

Aspergillus species in clinical specimen: a seven year prevalence study from eastern Nepal

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ABSTRACT

Aspergillus species is increasingly being associated with various clinical conditions. The isolation rate however varies from centre to centre owing to the difference in the patient population, the local prevalence of the fungus and the nature of the clinical specimens received in the particular mycology laboratory. The objective of this study was to find the prevalence of *Aspergillus species* in different clinical specimen submitted in our hospital. Prospective study of the specimens received in mycology laboratory to find the prevalence of *Aspergillus species* isolated from BP Koirala Institute of Health Sciences (BPKIHS) was done. The overall prevalence of *Aspergillus species* in clinical samples was 15.06%. *Aspergillus species* was most frequently isolated (73.21%) from samples from otitis externa. The most number (68/165) of *Aspergillus species* isolated in this study was from nail samples from cases of onychomycosis. *Aspergillus flavus* was the commonest species isolated. There was an increasing trend in the isolation rate from 2003 to 2009. This study being the first of its kind from Nepal shows that *Aspergillus species* is a common pathogen among the spectrum of diseases we encounter here. Mycology laboratories should incorporate reliable, rapid diagnostic tests for early diagnosis as well as antifungal susceptibility tests.

Keywords: Prevalence; *Aspergillus*; clinical specimen; Nepal.

INTRODUCTION

Aspergillus species is an ubiquitously present saprophytic fungi.¹ Since its first description as an opportunistic fungal agent in 1953,² it has been described as the cause of various chronic, saprophytic, allergic and invasive conditions. These days, the cases of aspergillosis are increasingly being reported as the number of predisposing conditions like immunosuppressive therapy for various medical conditions and immunocompromised states like HIV/AIDS are on the rise. There is a conspicuous lack of studies on its prevalence from Nepal. We have therefore tried to investigate the local prevalence of this emerging pathogen in different clinical specimens in our setup with the aim of aiding the management of these mycoses.

MATERIALS AND METHODS

This was a prospective analysis of specimens received in mycology laboratory over a period of seven years, starting from January 2003 to December 2009 in B.P Koirala Institute of Health Sciences (BPKIHS) Nepal. BPKIHS is a tertiary care hospital catering to the population of eastern Nepal and neighboring northern India. All *Aspergillus species* isolated from various clinical specimens during this period were included in the study.

SAMPLE PROCESSING AND IDENTIFICATION OF ISOLATES

Sample processing and identification of isolates was done according to standard mycological techniques.³ In brief, all the samples, except blood were examined for presence of yeasts and fungal hyphae by preparing a Gram stain and 10% KOH mount. A set of Sabourauds dextrose agar (SDA) tubes: one plain and one with gentamicin and cycloheximide were inoculated for each clinical specimen and incubated at 25°C and /or 37°C depending on the specimen. Blood and bone marrow were inoculated in biphasic SDA medium. They were examined regularly for a period of six weeks before declaring them as culture negative.

Those with fungal growth were identified as *Aspergillus species* by the macroscopic colony characteristics as well as the microscopic features on the lactophenol cotton blue mount made from the fungal growth. Whenever possible, an attempt was made to confirm the positive culture by isolation of the same fungus from repeated samples.

RESULTS

Out of the total of 1095 specimens received for fungal culture in mycology laboratory, over a period of seven years, 165 grew *Aspergillus species*. Table-1 shows the

Table-1: Distribution of specimens and percentage of samples yielding *Aspergillus species*

Specimen	Total	No. of <i>Aspergillus species</i> (%)
Nail	298	68 (22.81)
Ear	56	41 (78.21)
Eye	131	14 (10.68)
Respiratory	224	17 (07.58)
Skin	122	07 (05.73)
Tissue	102	11 (10.78)
Pus	68	02 (02.94)
Miscellaneous	94	05 (05.31)

distribution of different samples and the percentage of these which grew *Aspergillus species*. The miscellaneous samples included blood, bone marrow, hair, urine, and cerebrospinal fluid. Two blood, one bone marrow, one subdural hematoma and one hair sample grew *Aspergillus*.

Fig. 1. shows the overall distribution of different species of *Aspergillus* from all the clinical specimens. The most common was *Aspergillus flavus* (n=54). The other species were *A. fumigatus*, *A. terreus* and *A. clavatus*.

Of the 165 patients whose samples grew *Aspergillus species*, the male and female ratio was 76:89. The highest number of the positive samples was received from the dermatology followed by the ENT and the internal medicine departments.

The isolation rate from clinical samples showed an increasing trend with 5.5% of samples for fungal culture yielding *Aspergillus species* in 2003 to 26.96% in 2009 (Fig. 2).

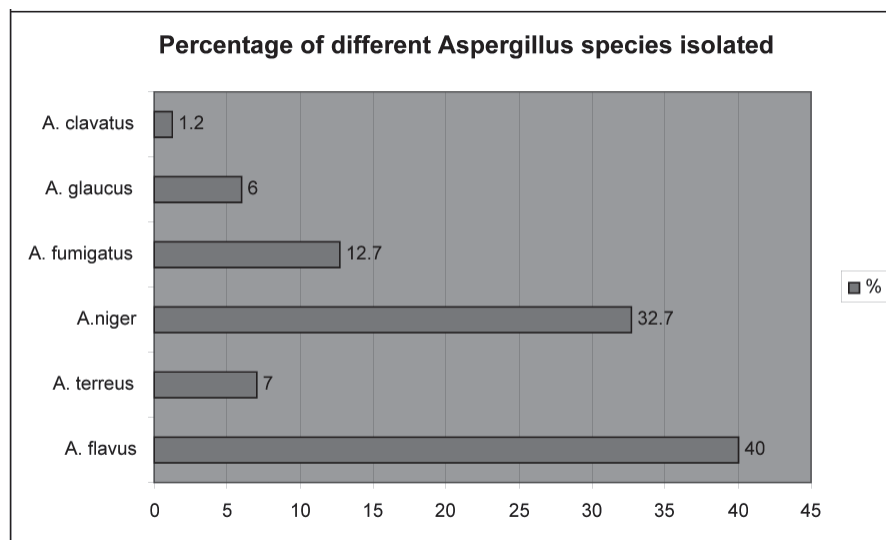


Fig. 1. Percentage of different *Aspergillus species* isolated

DISCUSSION

In the current study, 15.06% of the clinical samples grew *Aspergillus species*. Various studies have shown its prevalence ranging from <1% to 40%.⁴ The difference could have arisen from the difference in the patient population, the nature of clinical specimens and the different local prevalence of the fungus at different centres.

In our centre, the highest number of *Aspergillus species* isolated was from nail specimen from cases of onychomycosis (68/165). It represented 22.8% of all onychomycosis cases. An Egyptian study showed 47% of cases of onychomycosis to be due to *Aspergillus species* while a study from Italy had only 2.5% attributable to it. Onychomycosis is an opportunistic fungal disease usually caused by impaired barrier function in otherwise healthy individuals like trauma to the nail. *Aspergillus species* has been described as the second leading isolate among non dermatophytic agents of onychomycosis.⁵ Similar to other studies,^{6,7} the species frequently isolated in our study was *A. flavus* (24/68) followed by *A. niger* (20/68).

The highest rate of isolation of *Aspergillus species* was from debris, scrapings or exudates from external auditory canal from cases of otitis externa (73.21%). Otitomycosis or fungal otitis externa is described as fungal infection of external auditory canal with infrequent complications involving the middle ear. *Aspergillus species* and *Candida albicans* have been quoted as the most common causative agents of otomycosis in different studies.⁸⁻¹⁰ *Aspergillus species* is considered the predominant causal organism of otomycosis in the tropics and subtropics.⁹ Confirming to other studies,¹¹ our study showed *A. niger* as the predominant species (45.65%) followed closely by *A. flavus* (43.47%) in cases of otomycosis. Some others have found *A. flavus*¹² and *A. fumigatus*¹³ as the leading isolates from otomycosis followed closely by *A. niger*.

Seven point five percent of the respiratory samples grew *Aspergillus species*. The samples included sputum, bronchial lavage, nasal polyps and antrum puncture wash. The commonest was *A. flavus* (10/16). Xess *et al* found *A. flavus* as the major species from the paranasal sinus mycoses.⁷ In a previous study done in our centre, *Aspergillus*

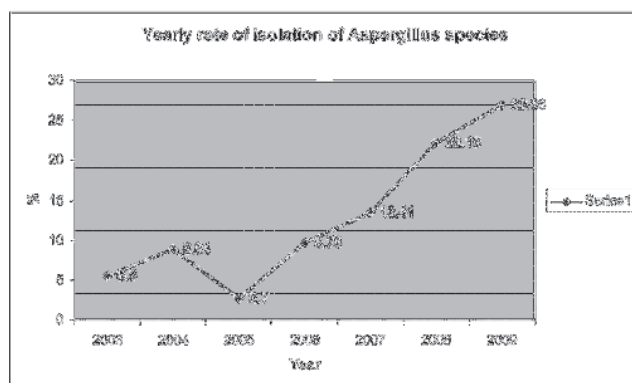


Fig. 2. Yearly rate of isolation of *Aspergillus* species from specimens received for fungal culture

species was the most common fungal pathogen in maxillary sinusitis.¹⁴ Invasive pulmonary aspergillosis is the commonest manifestation of aspergillosis.¹⁵ However, isolation of *Aspergillus* species from respiratory tract specimens from immunocompetent patients is not specific due to difficulties in distinguishing colonization from disease.¹⁵ The specificity of isolation of *Aspergillus* species is high in those with profound immunocompromise.

Ten point sixty eight percent of isolates of eye specimens and majority of corneal ulcer samples (33%) grew *Aspergillus* species with *A. flavus* as the predominant species. Similar high prevalence of *Aspergillus* species as causative agent of fungal keratitis was noted in studies from India and Nepal.^{16,17} *A. flavus*¹⁸ and *A. fumigatus*¹⁹ were among the leading species isolated from mycotic keratitis. Since *Aspergillus* species is ubiquitously present in the environment any break in or trauma to the corneal epithelium may inoculate this fungi and lead to invasive infection. Mycotic keratitis is difficult to treat and can lead to severe visual impairment or blindness.

The increasing trend of isolation of *Aspergillus* species from clinical specimens over the years reflects the changing populace of patients attending the hospital. This increase may also be because of the overall increase in the specimens received for mycological investigations and the disparity in the types of specimen received in different years.

It is clear from this study that *Aspergillus* species is one of the important causes of mycoses in our setup too. From superficial infections in the healthy to invasive diseases in the immunocompromised, it is increasingly being reported. The need for more research to understand the pathogenesis of *Aspergillus* species, to develop reliable diagnostic techniques and to formulate easy and

feasible antifungal susceptibility tests was felt. This kind of study which reveals the local prevalence of common mycological agents will help in the empirical management of cases of mycoses.

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