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Genetic diversity of the emerging human fungal pathogen *Pichia norvegensis*

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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

Objectives: *Pichia norvegensis* (= *Candida norvegensis*) is increasingly isolated from hospital settings, especially from immunocompromised patients. Understanding this 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 ($n = 236$; 90.8%) and environmental ($n = 24$; 9.2%) *P. norvegensis* isolates were subjected to Amplified Fragment Length Polymorphism (AFLP) fingerprinting and a novel six-loci microsatellite typing panel. Data were analyzed with BioNumerics and Structure. We applied a novel mating-type assay to determine the MAT α locus presence/absence.

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 (Simpson's D = 0.90), 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 distantly related. Structure analysis showed the presence of four genotypes, with signs of genetic admixture between geographic locations and environmental/clinical isolates. Nearly all isolates harbor the MAT α mating-type allele.

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

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First report of Mycetoma due to *Madurella fahalii* from India

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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

Objective: To describe the first case report of *Madurella fahalii* from India.

Methods: A 70-year-old non-diabetic man from Bhabrak Odisha who worked as a soldier in past came to us with a history of pus discharge containing black grains the size of mustard seeds from multiple sinuses in the posterior aspect of the thigh for last 5 years. He had been operated 2 years back for the same complaints, however, finding no relief he visited us. On examination, a depressed lesion measuring 10 × 10 cm, containing 6-8 sinuses which discharged pinkish yellow colored pus containing black grains measuring 0.5 × 1 cm was found on the posterior aspect of the thigh. The lesion was painless, doughy in consistency, and associated with enlarged and non-matted and non-tender inguinal lymph nodes measuring 2 × 2 cms. General physical examination and routine hematological laboratory examination revealed no abnormalities. Serological tests did not reveal the presence of HIV, Hepatitis B, or Hepatitis C infection. A clinical diagnosis of black grain mycetoma was made and the patient was sent to mycology laboratory for fungal culture. Black hard grains were found on KOH mount and culture on inhibitory mold agar grew a brown colony which diffused a brown pigment into the medium after 15 days of incubation. LPCB examination showed brown non-sporulating mold. DNA was extracted using phenol-chloroform isoamyl alcohol after grinding the mycelial mat in liquid nitrogen and subjected to PCR using ITS4 and ITS5 primers as described previously. The product was subjected to sanger sequencing and subjected to blast and it showed 99.45% similarity to *M. fahalii* (MF980633). 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 Mycetoma due to *M. fahalii* from India.

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Fungal isolates of the respiratory tract in symptomatic patients hospitalized in pulmonary units: A mycological and molecular epidemiology study

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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

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 Guilan 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 culture. Fungal identification was accomplished by internal transcribed spacer (ITS) and beta-tubulin sequencing. Also, in patients suspected of invasive pulmonary aspergillosis, BAL specimens were tested for galactomannan (GM) antigen.

Results: A total of 384 lung specimens (192 bronchoalveolar lavage (BAL) and 192 sputum samples) were obtained from symptomatic patients hospitalized in pulmonary units. Of these, 137 (35.67%) were positive in direct examination and culture. 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. Cough (94.16%), dyspnea (81.02%), purulent sputum (62.04%), and weight loss (56.2%) were the predominant symptoms and tuberculosis (24.81%), chemotherapy (21.89%), and diabetes mellitus (19.70%) were the predominant underlying conditions. Also, 5 cases of invasive pulmonary aspergillosis and 1 case of mucormycosis were diagnosed.

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

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Fungal and bacterial co-infections of the respiratory tract among patients with COVID-19 hospitalized in intensive care units

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Poster session 2, September 22, 2022, 12:30 PM - 1:30 PM

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/or fungal origins due to several risk factors. Consequently, the current study was conducted to evaluate the frequency, demographic characteristics, underlying conditions, and etiologic agents of fungal and bacterial co-infections of the respiratory tract among ICU patients with COVID-19 in Iran.

Materials and Methods: From May to October 2020, sputa and endotracheal 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 etiologic agents of bacterial co-infections were identified using the VITEK 2 identification method. For fungal identification, all samples were analyzed by direct microscopy using KOH 10% and culture. 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. Of these, in 15 cases (20.54%) fungal and/or bacterial co-infections were confirmed. Males were more infected (73.33%) and all of them were between 49 and 79 years. *Candida albicans* ($n = 8$, 61.53%) and Klebsiella pneumoniae ($n = 5$, 38.46%) were the most frequent etiologic agents related to fungal and bacterial co-infections, respectively. Pneumonia ($n = 15$, 100%) and diabetes mellitus ($n = 8$, 53.33%) were documented as the most prevalent underlying conditions. In the current study, 3 out of 15 patients (20%) died.

Conclusion: 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.