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Original Research Article

Isolation of *Aspergillus* Species from Various Clinical Samples in a Tertiary Care Hospital, Ahmedabad

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Abstract

Background: In recent years, fungi have been emerging as a common infection in hospitalized patients of tertiary care centres. Now adays it has become important to determine the prevalence of different *Aspergillus* species due to their ability to cause wide spectrum of also in the view of difference in antifungal susceptibility noted in some species.

Objective: To isolate different species of *Aspergillus* in various clinical samples received in mycology laboratory at our institute.

Methods: The retrospective study was conducted in the department of Microbiology for the period of three years (Jan 2019 –Dec 2021) . The samples were collected under aseptic precautions from the patients presenting clinically with suspected fungal infections and analysed by direct microscopy (KOH and Gram stain).Fungal culture was being done on SDA (Sabouraud dextrose agar) and the growth was identified by colony morphology, gram staining, Lectophenol cotton blue preparation and slide culture as per recommended procedures.

Results: Out of a total of 950 samples processed over a period of 3 years in our laboratory, 86 samples were culture positive for *Aspergillus* species. The highest number of isolates belongs to *A.fumigatus* (37.21%) which were from respiratory samples like sputum, ET secretions and BAL followed by *A. flavus*(23.25%). *A. clavatus* accounted for the least number of isolates (2.33%). Maximum numbers of isolates were from males(66.9%).

Conclusion: Local aetiology should be determined to know the common isolated fungal species in the given geographical area and early diagnosis of fungal infections using conventional and rapid tests(KOH) should be carried out for the prompt management of the cases by selecting empirical antifungal therapy and formulating prophylactic and pre- emptive stratergies.

Keywords: *Aspergillus* , clinical samples, fungal culture

Introduction

One of the oldest named genera of fungi, *Aspergillus* received its name from Micheli in 1729. In viewing the microscopic spore bearing structure, Micheli was reminded of advice used by the roman Catholic clergy to sprinkle holy water during the part of the liturgy called the asperges and the brush used for sprinkling is an aspergilla(aspergillum)

Aspergillus species are saprophytic thermotolerant fungi that are ubiquitous in

the air and environment. [1] Out of 185 species of genus *Aspergillus* about 20 can cause human infections. Though humans inhale *Aspergillus* spores at a rate of hundreds per day, they rarely experience complications.[2] However under special circumstances, *Aspergillus* species can produce a spectrum of diseases, including allergic bronchopulmonary aspergillosis, aspergilloma, chronic necrotisisng aspergillosis and life threatening invasive aspergillosis [3,4]. In addition,endocarditis, paranasal sinus granuloma, keratitis, otomycosis and onychomycosis caused by *Aspergillus* species have also been described in the literature [1,3].

The most common ones causing invasive disease include *A. fumigatus, A. flavus, A. niger, A. terrus* and *A. nidulans* [1]. *A. fumigatus* accounts for ~90% of cases of invasive aspergillosis though an increasing number of cases of aspergillosis due to species other than *A. fumigatus* is now being reported.

The study was undertaken to determine isolation of *Aspergillus* species from various clinical samples.

Material and Methods

The retrospective study was conducted in the Department of Microbiology in a tertiary care hospital over a period of 3 - years (Jan 2019 to Dec 2021).The specimens were received from inpatients and outpatient department of the hospital. The samples were collected from the patients with clinically suspected fungal infections .The various specimens received were sputum, urine, pus, BAL, ET secretions, nail clippings, gastric aspirates.

The specimens were collected using aseptic precautions and were subjected to microbiological examinations as follows.

Sample processing and identification of isolates was done according to standard mycological techniques.

Identification of *Aspergillus* species

Potassium hydroxide (KOH) wet mount

1. A drop of 10% KOH was taken on a clean microscopic slide with the help of sterile dropper.
2. The specimen was emulsified with the drop of KOH and covered with a cover slip.
3. The slide was examined under low (10X) and high power (40X) magnification after 10-15minutes for demonstration of fungal elements.

Culture on sabouraud's dextrose agar medium

The growth of fungal hyphae was taken with the help of L-shaped wire and inoculated on slant of the Sabouraud's dextrose agar containing chloramphenicol and kept in biological oxygen demand (BOD) incubator for 2 weeks at 25 ± 5°C and observed daily for growth. The details of growth characters are recorded.

LPCB mount was made for the identification of species.

Slide culture

1. From the Petri dish containing Sabouraud dextrose agar, a small block of agar for each slide culture is cut out.
2. With the flat side of a sterile L-shaped wire, or with a spatula, an agar block is placed in the centre of the slide culture set up.
3. With a probe, 3-4 fragments of the mould to be cultured are inoculated around the periphery of the agar block
4. With the forceps, place the coverslip is placed on the agar block the tips of which have been flamed.
5. Thoroughly moisten, but not to excess, the filter paper with sterile distilled water was placed in the petri dish and the slide culture was incubated at a room temperature.

6. When growth appears beneath the coverslip, a drop of LPCB was placed on the slide and the coverslip was placed on it removed from the block and examined.

Lactophenol cotton blue (LPCB) wet mount preparation

1. A drop of LPCB stain was taken on a clean glass slide.
2. Growth of fungus was taken with L-shaped wire and teased by the needle was placed in drop of LPCB stain.
3. The slide was examined microscopically after giving sufficient time for the structures to take up the stain, usually 30 minutes.

Results

Out of a total of 950 samples processed over a period of 3 years in our laboratory, 86 samples were culture positive for *Aspergillus* species. The highest number of isolates belongs to *A.fumigatus* (37.21%) followed by *A. flavus*(23.25%). *A. clavatus* accounted for the least number of isolates (2.33%).

Amongst the samples collected the highest number of isolates was from the cases of respiratory aspergillosis i.e, from Sputum, ET secretions and BAL(36%) and least numbers were from nail clippings and urine i.e, 20% and 15% respectively.

Sex wise distribution ratio was Male :Female (66.9:33.05)

Aspergillus species distribution amongst the various clinical samples showed highest isolate of *A.fumigatus* in sputum , ET Secreations, BAL and Pus , whereas in gastric aspirate and urine highest isolate was *A. niger* . In nail clippings isolated species were A.flavus and A.terrus

A.clavatus was also isolated from sputum and pus.

Table 1: *Aspergillus* isolates from various clinical samples are as below

Sample	No. of samples	No. of isolates	Percentage
Sputum	757	54	7
ET secretions	53	9	16
BAL	51	7	13
Pus	45	7	15
Gastric Aspirates	15	4	26
Urine	19	3	15
Nail clippings	10	2	20

Table 2: Distribution of various *Aspergillus* species in different clinical samples

Isolated species	Positive numbers (n=86)	Percentage
Aspergillus fumigatus	32	37.21
Aspergillus flavus	20	23.25
Aspergillus niger	15	17.4
Aspergillus terrus	10	11.6
Aspergillus nidulans	7	8.1
Aspergillus clavatus	2	2.3

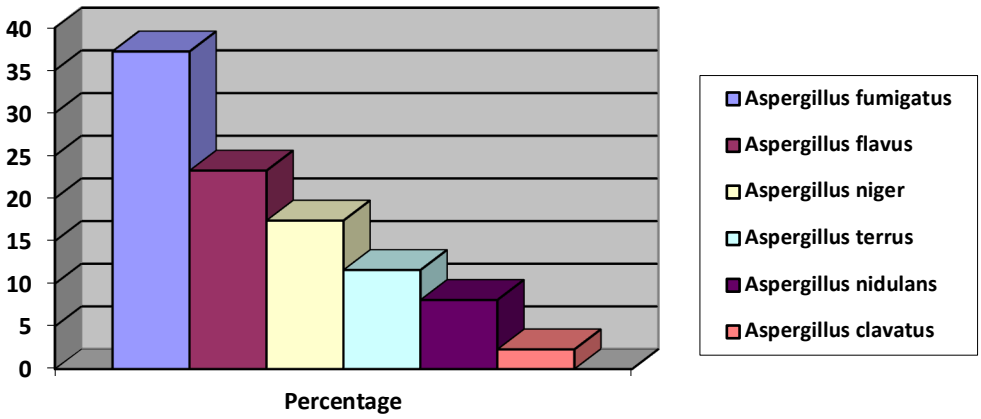

Figure 1: Distribution of various *Aspergillus* species in different clinical samples

Table 3: Sex wise distribution of *Aspergillus*

Sex	No. positive	Percentage
Male	636	66.9
Female	314	33.05
Total	950	100

Table 4: Distribution of *Aspergillus* spp. in various clinical samples

Sample	*A. fumigatus*	*A. flavus*	*A. niger*	*A. terrus*	*A. nidulans*	*A. clavatus*
Sputum	22	15	8	5	3	1
BAL	3	1	-	2	1	-
ET secretions	3	2	2	1	1	-
Nail clippings	-	1	-	1	-	-
Pus	4	-	1	-	1	1
Gastric Aspirates	-	1	2	-	1	-
Urine	-	-	2	1	-	-
Total	**32**	**20**	**15**	**10**	**7**	**2**

Discussion

Aspergillus species are prevalent airborne fungal pathogens, have caused an increased number of reported cases of infection during past decade [7-9,10]. Davis *et al*.[11], using the Health Care Cost and Utilization Project and National Sample from the Agency for Health Care Research and Quality, attempted to gauge the burden of aspergillosis. Furthermore, hospitalization for aspergillosis cost substantially more than non-aspergillosis related hospitalizations and were associated with lengthy hospital stays and a higher mortality rate [11].

According to CDC Journal Emerging Infectious Diseases, As many as 15% of patients with COVID 19 who are hospitalised in an ICU developed *Aspergillus* infection.

The incidence of invasive *Aspergillus* in different studies varies from <1% to 40% depending on the patient population. The rate of isolation of *Aspergillus* species observed in our hospital (9%) is similar to that seen in others.

Like other previously published reports, in our studies also *A. fumigatus* is the most

common isolated species thereafter *A. flavus* is the common isolated spp.

Allergic bronchopulmonary aspergillosis (ABPA) is a complex condition that results from hypersensitivity to the fungus. First described in United Kingdom in 1952, it has been estimated to occur in 1-2% of chronic asthmatics and up to 13.6% of patients with cystic fibrosis. Also, in our study we isolated the fungus from patients with chronic asthma and cystic fibrosis, pneumonia, bronchitis. The highest numbers are of *A.fumigatus* followed by *A.flavus*.

In bronchopulmonary aspergillosis and aspergilloma, inhalation is the most important method initiating infection from soil,rotting vegetables, leaf piles, dung heaps etc. In our study there was preponderance of aspergillosis in males and younger persons which leads to the possible explanation that males spend more time outdoors and in fields and thus acquire the agent from locally prevalent fungal flora.

In comparison with other studies like Dr. Mridula Mittal *et al* [12] showed highest numbers of *A.flavus* followed by *A.fumigatus* from sputum samples while in our study we found highest numbers of *A.fumigatus* followed by *A.flavus.* And also these results are similar to one of the study of Shrimali *et al* [13]. Other study like Alim A *et al* [14] showed highest numbers of *A.niger* followed by *A.fumigatus* from respiratory samples.

Mittal *et al* [12] showed no evidence of *Aspergillus spp.* from urine and nail clippings while in our study we isolated *A.niger* and *A. terrus* from urine and *A.flavus* and *A.terrus* from nail clippings.

Also from pus Mittal *et al* [12] showed *A.flavus* followed by *A.niger* while in our study we isolated *A.fumigatus, A.niger, A. nidulans, A.clavatus* [13-14].

Also we isolated *A.flavus, A. fumigatus, A. terrus, A.nidulans* from the rare samples like BAL and gastric aspirates.

Conclusion

There is a need to develop a greater understanding of the pathogenesis of the disease, formulate better and more sensitive diagnostic techniques, develop superior antifungal agents and increase an awareness of the disease among clinicians. More important is the need to develop antifungal susceptibility testing for *Aspergillus* species and means to prevent occurrence of the disease specially in immunocompromised individuals. A knowledge of local patterns of infection and antifungal susceptibility would prove useful in selecting empirical therapy and formulating prophylactic and pre-emptive strategies.

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