Section: Microbiology

Research

A STUDY ON CYSTIC FIBROSIS AND ASPERGILLUS SPECIES DONE IN A MEDICAL COLLEGE OF BIHAR

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Abstract

Background: Cystic fibrosis (CF), a life-limiting, hereditary disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encodes a chloride-conducting transmembrane channel regulating anion transport and mucociliary clearance in the airways. The aim of the study was to characterize the diversity of colonizing fungi and to determine the frequency of azole resistance in A. fumigatus. **Materials and Methods:** An observational study was carried out over duration of One year, between February 2022 to January 2023, by the Department of Microbiology, Darbhanga Medical College, Darbhanga, Bihar. During the course of the study, a total of 50 respiratory samples were collected from confirmed cases of CF during their routine visits, clinical deterioration and hospitalization. Permission was obtained from the Institutional Ethics Committee. Data were anonym zed prior to analysis. Statistical analysis was done using Microsoft excel. Samples were inoculated onto Sabouraud dextrose agar (SDA, Oxoid, and Basingstoke, UK) and Medium B+ agar plates. All inoculated plates were incubated aerobically at 30 °C for three weeks and checked the first week daily and the second and third week twice weekly for growth. **Result:** Around 200 suspicious samples were obtained, out of which, 50 (25%) samples yielded one or more fungi. A. fumigatus was most prevalent fungus. Other isolated fungi were Penicillium species, non-fumigatus Aspergillus species and Scedosporium species. A. flavus and A. terreus were the most frequent Aspergillus species besides A. fumigates, Penicillium spinulosum was the most cultured Penicillium species. **Conclusion:** To conclude, we found a vast diversity of fungal species in CF airways. A. fumigatus was the most prevalent fungus, followed by Penicillium species and Scedosporium species.

INTRODUCTION

Cystic fibrosis (CF), a life-limiting, hereditary disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encodes a chloride-conducting transmembrane channel regulating anion transport and mucociliary clearance in the airways.[1] Dysfunctional CFTR leads to mucus retention, resulting in chronic infection and local inflammation of the airways. In the course of time, this causes progressive, irreversible damage to the lungs which ultimately leads to death of the patient.[1] The progressive lung damage is accompanied by a shift of causal pathogens of infection. At younger age destructive infections are primarily caused by bacteria, in particular Staphylococcus aureus and Haemophilus influenzae.[1] As the disease progresses, bronchiectasis develops and CF patients become more susceptible to a range of Gram-negative bacteria including Pseudomonas aeruginosa.[1] As a result of improved health-care systems and a consequent increase in life expectancy, this paradoxically results in a larger number of patients with advanced disease.[2] This alteration of the patient population offers new challenges and clinical dilemmas, among them the increasing proportion of patients with growth of filamentous fungi in their respiratory samples.[3] At present there is no clear understanding of the clinical significance of fungal colonization and whether treatment should be advised,[4] but a well-balanced decision is crucial as unnecessary treatment can be harmful for the patient.

Several epidemiological studies have been performed, though most were limited by small sample sizes and/or heterogeneous culture protocols.[5-8] These studies all point towards

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Aspergillus fumigatus as the most prevalent mold in the CF patient population and additionally show a considerable diversity of other fungi. However, analyzing the full extent of diversity is challenging, often due to the limitations of (phenotypic) identification methods. A. fumigatus is a medically important fungus recognized to cause a range of diseases in humans,[9,10] including multiple cases of significant infections in CF.[11,12] Azoles are the first-choice drug for treating Aspergillus disease.[13] However, therapy is increasingly compromised due to rising rates of azole resistance.[14-16] The aim of the study was to characterize the diversity of colonizing fungi and to determine the frequency of azole resistance in A. fumigatus.

MATERIALS AND METHODS

An observational study was carried out over duration of One year, between February 2022 to January 2023, by the Department of Microbiology, Darbhanga Medical College, Darbhanga, and Bihar. During the course of the study, a total of 50 respiratory samples were collected from confirmed cases of CF during their routine visits, clinical deterioration and hospitalization. Permission was obtained from the Institutional Ethics Committee. The diagnosis of CF disease was based on typical clinical characteristics alongside a positive sweat test (chloride >60 mmol/l) and/or the presence of two known pathogenic CFTR mutations. Data were anonym zed prior to analysis. Statistical analysis was done using Microsoft excel. Samples were inoculated onto Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK) and Medium B+ agar plates.[17] Medium B+ consists of glucose (16.7 g/l), yeast extract (30 g/l), peptone (6.8 g/l) and agar (20 g/l) and is supplemented with ceftazidime (32 mg/l), chloramphenicol (50 mg/l), colistin (24 mg/l) and cotrimoxazole (128 mg/l) to suppress the growth of bacterial co-habitants in CF sputa, most notably the Gram-negative organisms. All inoculated plates were incubated aerobically at 30 °C for three weeks and checked the first week daily and the second and third week twice weekly for growth. Fungal spores and/or hyphae were harvested and suspended into 400 µl Bacterial Lysis Buffer that was present in Green Beads Tubes, processed in a MagNA Lyzer. They were centrifuged for 2 min at 13.362 g, and heat-inactivated at 100 °C for 15 min. After cooling down to room temperature a 200 µl suspension was used for genomic DNA extraction by using the MagNA Pure 96 automatic platform. All identifications were performed using Amplified Fragment Length Polymorphism (AFLP) fingerprinting according to previously described methods.[18-20] Azole resistance in Aspergillus fumigatus A. fumigatus was screened for azole resistance using a mixed format real time PCR assay.[21]

RESULTS

A total of 50 samples that yielded single or multiple fungi strains evaluated during the course of the study were included in the study. Majority of the samples were obtained from the adult male patients. Around 200 suspicious samples were obtained, out of which, 50 (25%) samples yielded one or more fungi. A. fumigatus was most prevalent fungus. Other isolated fungi were Penicillium species, non-fumigatus Aspergillus species and Scedosporium species. A. flavus and A. terreus were the most frequent Aspergillus species besides A. fumigatus. Penicillium spinulosum was the most cultured Penicillium species. Out of 50 positive samples, 7 (14%) showed a mixed fungal population. The mixed population consisted of 2 (n = 4), 3 (n = 1), 4 (n = 1) or 5 (n = 1) different fungal species. Cases with Scedosporium species revealed that all the cultures containing a Scedosporium species also contained another fungal species, majority being A.fumigatus. In total 50 patients we had 63 isolates. Overall, 11 azole-resistant isolates were found in 7 patients.

Table 1: Prevalence of filamentous fungi cultured in respiratory samples

Isolates	Number
fumigatus	28 (44.5%)
Penicillium species	15 (23.8%)
Non-fumigatus aspergillus species	12 (19.1%)
Scedosporium species	8 (12.7%)

DISCUSSION

In this study we demonstrated a vast diversity of fungal species in the respiratory samples of CF patients. Aspergillus species, in particular A. fumigatus, showed the highest prevalence, followed by Penicillium species and Scedosporium species. Azole resistance was observed in 17.5% of culture positive patients. An important question is whether all cultured fungi represent colonization of the respiratory tract. Inhaled fungal spores can lead, when secreted in mucus, to positive cultures in the laboratory without actual (sustained) hyphal growth in the airway. It is quite possible that the observed fungal diversity in the respiratory samples is, at least in some instances, a representation of fungal spores in ambient air that are trapped in mucus of CF patients. Recently Schwarz et al. reviewed fungal epidemiology of CF in multiple European countries,[22] and they observed considerable geographical differences and stressed the need for locally conducted studies. Penicillium is the second

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most cultured fungal genus. The prevalence of individual species however is low, due to the heterogeneousness of this group. The general opinion is that Penicillium species are not associated with clinical deterioration and arise from environmental sources. This view is strengthened by ours and others,[5] observation that each Penicillium species is hardly found more than once in a patient. In contrast, there are reports of Penicillium species causing post-transplant,[23] and other invasive infections,[24] in addition to allergic bronchopulmonary mycosis.[25] We found a mean Scedosporium species prevalence of 12.5% in the study period making it the third most prevalent fungus. However, the prevalence of Scedosporium species and other slow growing fungi might have been even higher if a fungal selective medium like Scesel+ medium had been used. This medium developed in 2007,[26] inhibits fast growing fungi like Aspergillus species and Penicillium species and has shown to increase the yield of Scedosporium species in clinical specimens.[27,28] Simultaneous growth of Scedosporium species and Aspergillus species is possible. Nevertheless, the lack of a selective culture medium for Scedosporium species in our culture protocol might have lead to underreporting of this fungus. It has been suggested that Scedosporium species are more virulent than Aspergillus species in CF, as Aspergillus species are detected more often in respiratory samples but comparatively cause less infections.[11] Our study confirms and extents a recent study from Germany which reported an azole resistance prevalence of 5.3%.[16] Overall 11 azole-resistant isolates were recovered. The mean resistance of 17.4%, is higher than the majority of resistance rates reported in Europe (range 0%–8%),[15] but comparable to the national resistance rate of all clinical A. fumigatus isolates in 2013 and 2014.[15,29] The present study strengthens the general recommendation to routinely perform antifungal susceptibility testing in all (CF) patients requiring antifungal treatment.

CONCLUSION

To conclude, we found a vast diversity of fungal species in CF airways. A. fumigatus was the most prevalent fungus, followed by Penicillium species and Scedosporium species. We observed a mean azole resistance prevalence of 17.4%.

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