Identification of Cryptic species of Aspergillus using Beta-Tubulin gene

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Objectives: To identify cryptic species of the genus Aspergillus using Beta-Tubulin gene by sequence typing.

Methods: Aspergillus grown from various clinical samples (Ear swab, Bronchial wash, Endotracheal Aspiration, Paranasal sinuses, BALT. Systemic was subcultured on Sabouraud’s Dextrose Agar/Tween Agar.

Tissue mount slide culture was done to study the morphological features of the hyphae, size, shape, and arrangement of the conidia.

DNA Extraction: using phenol-chloroform method. DNA was extracted and purified directly from 4-day-old cultures and used as a template for polymerase chain reaction (PCR) amplification.

DNA Amplification: A fragment of the target gene were amplified using PCR.

The primer pairs used for Beta-Tubulin gene were Bt2a (Forward primer) and Bt2b (Reverse primer).

Beta-tubulin:

| Primer | Sequence |
|--------|----------|
| Bt2a F | GGTAAACAAATGCGTGCTGTTTC |
| Bt2b R | ACCCTCAGTGATGACCCCTGGC |

DNA Purification: The PCR product was purified with multi-screen filter plates.

Gel electrophoresis: The amplified DNA product was subjected to agarose gel electrophoresis and specific band formation was to be observed for the species of Aspergillus.

DNA sequencing: The purified product will be used as a template for sequencing. An automated sequencer 3730 sequencer will be used to obtain DNA sequences.

Phylogenetic Analysis: Parsimony analysis of individual and combined matrices will be conducted using PAUP version 4.0b10 software with searches at phylogeny by the maximum parsimony (MP) and Neighbor-joining methods.

The results will be represented using a dendrogram.

Results: A total of 30 Aspergillus isolates were collected and identified using Phenotypic methods.

The DNA extraction and PCR amplification were done and sent for Sequencing. The results of sequence typing are awaited.

Conclusion: Cryptic species are morphologically indistinguishable forms of Aspergillus and their identifications can be confined exclusively by using molecular techniques which have led to the description of previously unknown or rare species among different Aspergillus species complex. According to studies, the frequency of such species was found to be 10% in TRANSMART study 85 and 12% in HELPPOP study Brazil. Most of these species are less susceptible and certain species are multidrug-resistant (azole and amphotericin B) and the emergence of antimicrobial resistance among the Aspergillus species forms the importance in identifying Cryptic species.

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Identification of Cryptic species of Aspergillus using Beta-Tubulin gene in a tertiary care center in South India

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Defung: direct mycological examination of microscopic fungal images

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Objectives: To classify three fungal types using two different deep learning approaches and three different convolutional neural network models, VGG16, Inception V3, and ResNet50.

Method: A mycological laboratory in Colombia donated the images used for the development of this research work. They were manually labeled into five classes and curated with subject matter expert assistance. The images were later cropped and modified with automated scaling routines to produce the final dataset.

Results: We present experimental results classifying five types of fungi using different deep learning approaches and three different convolutional neural network models, VGG16, Inception V3, and ResNet50. The first approach benchmarked the classification performance for the models trained from scratch, while the second approach benchmarked the classification performance using pre-trained models based on the ImageNet dataset. Using a fold cross-validation testing on the 5-class dataset, the best performing model trained from scratch was Inception V3, reporting 73.5% accuracy. Likewise, the best performing model using transfer learning was VGG16, with 85.04% accuracy.

Conclusion: The statistics provided by the two approaches serve as initial benchmarks to encourage further research work to improve classification performances. Furthermore, the dataset built is published on Kaggle and GitHub to encourage future research.

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Defung: direct mycological examination of microscopic fungal images
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Clinical correlation of beta galactomannan with culture and patient outcome in a tertiary care center in south India

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Background: Invasive aspergillosis has increased in the last decade. They mainly occur in neutropenic patients (following antinecancer treatment) and in patients treated with immunosuppressants and corticosteroids. The galactomannan antigen in serum appears to be a serological method able to aid in the diagnosis of invasive aspergillosis also the antigen detection in bronchoalveolar lavage has proven to be advantageous for the diagnosis of invasive aspergillosis.

Objectives:
- To perform galactomannan test on BAL and serum samples.
- To correlate the galactomannan results with culture.
- To correlate the galactomannan positive patients with the clinical outcome.

Methods: A total of 175 samples were collected from patients suspected to have fungal infections from the period of January 2018 to March 2022 from a tertiary care center in South India. The galactomannan assay and culture were done for these samples. Data of age, sex, gender, diagnosis, underlying conditions, antifungal treatment, and outcomes were collected.

Results: Out of 175 samples collected from patients suspected with Aspergillus, 120 were males and 55 were females (7 were repeat samples).

The major underlying conditions were diabetes and hypertension, coronary artery disease (CAD), and Hematopoetic malignancy such as B Cell lymphoma, ALL, and AML (Fig. 1).

- The samples were for galactomannan assay were serum (107 samples), Bronchial wash (74 samples), BAL (4 samples), and tracheal aspirates (3 samples). The test was performed and cultured (Table 1). The cutoff point for BAL/sera was taken as an index > 0.50
- A total of 90 patients were galactomannan positive. In which, 15 patients were only KOH positive and 6 were KOH and culture positive.
- In all, 6 cultures grew Aspergillus sp (1), Aspergillus flavus (2), Aspergillus fumigatus (1), Aspergillus fumigatus (2).
- A total of 56 patients out of 90 with galactomannan positive were treated with antifungals like liposomal amphotericin B, voriconazole, amphoterin, and fluconazole.
- In all, 30 patients were discharged, 10 were discharged at request, 66 left against medical advice, 18 patients succumbed to infection, and 12 were outpatients lost to follow-up.

Conclusion: Galactomannan alone cannot be taken as a diagnostic marker. Clinical correlation, radiologic findings, as well as underlying risk factors play a major role for decision on the initiation of empiric treatment.