Original Article

Clinical Spectrum Of Pulmonary Aspergillosis

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Abstract

Background: Invasive fungal infections are not only responsible for the increased morbidity and mortality in human population but are also a major concern throughout the world because of the increased antifungal resistance.

Objective: The study was conducted to find out the prevalence of Aspergillus species endemic in our area along with the co-morbidities in suspected cases of Pulmonary Aspergillosis.

Materials and methods: In this cross-sectional study, 73 cases of Pulmonary Aspergillosis were included. Aspergillus species were isolated from the pulmonary specimens of those patients who were visiting the Out Patient Department (OPD) or were admitted to Lahore General Hospital, Lahore.

Results: In this study, the most prevalent specie of Aspergillus to be isolated from the pulmonary specimens was found to be Aspergillus flavus 39 (53.4%) followed by Aspergillus niger 21 (28.8%), Aspergillus fumigatus 12 (16.4%) and Aspergillus terreus 1 (1.4%). Co-morbidities of the patients were greatest with Asthma 29 (40%), followed by Tuberculosis 22 (30.1%) and Chronic Obstructive Pulmonary Disease (COPD) 22 (30.1%) .

Conclusion: The data of the present study can serve as a basis for future surveillance of clinically significant and prevalent Aspergillus species. Correlation with co-morbidities could play a valuable role in better understanding of the disease pathophysiology.

Introduction

Fungal infections in humans can be classified as superficial and invasive. Superficial fungal infections (SFI) typically affect the outer layers of the skin, hair, and nails. These are caused by dermatophytes, yeast and non-dermatophyte filamentous fungi (Gupta et al., 2021, Khodadadi et al., 2021). Invasive fungal infections (IFIs) have a lower incidence than invasive bacterial infections but fungal infections that invade, are of greater concern because the mortality rate usually exceeds 50%, approximately killing 1.5 million people annually (Williams et al., 2020). There are many different types of fungi that can cause invasive fungal infections, including Aspergillus, Candida, Cryptococcus, and Mucorales. These fungi attribute to almost 90% of the fungal infections. These can affect many different organs and systems in the body, including the lungs, brain, liver, and kidneys (Leroy-Freschini et al., 2018). The filamentous fungi consist about 250 species and Aspergillus is the largest genera which cause human diseases. Globally, the most common cause of invasive aspergillosis is Aspergillus fumigatus and it has been studied and reviewed widely. Infection due to A. flavus is more common in Africa, Asia, and the Middle

East because it has the ability to survive in dry climatic and hot conditions as compared to other Aspergillus species (Friedman and Schwartz, 2019). Pulmonary Aspergillosis is a type of invasive fungal infection that affects the lungs (Kousha et al., 2011). There are several types of pulmonary aspergillosis, including allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), and invasive pulmonary aspergillosis (IPA) (Tillie-Leblond and Tonnel, 2005). Pulmonary aspergillosis (PA) is caused by A. fumigatus, A. niger, A. flavus and A. terreus. A. flavus is mainly reported to cause invasive forms of aspergillosis (Zanganeh et al., 2018). Chronic pulmonary aspergillosis (CPA) is a type of pulmonary aspergillosis caused by infection with Aspergillus spp., most commonly Aspergillus fumigatus complex. Unlike invasive pulmonary aspergillosis (IPA), which is typically an acute and rapidly progressive infection that affects immunocompromised patients, CPA is a chronic and slowly progressive infection that can occur in people with relatively minor immunosuppression and pre-existing co-morbidities (Kosmidis and Denning, 2015). Chronic Pulmonary Aspergillosis(CPA) has an effect on individuals with previous mycobacterial lung disease or chronic structural lung disease. Aspergillus bronchitis has an effect on individuals with bronchial lung disease such as bronchiectasis (Hosseini-Moghaddam et al., 2020). Multiple co-morbidities can exist along with CPA like Tuberculosis TB, Diabetes, Asthma and any malignancy but the most common is TB worldwide (Hayes and Novak-Frazer, 2016). In nations with a high prevalence of tuberculosis (TB), the rate of chronic pulmonary aspergillosis (CPA) is growing at an exceptionally alarming rate. In spite of the fact that Pakistan is thought to have a high pulmonary aspergillosis burden, there is lack of actual data. A recent CPA burden assessment from Pakistan indicated a high frequency of 39 cases for every 0.1 million people in the country (Iqbal et al., 2020). This study has focused on overall prevalence of Aspergillus species endemic in our area in suspected cases of pulmonary aspergillosis and the co-morbidities in patients along with pulmonary aspergillosis.

Methodology

This cross-sectional study was conducted at Microbiology department of Lahore General Hospital between the time period of January, 2022 to October, 2022. The study protocol was approved by the Advanced Studies and Research Board of University of Health Sciences, Lahore and Ethical Committee of Postgraduate Medical Institute, Lahore.

Sampling techniques

Convenient sampling technique was used. Clinical specimens of patients admitted to /visiting in Out Patient Department (OPD) in Lahore General Hospital, Lahore yielding Aspergillus species from pulmonary samples including bronchoalveolar lavage fluid, sputum and tracheal aspirates. Duplicate samples were excluded from study.

Sample collection procedure

Samples were collected under supervision of a trained doctor following aseptic conditions and sent to the laboratory within two hours. Each sample was processed according to predefined Standard Operating Procedures in the Microbiology laboratory of Pathology Department, Lahore General Hospital. Following protocols were followed

Sputum; Sterile containers were used. Patients were asked to expectorate sputum by deep cough. Patient information was labelled on the container.

Tracheal aspirate; Tracheal aspirates were collected through a catheter from an endotracheal tube or from tracheotomised patients, directly by tracheal suction in sterile containers. Patient information was labelled on the container.

Bronchoalveolar lavage BAL; BAL fluid samples were collected from patients undergoing bronchoscopy procedure and collected in sterile and labelled containers.

Sample processing

All respiratory samples were mixed with sputasol reagent which liquefies the mucus elements. Samples were then centrifuged at 1200 RCF for ten minutes. Direct examination was done to observe fungal hyphae by making (Potassium hydroxide) KOH preparation.

10 % KOH preparation

10% KOH has the ability to emulsify the non-fungal elements like cellular material and it was used to purify the fungal elements. After a minute, 1-2 drops of Lactophenol cotton blue (LPCB) stain were added for clear demonstration of fungal elements. The slide was then observed under 40X lens.

Culture Inoculation

Sabouraud's Dextrose Agar was used for culture inoculation. Sterilized loop was used for inoculation. Already prepared sediment after sputasol treatment was used for inoculation. The culture plates were aerobically incubated at 35 °C for a total of ten days. The plates were observed daily for any fungal growth. Plates showing no fungal growth after an incubation of ten days were discarded (Waqas et al., 2021).

Identification techniques

Macroscopic Characteristics:

Growth and morphology (Walsh et al., 2018)

Rate of growth was observed as: Rapid (mature within 3 to 4 days), Moderate (mature within 5 to 10 days) or Slow (mature in more than 10 days)

Pigmentation on the surface and on reverse was observed, such as Aspergillus Flavus (Front: yellow to green; Reverse: golden to red brown), Aspergillus Fumigatus (Front: Dark green to grey; Reverse: White to tan), Aspergillus Niger (Front: Black; Reverse: White to yellow), Aspergillus Terreus (Front: Cinnamon Brown; Reverse: Yellow to brown)

Surface texture of colonies grown on agar plates was note as: glabrous, waxy, powder, velvety, granular or fluffy.

CELLOPHANE TAPE PREPARATION (Koneman et al., 2006)
LactophenolCotton Blue Staining:

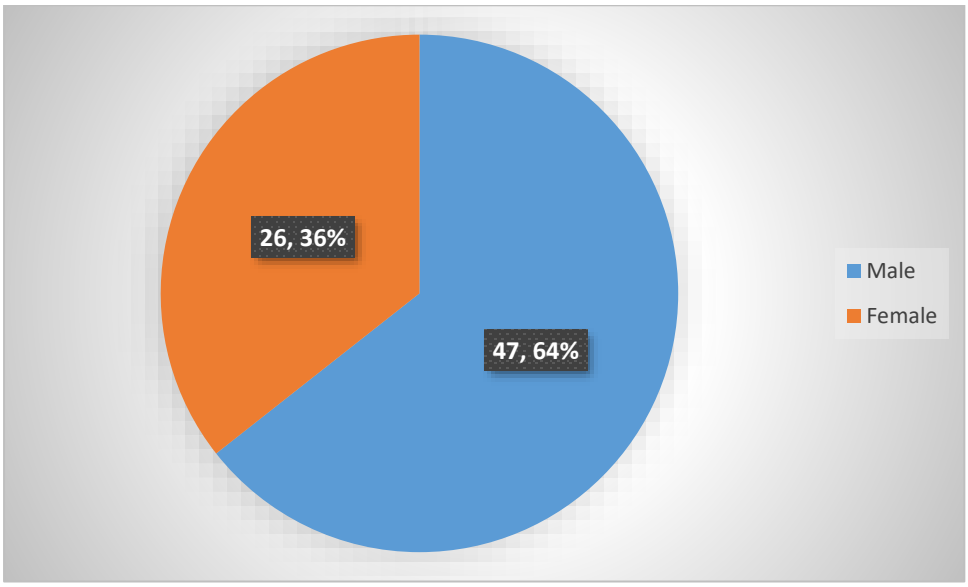
Tape preparations were made from the culture plates after growth of the fungus to identify the Aspergillus upto species level. Preparations were observed microscopically first under lower power 10X objective and then under high power 40X. The fungal identification was done upto species level.

Data collection and analysis

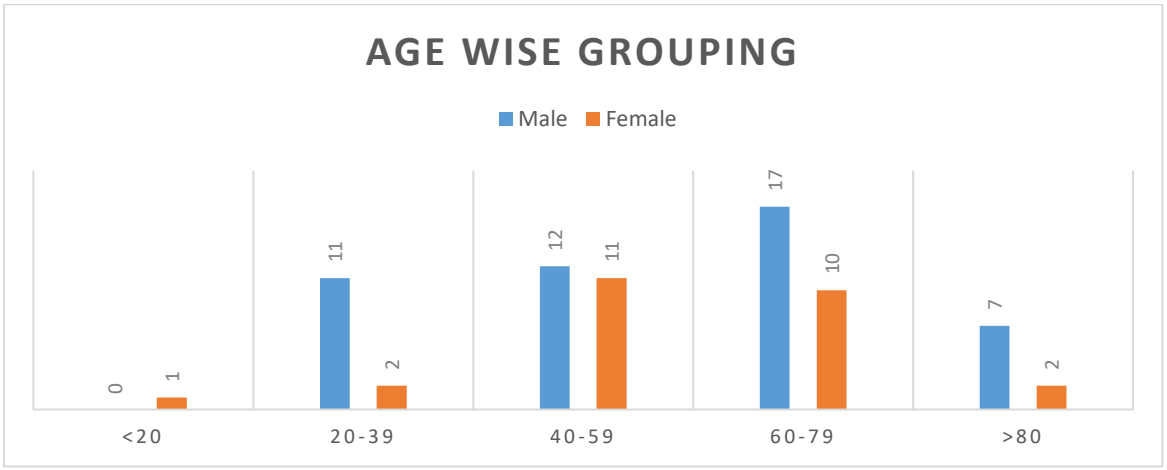
The patient's required details were collected via profoma. The parameters included in the proforma were the type of specimen, date of sample collection, patient's name, patient's age and gender, medical record number (MR number), presenting complaints of the patient, previous medical history and the provisional diagnosis. The data was analyzed statistically using SPSS 25.0. The descriptive statistics including mean and standard deviation were calculated for numeric data. Frequency percentage was used for Aspergillus species and their antifungal susceptibility patterns.

Results

The present study was conducted on 73 clinical isolates of Aspergillus species. The Aspergillus species in the study were isolated from the pulmonary specimens of patients visiting the Outpatient Department or admitted to Lahore General Hospital, Lahore. Out of the total 73 Aspergillus isolates yielding positive specimens, 47 (64.4%) patients were males and 26 (35.6%) were females as shown in figure 1.



The patients included in the study were divided into five categories according to age groups. The mean age of the patients was 56.4±17.1, ranging from 18 to 90 years. Only one patient was below 20 years of age. The maximum number of patients were from the age group 60-79 years (N=27) shown in figure 2



Direct examination of the specimens was done by making 10% KOH wet mount preparations. On KOH preparation, septate hyphae were seen in 70 (95.9%) of the total samples and no hyphae were observed in 3 (4.1%) samples. Shown in table 1

KOH preparation	**Frequency**	**Percentage**
	70	95.9

Fungal Hyphae seen		
No Fungal Hyphae seen	3	4.1

Growth and colony morphology

Colonies were confirmed under microscope by making Lactophenol cotton blue tape preparation of growth isolated in positive cultures. On the basis of color, appearance and growth rate following isolates were obtained.

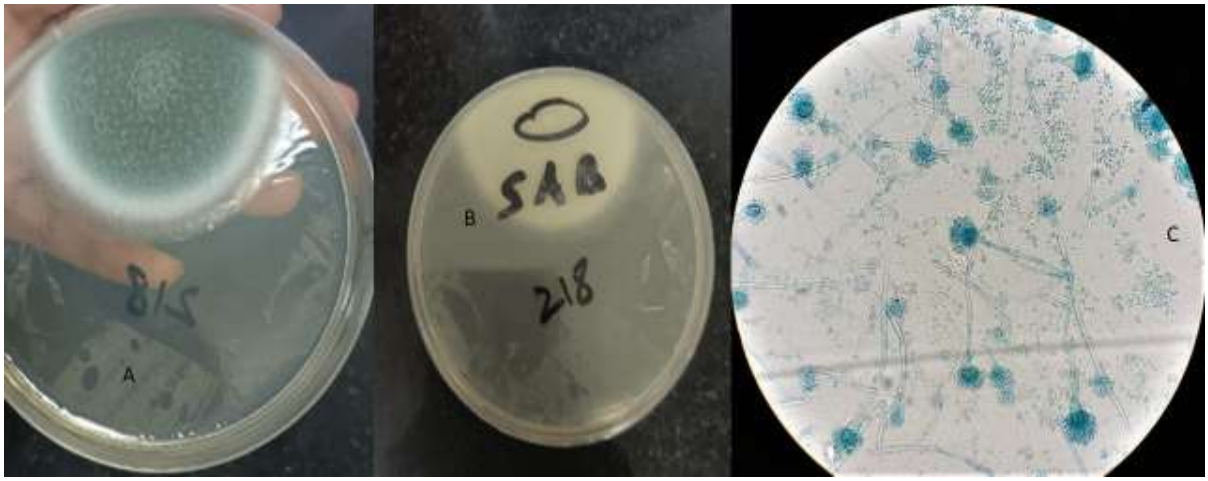


Figure 3 Aspergillus fumigatus; A front texture of colony, B back texture of colony, C Lactophenol blue staining results.

Aspergillus fumigatus appeared to be velvety or powdery; at first white, then turned dark greenish to gray on culture plate. Reverse was white to tan. Microscopically, conidiophores expanded into a large dome shaped vesicles that had bottle shaped phialides usually on upper two-thirds of the surface.

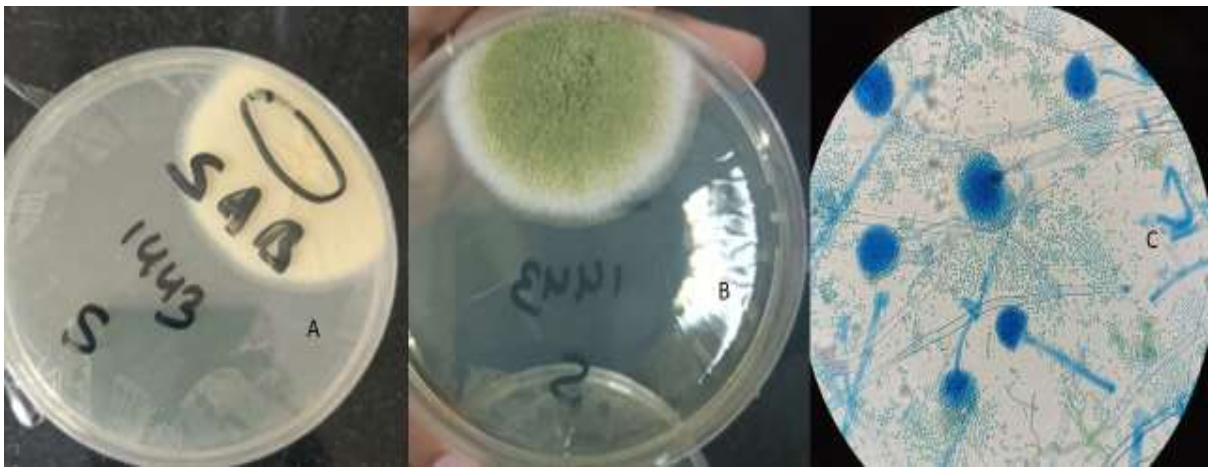


Figure 4 Aspergillus flavus; A back texture of colony, B front texture of colony, C Lactophenol blue results.

Aspergillus flavus appeared to be velvety; yellow to green or brown. Reverse was goldish to red-brown. Microscopically, vesicles were globose and phialides which covered entire vesicle pointing out in all directions.

Aspergillus niger on culture plate had a deep cottony appearance which was white to yellow at first and then turned black. Reverse was white to yellow. Microscopically it was biseriate with smaller phialides that covered the entire vesicle.

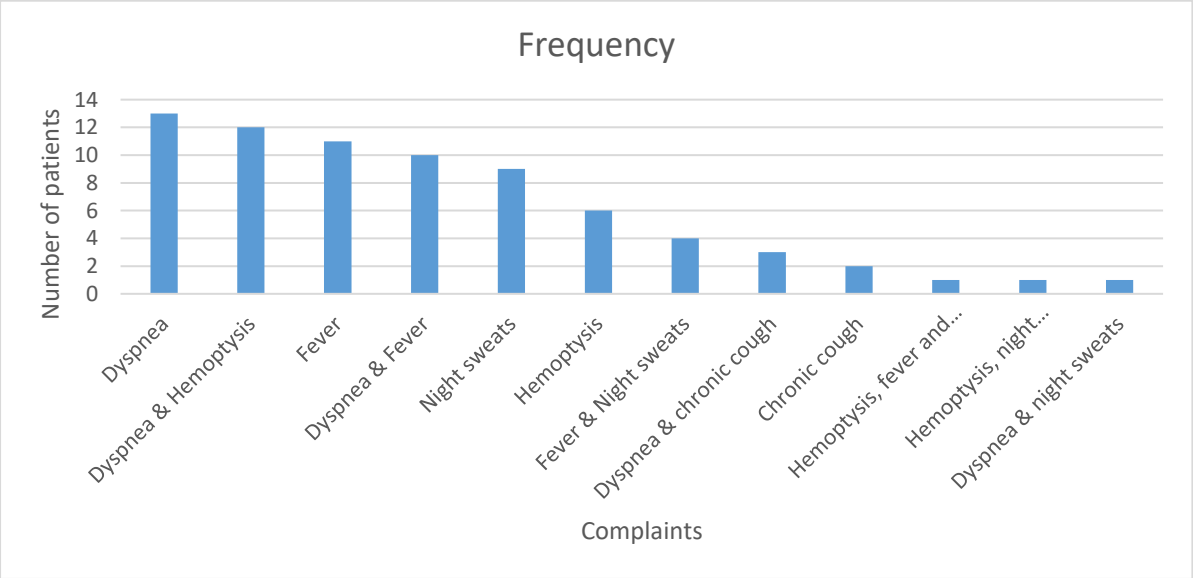
Aspergillus terreus on culture plate had a velvety; cinnamon-brown colour appearance. Reverse was yellow to brown. Microscopically, vesicles were hemispherical and phialides covered the entire surface.
Number of isolates obtained were as Aspergillus flavus 39 (53.4%), Aspergillus fumigatus 12 (16.4%), Aspergillus niger 21 (28.8%) and only one (1.1%) Aspergillus terreus.

Co-morbities status

The patients had different presenting complaints and previous medical histories. COPD was found to be the most common comorbid condition in the patients included in this study (Table 3). Patients presented with an array of presenting complaints. Most commonly, the patients presented with dyspnea only followed by dyspnea along with hemoptysis (Table 2)

Comorbid	**Frequency**	**Percentage**
TB	22	30.1
Asthma	22	30.1
COPD	29	40%
Total	73	100.0

All patients visited the clinicians with different complaints (Figure 5)



Discussion

Present study has focused on prevalence of Aspergillus species and co-morbidities in patients suspected to have pulmonary aspergillosis. In this study, the sample size used was 73 and Aspergillus species were isolated from the pulmonary specimens of patients visiting the OPD or admitted in Lahore General Hospital. The demographic details of the patients were noted. These included gender, age and Medical Record number of the patient. The type of clinical specimen, complaints with which the patient presented to the doctor and significant past clinical history of the patient was also noted. In another study conducted by Waqas Akram on Aspergillosis in 2021, same characteristics were taken into consideration (Waqas et al., 2021). In a similar way, (Machado et al., 2021) studied about aspergillosis but in contrast to the present study, all patients included in this study were COVID-19 positive. In the present study, the specimens that were included to isolate the Aspergillus species were sputum, tracheal aspirate and bronchoalveolar lavage. Out of the three specimens, sputum was the most commonly encountered specimen in this study. Frederick Lamoth in 2020 also performed a study to isolate the Aspergillus species from pulmonary specimens of the patients. However, the patients included in his study were those who were suffering from COVID (Lamoth et al., 2020).

Distribution of Aspergillus species isolates were more among males 47(64.4%) than females 26(35.6%). Thomas Maitre in 2021 in France to determine the prevalence of pulmonary aspergillosis showed the similar patteren of prevalence among males which described the predominance of male patients (63.5%) as compared to the female patients (37%). Another meta-analysis performed by Shi et al (2022) including fourteen studies also showed that pulmonary aspergillosis was more common among males than females (Shi et al., 2022).

On the basis of age, five groups were made to understand the distribution of aspergillosis age wise and the data present the fact that most patients infected with Aspergillus belong to the older age group. Only fourteen patients were below the age 40. A study conducted in Iraq on pulmonary aspergillosis also showed that pulmonary aspergillosis was more prevalent among people who are above 60 years of age (Jasim and Klaaf, 2021). Another multinational observational study by (Prattes et al., 2022) on pulmonary aspergillosis comprised of data from twenty centres belonging to nine countries which had concluded that pulmonary aspergillosis was significantly more common in the elderly age group. Multiple reasons could be there to explain the high rate of infection in old age incuding poor metabolism, low immunity, poor hygiene and lack of appetite (Hof, 2010).

Hyphae were observed in 95% (n=70) of the total specimens under direct examination with KOH wet mount preparation. No hyphae could be seen in the rest of the specimens. Manikandan et al in India (2019) studied fungal keratitis. According to the results of this study as well, KOH wet mount was found to be 96% sensitive in cases of fungal pathogens (Manikandan et al., 2019). Malik (2018) studied the comparison of different staining techniques and culture media. According to this study, the sensitivity of KOH wet mount preparation was found to be 76% for diagnosing fungal pathogens (Malik et al., 2018). Venugopal et al in Riyadh, Saudia Arabia (2020) conducted a study to evaluate candidiasis. According to this study, 26 isolates were positive for direct microscopy using 10% KOH out of the total 37 isolates which were culture positive showing 70% sensitivity (Venugopal et al., 2021). We concluded from the results of the present study that diagnosis should not only include clinical assessment and direct microscopy but culture as well. Co-relating the results of direct microscopy with the culture results helped in the identification of the Aspergillus species for the correct diagnosis

In this study, the most prevalent Aspergillus specie to be isolated from the pulmonary specimens was Aspergillus flavus followed by Aspergillus niger. Aspergillus fumigatus was found to be the third most common and Aspergillus terreus was found to be the least common among the four species. A similar retrospective review study was conducted by Nousheen Iqbal at Aga Khan University Hospital, Karachi, Pakistan from January 2012 to December 2017. This was also conducted on pulmonary specimens (Iqbal et al., 2020). Aspergillus flavus was noted to be the most common specie isolated according to this study.

A study was conducted at the Agha Khan Hospital, Karachi, Pakistan in 2022 by Nosheen Nasir who studied the association of COVID and CAPA. Aspergillus flavus was found to be the most prevalent specie according to this study as well, followed by Aspergillus niger. Another study was conducted in Gulab Devi Chest Hospital, Lahore, Pakistan by Waqas Akram in the year 2021 (Waqas et al., 2021). In contrast to the present study, the most prevalent specie to be noted in this study was Aspergillus fumigatus. The reason for this predominance could possibly be due to the ability of A.flavus to be able to survive in hot and dry climatic conditions in comparison to other Aspergillus species (Rudramurthy et al., 2019).

Conclusion: The data of the present study can serve as a basis for future surveillance of clinically significant and prevalent Aspergillus species. Correlation with co-morbidities could play a valuable role in better understanding of the disease pathophysiology.

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