing black grains of the size of mustard seeds without any pus in the posterior aspect of the thigh for last 5 years. He had been operated twice back for the same complaints, however, finding no relief he visited us. On examination, a deformed bone measuring 10 × 10 cm, containing 6-8 masses which discharged yellowish pus containing black grains measuring 0.5 × 1 cm was found on the posterior aspect of the thigh. The lesion was painless, devoid of any Driscoll’s sign and associated with enlarged and non-tender and non-indurated inguinal lymph nodes measuring 2 × 2 cm. General physical examination and routine hematology laboratory examination revealed no abnormalities. Serological tests did not reveal the presence of HIV, Hepatitis B, or Hepatitis C. A clinical diagnosis of black grain mycetoma was made and the patient was sent to microbiology laboratory for fungal culture. Black hard grains were found on KOH mount and culture on subculture media agar grew a brown colony which differed a brown pigment into the medium after 15 days of incubation. LSPC examination showed brown non-spore forming mold. DNA was extracted using phenol-chloroform isoamyl alcohol after grinding the sessile mus in liquid nitrogen and subjected to PCR using ITS4 and ITS5 primers as described previously. The product was subjected to agarose sequencing and subjected to Blast and it showed 99.41% similarity to M. fajalii (MJP9863). The patient was started on Itraconazole in lieu of voriconazole as the patient could not afford the drug. While there is no reduction in the size of the lesion the patient reported symptomatic relief and is still on follow-up.

Conclusion: To the best of our knowledge this is the first case of Mycosoma due to M. fajalii from India.

P210
Fungal Isolates of the respiratory tract in symptomatic patients hospitalized in pulmonary units: A mycological and molecular epidemiologic study
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Objective: Fungal respiratory infections are being recognized with increasing frequency in parallel with an expanding population of immunocompromised patients. In most cases, colonization is the first step in the progression to pulmonary fungal infection. This study was designed to evaluate the distribution of fungal elements in the respiratory tract of symptomatic patients hospitalized in pulmonary units.

Methods: This descriptive cross-sectional study was carried out over a period of 2 years, from October 2017 to October 2019 in Golestan province, located in Iran’s northern region. In the current study, bronchoalveolar lavage or sputum specimens were collected. All samples were analyzed by direct microscopy using KOH 10% and cultures. Fungal identification was accomplished by internal transcribed spacer (ITS) and beta-tubulin sequencing. Also, in patients suspected of invasive pulmonary aspergillosis, BAA specimens were tested for galactomannan (GM) antigen.

Results: A total of 184 lung specimens (192 bronchoalveolar lavage (BAL) and 192 sputum samples) were obtained from symptomatic patients hospitalized in pulmonary units. Of these, 137 (53.67%) were positive in direct examination and cultures. Among the 137 positive cases, most isolates were from male patients 86 (62.77%) and most of them were between 46 and 72 years. Candida albicans (37.22%) and C. tropicalis (21.89%) represent the two most commonly isolated species in the current study. C. albicans (84.16%), C. guilliermondii (32.02%), parakar species (62.04%), and weight loss (54.2%) were the predominant symptoms and complications (24.81%), keratitis (21.89%), and diabetes mellitus (19.70%) were the predominant underlying conditions. Also, 5 cases of invasive pulmonary aspergillosis and 3 cases of mucormycosis were diagnosed.

Conclusions: Candida albicans was the most common fungal species isolated from symptomatic patients hospitalized in pulmonary units. Tuberculosis, chemotheraphy and diabetes mellitus were important underlying conditions for pulmonary fungal colonisation and/or infection.

P211
Fungal and bacterial co-infections of the respiratory tract among patients with COVID-19 hospitalized in intensive care units
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Backgrounds: The pandemic of COVID-19 has created a global public health crisis. ICU patients with COVID-19 are prone to infections of bacterial and fungal origins due to several risk factors. Consequently, the current study was conducted to evaluate the frequency, demographic characteristics, underlying conditions, and etiological agents of fungal and bacterial co-infections in the respiratory tract among ICU patients with COVID-19 in Iran.

Materials and Methods: From May to October 2020, sputum and multidrugs aspirates were collected from ICU patients hospitalized with COVID-19 who also were suspected of bacterial and/or fungal co-infections according to inclusion criteria. The etiological agents of bacterial co-infections were identified using the VITEK 2 identification system. For fungal identification, all samples were analyzed by direct microscopy using KOH 10% and cultures. Furthermore, all isolates were subjected to the sequencing method.

Results: A total of 73 lung specimens were obtained from patients who met the inclusion criteria. All these, in 35 cases (54.05%) fungal and/or bacterial co-infections were confirmed. Male were more infected (75.33%) and all of them were between 49 and 72 years. Candida albicans (n = 8, 41.43%) and Klebsiella pneumoniae (n = 7, 38.46%) were the most frequent etiologic agents related to fungal and bacterial co-infections, respectively. Pseudomonas (n = 15, 100%) and diabetic mellitus (n = 8, 53.33%) were documented as the most prevalent underlying conditions. In the current study, 5 out of 15 patients (20%) died.

Conclusions: The frequency of bacterial co-infections of the respiratory tract in ICU patients hospitalized with COVID-19 was relatively high. According to the results, one of the causes of death in these patients could be a secondary infection.
Table 1.
GenBank accession numbers of DNA sequences included in this study.

| Isolate | Molecular identification | GenBank accession number |
|---------|--------------------------|-------------------------|
| SP1^b   | *Candida glabrata*       | MT772038                |
| EA1^c   | *C. glabrata*            | MT772039                |
| EA2     | *Candida albicans*       | MT772040                |
| EA3     | *Candida krusei*         | MT772041                |
| EA4     | *C. albicans*            | MT772042                |
| EA5     | *C. krusei*              | MK793223                |
| EA6     | *C. albicans*            | MT772043                |
| SP2     | *C. albicans*            | MT772044                |
| EA7     | *C. albicans*            | MT772045                |
| EA8     | *C. albicans*            | MT772046                |
| SP3     | *C. glabrata*            | MK793225                |
| EA9     | *C. albicans*            | MT772047                |
| EA10    | *C. albicans*            | MT772048                |

ITS ^a: internal transcribed spacer; SP ^b: Sputum; EA ^c: Endotracheal aspirate.

Table 2.
Detailed information of each ICU patient hospitalized with COVID-19 who had positive results for fungal and/or bacterial co-infections of the respiratory tract.

| Patient number | Age (years) | Gender | Underlying diseases | Sample | CT scan^1 | Fungal culture and molecular detection | Bacteria | Antifungal and/or antibacterial given for curative intent | Outcome |
|----------------|-------------|--------|---------------------|--------|-----------|----------------------------------------|----------|--------------------------------------------------------|---------|
| 1              | 51          | Male   | Pneumonia, heart failure and hypertension, lung cancer | Sputum |           | Candida glabrata                        | Streptococcus viridans | Caspofungin + Meropenem | Recovered |
| 2              | 51          | Male   | Pneumonia, diabetes mellitus, AIDS^b | Endotracheal aspirate | Nodular infiltrates | Candida glabrata | Klebsiella pneumonia | Amphotericin B + Meropenem | Recovered |
| 3              | 53          | Female | Pneumonia, diabetes mellitus, systemic lupus erythematosus | Sputum |           | None | K. pneumonia | Meropenem | Recovered |
| 4              | 74          | Male   | Pneumonia, rheumatoid arthritis | Endotracheal aspirate | Nodular infiltrates | Candida albicans | K. pneumonia | Fluconazole + Meropenem | Recovered |
| 5              | 73          | Male   | Pneumonia, prostate cancer | Endotracheal aspirate | - | Candida krusei | Acinetobacter baumannii | Meropenem | Recovered |
| 6              | 49          | Male   | Pneumonia, rheumatoid arthritis | Endotracheal aspirate | - | None | K. pneumonia | Meropenem | Recovered |
| 7              | 79          | Male   | Pneumonia, diabetes mellitus | Endotracheal aspirate | - | C. albicans | A. baumannii | Fluconazole + Meropenem | Died |
| 8              | 67          | Male   | Pneumonia, multiple sclerosis | Endotracheal aspirate | Nodular infiltrates | C. krusei | None | Fluconazole + Meropenem | Recovered |
| 9              | 60          | Female | Pneumonia, diabetes mellitus, prostate cancer | Endotracheal aspirate | - | C. albicans | S. viridans | Fluconazole + Meropenem | Recovered |
| 10             | 74          | Male   | Pneumonia, diabetes mellitus, prostate cancer | Sputum | - | C. albicans | P. vulgaris | Fluconazole + Meropenem | Recovered |
| 11             | 66          | Female | Pneumonia, Lung cancer | Endotracheal aspirate | - | C. albicans | None | Fluconazole + Meropenem | Recovered |
| 12             | 57          | Female | Pneumonia, diabetes mellitus, breast cancer | Endotracheal aspirate | Nodular infiltrates | C. albicans | A. baumannii | Fluconazole + Meropenem | Died |
| 13             | 55          | Male   | Pneumonia, diabetes mellitus, rheumatoid arthritis | Sputum | - | Candida glabrata | Klebsiella oxytoca | Fluconazole + Meropenem | Recovered |
| 14             | 46          | Male   | Pneumonia, diabetes mellitus, prostate cancer | Endotracheal aspirate | Bilateral ground-glass opacities | C. albicans | A. baumannii | Fluconazole + Meropenem | Recovered |
| 15             | 60          | Male   | Pneumonia, diabetes mellitus, prostate cancer | Endotracheal aspirate | - | C. albicans | K. pneumonia | Fluconazole + Meropenem | Recovered |

^a AIDS, Acquired immunodeficiency syndrome.
^b CT scan, computerized tomography.