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Original Research Article

Identification and antifungal susceptibility profile of filamentous fungi isolated from cases of healthcare associated infections

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ABSTRACT

Background: Although, bacteria have been considered as most common cause of healthcare associated infections (HCAI), however recent years have witnessed increased isolation of fungal pathogens. *Candida* species, Mucorales, *Aspergillus* spp., *Fusarium* spp. and *Scedosporium* spp. are predominant fungal pathogens isolated from cases of HCAI. As compared to bacterial nosocomial infections, fungal infections are generally difficult to diagnose and treat. Nosocomial mycoses are associated with high mortality and morbidity. The present study was conducted in the tertiary care academic hospital with an aim to identify filamentous fungal pathogen isolated from HCAI and study its antifungal susceptibility profile.

Materials and Methods: Filamentous fungi isolated from cases of HCAI from intensive care unit (MICU) were included. They were identified by standard mycological techniques and antifungal susceptibility profile was studied.

Results: Out of 50 fungal pathogens, *Candida* spp. were isolated from 21 (42%) cases, whereas 29 (58%) isolates were filamentous fungi. *Aspergillus* spp. (51.7%) were predominant among filamentous fungal pathogen. *A. fumigatus* (27.6%) was the predominant isolate. Use of broad-spectrum antibiotics followed by neutropenia and presence of indwelling medical devices were common risk factors associated with HCAI due to filamentous fungi. Amphotericin B resistance was observed in 6.9% of isolates whereas 33.3% were resistant to fluconazole.

Conclusion: Invasive fungal infection (IFI) though less common is associated with increased morbidity and mortality in patients admitted to ICU. Neutropenia and use of broad spectrum antibiotics are important risk factors for IFI. *Aspergillus fumigatus* is the most common filamentous fungus cause of IFI. More emphasis should be given on rapid diagnosis, prompt treatment and strict compliance with infection prevention and control practices.

1. Introduction

Over the period of last four decades there has been a drastic increase in the incidence of fungal infections. A vast number of factors are implicated for increased incidence of fungal infections but advent of HIV/AIDS, aging population and certain medical practices like widespread use of immunosuppressive therapies, use of medical devices for diagnosis and treatment of various ailments, and use of broad-spectrum antibiotics are more significant.[1]

In additional to community acquired infections, incidence of healthcare associated fungal infections are also increased. As per National Healthcare Safety Network (NHSN) surveillance definition, healthcare associated infections (HCAI), are localized/systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin (s)that was neither present nor in incubation at the time of admission to healthcare facility.[1]

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Traditionally, bacteria have been considered as most common cause of HCAI, however recent years have witnessed increased isolation of fungal pathogens.[2] In addition to various host factors, advancement in the domain of diagnostic mycology particularly use of molecular techniques is important for increased recognition of fungi as an etiology of HCAI.[3]

Candida species, *Mucorales, Aspergillus* spp., *Fusarium* spp. and *Scedosporium* spp. are predominant fungal pathogens isolated from cases of HCAI.[3] As compared to bacterial nosocomial infections, fungal infections are generally difficult to diagnose and treat. Nosocomial mycoses are associated with high mortality and morbidity. The limited antifungal armamentarium, emergence of treatment resistant fungal strains and resource constrained mycology complicates the treatment and outcome of nosocomial mycoses.

As compared to *Candida* spp., there is less information available related to filamentous fungal pathogens isolated from cases of HCAI. The present study was conducted in the tertiary care academic hospital with an aim to identify filamentous fungal pathogen isolated from HCAI and study its antifungal susceptibility profile.

2. Materials and Methods

The present study is a part of a Ph.D. thesis carried out in the Department of Microbiology, at Maharao Bhimsingh Hospital, Nayapura, which is affiliated with Government Medical College, Kota, Rajasthan. Descriptive cross-sectional study was conducted for a period of one year.

Filamentous fungi isolated from cases of HCAI from intensive care unit (MICU) were included. The demographical and clinical features of the patients suspected for HCAI were recorded and analyzed. The protocol of the study was approved by Institutional Ethics Committee.

Specimens collected from suspected for HCAI were processed as per standard mycological protocol. The specimens were examined microscopically by potassium hydroxide (KOH) preparation for presence of fungal elements. For culture, the specimens were inoculated onto a pair of Sabouraud dextrose agar (SDA) slant without antibiotics. One set of SDA was incubated at 37°C while other was incubated at room temperature.

After initial inoculation and incubation, cultures were examined for growth every 2-3 days during the first week and weekly thereafter. If fungal growth was observed, the texture and surface color of the fungal colony were carefully noted. The color of the reverse (underside) of the colony was also recorded along with any pigment diffusing in the medium. SDA slants were incubated for 4 weeks before being considered negative for fungus.

Lactophenol cotton blue (LPCB) mount was prepared to study microscopic features of filamentous fungi. For preparation of LPCB mount, a drop of LPCB was placed on a clean glass slide. A small portion of the colony was removed aseptically using a sterile loop and placed on the drop of LPCB. The colony was gently teased using two dissecting needles and coverslip was placed on the preparation. The LPCB was observed under the microscope with low-power and high-dry magnifications for the pattern of conidiation. The preparation of LPCB mount was done in a class II biosafety cabinet.

Additionally slide culture was performed for studying the fine points of the microscopic morphology of fungi. The isolation of fungi was considered significant only if it was demonstrated by microscopy and isolated in culture.

The method used to determine the antifungal susceptibilities of molds/filamentous fungi was based on the Clinical and Laboratory Standard Institute (CLSI) approved procedure M38-A2. The minimum inhibitory concentration (MIC) was detected for antifungal drugs like amphotericin B and fluconazole. The antifungal drugs were procured from Himedia Laboratories Pvt Ltd Mumbai as a pure standard compound.

3. Results

During the study period a total of 26643 patients were admitted to different intensive care unit of the hospital. Out of these 26643, a total of 1868 (7.1%) patients developed HCAI. Bacterial pathogens were isolated from 1818 (97.3%) cases whereas fungi were isolated from 50 (2.7%) cases. *E. coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were common bacterial pathogens.

Out of 50 fungal pathogens, *Candida* spp. were isolated from 21 (42%) cases, whereas 29 (58%) isolates were filamentous fungi. *C. albicans* were isolated from 7 (33.3%) cases, whereas 14 (66.7%) isolates belonged to non albicans *Candida* (NAC) spp. which included 10 isolates of *C. tropicalis* and 4 isolates of *C. krusei*.

The distribution of filamentous fungi is shown in Table 1. *Aspergillus* spp. were predominant among filamentous fungal pathogen. They were isolated from a total of 17 (58.6%) cases. *A. fumigatus* (27.6%) was the predominant isolate. Mucorales were isolated 08 (27.6%) cases.

The demographic and clinical features of patients showing having HCAI due to filamentous fungi is shown Table 2. Male predominance was noted in the present study. Use of broad-spectrum antibiotics followed by neutropenia and presence of indwelling medical devices were common risk factors associated with HCAI due to filamentous fungi.

Antifungal susceptibility profile of filamentous fungi is shown Table 3. A total of 2 (6.9%) isolates were resistant to amphotericin B. These include 1 isolate each of *A. niger* and *A. flavus*. Out of 21 filamentous fungi tested for fluconazole

Table 1:

Filamentous fungi	Number (%)
Aspergillus spp.	
fumigatus	08 (27.6)
flavus	05 (17.2)
nidulans	02 (6.9)
niger	02 (6.9)
Mucorales	
Mucor spp.	03 (10.3)
Rhizopus	02 (6.9)
Rhizomucor	02 (6.9)
Syncephalostrum racemosum	01 (3.4)
Scedosporium spp.	01 (3.4)
Fusarium spp.	03 (10.3)
Total	29

Table 2:

Demographic/clinical feature	Number (%)
Gender	
Male	22 (75.9)
Female	07 (24.1)
Mean age in years ±SD	51.9±5.5
Indwelling medical device	18 (62.1)
Major surgery	15 (51.7)
Neutropenia	23 (79.3)
Malignancy	09 (31.1)
HIV/AIDS	07 (24.1)
Broad spectrum antibiotics	25 (86.1)
Burn	11 (37.9)
Immunosuppressive therapy	12 (41.4)
Prophylactic antifungal therapy	06 (20.7)
Haemodialysis	10 (34.5)
Diabetes	12 (41.4)

Table 3: Antifungal susceptibility profile of filamentous fungi

Filamentous fungi (N)	Amphotericin B		Fluconazole	
	Susceptible (%)	Resistant (%)	Susceptible (%)	Resistant (%)
A. fumigatus (8)	07 (87.5)	01 (12.5)	05 (62.5)	03 (37.5)
A. flavus (5)	04 (80)	01 (20)	04 (80)	01 (20)
A. nidulans (2)	02 (100)	-	01 (50)	01 (50)
A. niger (2)	02 (100)	-	02 (100)	-
Mucor spp (3)	03 (100)	-	Not tested	
Rhizopus (2)	02 (100)	-	Not tested	
Rhizomucor (2)	02 (100)	-	Not tested	
Syncephalostrum racemosum (1)	01 (100)	-	Not tested	
Scedosporium spp. (1)	01 (100)	-	01 (100)	
Fusarium spp. (3)	03 (100)	-	01 (33.3)	02 (66.7)
Total (29)	27 (93.1)	02 (6.9)	14 (66.7)	07 (33.3)

susceptibility, a total of 14 isolates were susceptible to fluconazole (66.7%) whereas 7 (33.3%) were resistant.

4. Discussion

HCAIs are one of the most common adverse iatrogenic events experienced by patients admitted to healthcare setup for diagnosis and or treatment of various diseases. It one of the important causes of morbidity and mortality particularly in ICU patients. Various factors like high prevalence of invasive procedures and devices, acquired and/or induced immunosuppression, comorbidity, frailty and advanced age are associated with high risk of acquisition of HCAI in ICU patients.[4,5]

The rate of HCAI varies greatly as per country, the type of healthcare setup and types of patients care for. In general, ICU patients are twice as likely to acquire HCAI as patients admitted to general wards.[6] Worldwide the prevalence of ICU acquired infections is significantly high. It is estimated to be as high as 51.4%. In Europe and the USA, the prevalence of HCAI acquired in ICU is estimated to range between 9 to 37% R3, R4, R5. However, there is dearth of information regarding ICU-acquired infections from developing countries like India. In the present HCAI was noted in 7.1% of patients admitted to ICU.

In ICU, although bacteria are important of infections, fungal infections represent as a grave problem and are associated with high morbidity and mortality rates, increased hospital stay and high health care cost.[7] Although vast majority of these infections are caused by *C. albicans* and *A. fumigatus*, in recent years new opportunists including yeast-like and filamentous fungi has emerged as an important cause of ICU-acquired invasive fungal infections.[8]

In the present study, 42% of invasive fungal infections (IFI) were due to *Candida* spp. In Europe, *Candida* spp., is the fourth most common nosocomial pathogen isolated after *S. aureus, P. aeruginosa* and *E. coli.*[8] Although, *C. albicans* is considered as the most pervasive pathogen. Recent studies have highlighted emergence of cryptic NAC spp. In the present study, 66.6% of *Candida* isolates belonged to NAC spp. As NAC spp. are either innately resistant or acquire resistance to antifungal drugs, infection due to these species are usually difficult to treat.[2]

Aspergillus spp. was predominant filamentous fungi isolated in the present study. Opportunistic Invasive aspergillosis usually occurs in high-risk severely immunocompromised patients. *Aspergillus* spp. causes invasive pulmonary and disseminated infections in hospitalized patients. It is an important cause of morbidity. The mortality of invasive aspergillosis ranges between 65-92% in high-risk population.[9,10] *A. fumigatus* was commonest species. *A. fumigatus* is most common species associated with invasive aspergillosis, although isolation of other species like *A. flavus, A. niger,* and *A. terreus* have also been reported.

In the present study, a total of 08 fungi belonging to mucorales were isolated. As spores of this group of fungi are dispersed, infection generally occurs through inhalation of fungal spores which results in sinopulmonary disease. Systemic infection can result from inoculation of the skin or gastrointestinal mucosa. Although infection caused by Mucorales is less common, it is often a more.[11]

In recent years, antifungal susceptibility testing is becoming an increasing important tool for various purposes including guiding patient therapy, tracking rates of antifungal resistance and for epidemiological studies.[12] Antifungal susceptibility testing is particularly important when fungal infection is invasive, development of resistance during course of therapy is suspected and patient is not responding to the therapy. In the present study, amphotericin B resistance was observed in 6.9% of isolates whereas 33.3% were resistant to fluconazole. Although amphotericin B resistance was less, it is of great concern because amphotericin B is gold standard antifungal drug used for treating serious fungal infections.[13]

5. Conclusion

Invasive fungal infection (IFI) though less common is associated with increased morbidity and mortality in patients admitted to ICU. Neutropenia and use of broad-spectrum antibiotics are important risk factors for IFI. *Aspergillus fumigatus* is the most common filamentous fungus cause of IFI. More emphasis should be given on rapid diagnosis, prompt treatment and strict compliance with infection prevention and control practices.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

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