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IP Indian Journal of Immunology and Respiratory Medicine

Journal homepage: https://www.ijirm.org/

Original Research Article

Clinico-mycological profile of fungal infections in a tertiary care hospital

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ARTICLE INFO

Article history:
Received 29-08-2023
Accepted 09-11-2023
Available online 27-01-2024

Keywords:
Fungal infections
Antifungal susceptibility
Candidiasis
Aspergillosis

ABSTRACT

Background: The fungal infections are increasing at high rate especially in immune-compromised patients and elderly population. In high risk population, antifungal resistance is becoming a major concern. Antifungal susceptibility testing is important for appropriate management and better outcome of patients.
Aim: The aim of the study was to know clinico-Mycological profile of fungal infections in admitted patients.
Materials and Methods: This prospective study was carried out in a tertiary care hospital. Various samples were received from patients with suspected fungal infections. All samples were inoculated on SDA except blood & body fluids which were directly inoculated into blood culture bottles and processed in BACTEK/BacTAlert system. Fungal infections were characterized and antifungal susceptibility was done for yeast isolates with VITEK-AST panel.
Results: Out of total 19698 patients suspected of fungal infection, fungal infection was seen in 365 patients *Candida albicans*, 100% susceptibility was seen to Fluconazole and Echinocandins. Whereas in NAC (non-albicans *candida*), 72.5% susceptibility was seen to fluconazole and Voriconazole (82.5%).
Conclusion: Antifungal resistance is major cause of morbidity and mortality. Prompt diagnosis and antifungal susceptibility will offer the early appropriate treatment and better clinical outcome of patient.

1. Introduction

Fungal infections (FI's) are emerging as world-wide healthcare problem. They are extremely common and can have varied clinical presentations. Recently invasive fungal infections have emerged as an important cause of morbidity and mortality. The economic burden of these infections are significant with increase in cost of care and prolonged hospital stay.[1] The rising prevalence of these infections is related to several host factors, including elderly or critically ill patients, immunocompromised individuals secondary to malignancies, solid organ transplants, HIV and patients on immunosup pressants. Prolonged use of broad spectrum antibiotics in chronic patients may stimulate the growth and virulence of fungal infections by destruction of bacterial flora.[1]

The overall incidence rate of IFI (Invasive fungal infections) varies from 3% to 20%.Opportunistic pathogens such as *Candida spp*., *Cryptococcus spp*., *Aspergillus spp*., and *Mucorales* are the most common causative agents and other hyalohyphomycete, phaeohyphomycetesand basidiomycetous are also known to cause fungal infections. Candidemia is the most common infection encountered with different types of non-albicans *candida* as a major cause.[2,3] In critically ill patients, coinfections are also observed that further complicates and delay the diagnosis.[4,5]

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https://doi.org/10.18231/j.ijirm.2023.027

Recently, the burden of antifungal resistance is becoming a major concern in high risk population. Resistance to the azole group of antifungal agents has been observed because azoles like fluconazole are the most commonly used antifungals.[6] Resistance represents a major obstacle against successful empirical, therapeutic and prophylactic strategies. Acquired resistance to azoles also seen in aspergillosis due to sustained exposure of fungi to azoliccompounds.[7] Antifungal susceptibility testing can manage the selection of adequate therapy and provide an estimate to antifungal efficacy. Though recent guidelines are there to standardize antifungal susceptibility testing, but still to incomplete correlation between in vitro susceptibility and clinical response to treatment. With this aim the prospective study was undertaken to know the incidence, types of fungal infections, to study the antifungal susceptibility and to correlate it with the clinical outcomes in admitted patients

2. Material and Methods

This prospective study was conducted in the Department of Microbiology, for the period of 1 year. Patients with suspected fungal infections were included and detailed history was recorded.

Various Samples like blood, body fluids, respiratory samples, pus, tissue, skin scrapings, corneal scarpings, biopsy etc were included in the study. Microscopic examination with KOH mount was done for all samples except blood samples. The samples were inoculated on two tubes of Sabouraud dextrose agar (SDA) with antibiotics and cycloheximide and two tubes of SDA with antibiotics but without cycloheximide. The growth obtained was identified on the basis of colony morphology, pigment production and microscopic examination with KOH mount and lactophenol cotton blue (LCB). Isolation of candida species from respiratory samples (sputum, endotracheal aspirate, bronchoalveolar lavage, suction tip) and urine were considered as fungal colonization.[8]

Blood and body fluid culture were done by BAC-T alert or BACTEC automated systems. The blood culture bottles were incubated till the bottle indicate positive by the system for maximum period of 7 days. Identification and antifungal susceptibility for yeasts was done by VITEK-2 technology. Serological correlation was done with (1-3)-β-D Glucan assay with Fungitell Kinetic Assay and value of <60pg/mL was taken as negative, 60 to 79 pg/mL indeterminate and ≥ 80 pg/mL positive. Serum galactomannan assay was done using Platelia *Aspergillus* EIA (enzyme immunoassay) kit (Bio-Rad, France) and value of < 0.50 were considered as negative and ≥ 0.50 were considered as positive. Invasive aspergillosis cases were classified according to revised definitions given by Invasive Fungal Infections Co-operative Group (IFICG) of the European Organization for Research and Treatment of Cancer and Mycoses study group case definitions (EORTC/MSG) into proven, probable and possible invasive aspergillosis based on host, clinical and mycological factors.[8]

2.1. Statistical analysis

The data was recorded on the proforma enclosed. All statistical calculations were done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Window.

Ethical approval was taken from institutional ethical committee.

3. Results

In 1 year study, out of 19698 samples received, 372 isolates were obtained from 365 patients. Out of 372 isolates, 97(0.63%) were obtained from 15380 blood sample and 126 (29.2%) from other 4318 samples. Candida isolates (149) from respiratory samples and urine samples were considered as colonizers and rest 223 were labelled as pathogens.(Table 1) These 223 isolates were obtained from 216 patients. Male preponderance was seen 141(65.3%) as compared to females 75(34.7%)Majority of patients belong to 51-60 years (23.1%), followed by 61-70 years (21.8%)(Figure 1).

Maximum no. of patients with fungal infections were from medicine wards (59.3%) followed by ICU"s (22.7%), Surgery 11.5% and paediatrics 6.5%.Steroid use (21.3%) was the most common risk factor observed followed by diabetes mellitus (18.1%) (Figure 2).

Majority of patients presented with fever (65.3%) followed by shortness of breath (41.2%) and cough (23.1%).

Most common fungal infection was *Candidiasis* 131(60.6%) followed by Aspergillosis 63(29.2%) and Mucormycosis 13(6.0%) and Cryptococcosis 5(2.3%). Among 2 cases of *Trichosporon* infection, 1 was *trichosporonemia* and other was *Trichosporon* pneumonia. 1 case of Fusarium keratitis was observed.(Table 2)

Among 131 patients with *candidiasis*, invasive *candidiasis* was seen in 128(97.7%) patients & cutaneous *candidiasis* seen in 3(2.3%) patients. *Candida tropicalis* 86(62.7%) was most common isolate followed by *Candida albicans* 17(12.4%) & *Candida gulliermondi* 12(8.7%)(Figure 3)

In C. albicans 100% susceptibility was seen to Fluconazole and Echinocandins. In NAC, maximum susceptibility was seen to Amphotericin-B (89.2%) followed by Voriconazole (82.5%). Susceptibility to fluconazole and micafungin in C. albicans was statistically significant as compared to NAC.(Table 3)

Out of 5 *cryptococcus* species, 4 isolates of *Cryptococcus neoformans* and 1 *Cryptococcus laurentii* was seen. All the 4 patients with cryptococcal meningitis were HIV reactive.

Out of 63 cases of aspergillosis, most common infection was pulmonary aspergillosis 45(71.4%) followed by sino-

nasal aspergillosis 12(19.1%). *Aspergillus flavus* 49(77.8%) was predominant species followed by *Aspergillus fumigatus* 14(22.2%).Among the cases of pulmonary aspergillosis, most common radiological findings were consolidation 7(21.9%) and consolidation with ground glass opacity 7(21.9%). Out of 45 cases of pulmonary aspergillosis, galactomannan assay was received in 38 patients, out of which 20 were positive. Percentage positivity of 52.6% was observed.

In the present scenario, suspicion of Invasive pulmonary Aspergillosis (IA) were categorized. Cases of A were further classified as probable IA-32 and possible IA-45 and no case was as proven as per European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria.

Out of total 13 cases of mucormycosis, most common infection was Sino-nasal mucormycosis 8(61.5 %) followed by cutaneous mucormycosis 4(30.8%). Most commom species observed was *Rhizopus* 8(57.2%) followed by *Mucor* 6(42.8%). Out of 216 patients with fungal infections, 204(94.4%) patients got discharged and 12(5.6%) expired.

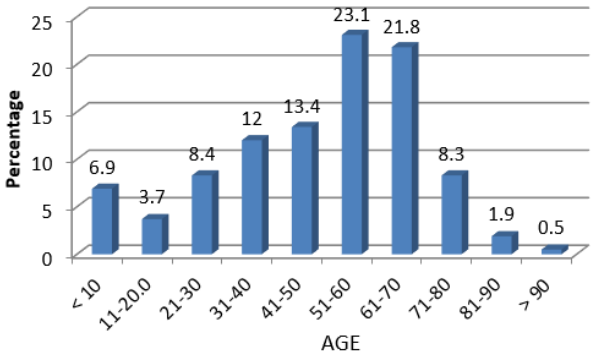

Figure 1: Age wise distribution of patients with fungal infections (n=216)

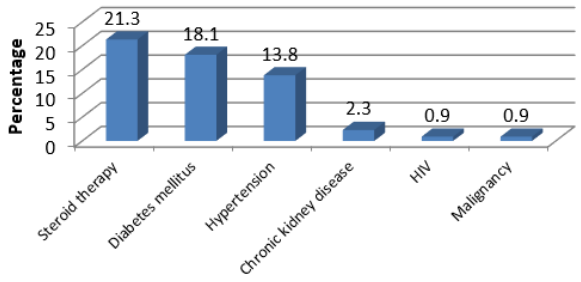

Figure 2: Distribution of risk factors in patients with fungal infections (n=216).

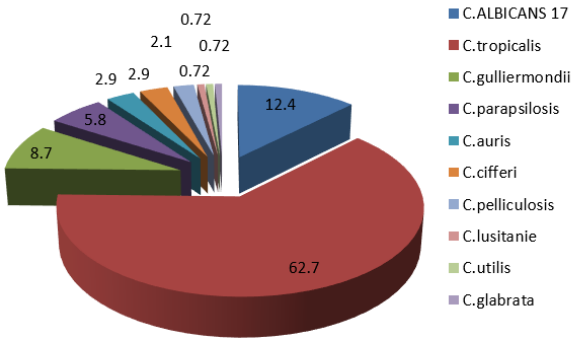

Figure 3: Species wise distribution of *candida* (n=137)

4. Discussion

Out of total, fungal infection was seen in 1.88% (372) samples. Whereas in a study conducted by Ahir HR et al[9] higher positivity (6.7%) of fungal isolates can be seen. 372 isolates were obtained from 365 patients. Out of total isolates, 198 were from respiratory samples, 97 from blood, 42 from body fluids, 19 from sino nasal mucosa, 9 from tissue, 2 from frank pus, 1 from urine and 4 from other samples. Table 1 shows the sample wise distribution of fungal isolates. Out of total samples, positivity of fungal infections in blood was 0.63% and 29.2% from others.

Fungal infections were more common in males 141(65.3%) as compared to females 75(34.7%) in our study. This can be explained by the fact that the males are more exposed to fungal spores due to outdoor activities. These findings were more accordant with the study conducted by Lin SJ et al.which showed out of 225, 162 (72%) were males and 63 (28%) were females.[10]

Majority of patients belonged to elderly age group 51-60 years (23.1%) in our study. This can be compared with the study done by Marrin K et al[11] where 50-70 years age group was most common age group. (range 20-95 years).

Majority of the patients were admitted in medical wards (59.3%) followed by ICU (22.7%). Similarly, Chen S et al[12] reported majority of fungal infections in medical wards 390 [35.6%] followed by critical care units 273 [24.9%] and surgical wards [17.3%]. In contrast to this, some studies showed a significant increase in candidemia incidence in ICU's.[13,14]

Chronic steroid use was most common risk factor (21.29%) followed by diabetes mellitus (18.05%). Majority of immunocompromised individuals were started with steroid therapy. Uncontrolled diabetes mellitus is the most important co-morbid condition associated with mucormycosis. This can be associated with lack of regular health checkups in Indian population. Similarly in a study by Ahmadikia K et al[15] in 2021 the use of steroids therapy was a prominent risk factor. In a study by, Singh G et al[16] most common risk factor was diabetes mellitus (41.9%)

Table 1: Sample wise distribution of fungal isolates(n=223)

Sample	***Candida***	***Cryptococcus***	***Trichosporon***	***Aspergillus***	***Mucor***	**Rhizopus**	**Fusarium**	**Total**
Blood	96	-	1	-	-	-	-	97
Respiratory samples	148	1	1	46	1	1	-	198
Bodyfluids	37	4	-	1	-	-	-	42
Tissue	2	-	-	4	1	1	1	9
Sino-nasal mucosa	-	-	-	11	3	5	-	19
Pus	1	-	-	-	-	1	-	2
Urine	1							1
Others	1	-	-	2	1	_	-	4
	286	5	2	64	6	8	1	372

Table 2: Clinical categorization of fungal infections. (n=216)

Clinical categorisation	**n=216**	**Percentage (%)**
Candidiasis	**131**	**60.6%**
Candidemia	93	43.1
Invasive *Candidiasis*	35	16.1
Cutaneous *Candidiasis*	3	1.4
Cryptococcosis	**5**	**2.3%**
Cryptococcal Meningitis	4	1.9
Cryptococcal Pneumonia	1	0.5
Trichosporonosis	**2**	**0.9%**
Trichosporinemia	1	0.5
Trichosporon pneumonia	1	0.5
Aspergillosis	**64**	**29.6%**
Pulmonary Aspergillosis	46	21.3
Sino-nasal Aspergillosis	12	5.5
Cutaneous Aspergillosis	5	2.2
Ocular Aspergillosis	1	0.5
Mucormycosis	**13**	**6.1%**
Sino-nasal Mucormycosis	8	3.6
Cutaneous Mucormycosis	4	1.9
Pulmonary Mucormycosis	1	0.5
Fusarium Keratitis	**1**	**0.5%**

Table 3: Anti fungal susceptibility profile of *Candida species*.(n=137)

Species	**Fluconazole**	**Voriconazole**	**Amphotericin-b**	**Caspofungin**	**Micafungin**
C.albicans (17)	100%	88.20%	94.10%	100%	100%
C.tropicalis (86)	79.90%	87.30%	96..2%	93.60%	89.90%
C.parapsilosis (8)	62.50%	87.50%	100%	100%	87.50%
C.cifferi (4)	-	75%	75%	-	-
C.pelliculosis (3)	66.70%	100%	100%	/	/
C.lusitanie (1)	100%	100%	100%	100%	100%
C.utilis (1)	100%	100%	100%	-	-
C.glabrata (1)	-	100%	100%	100%	100%

followed by mechanical ventilation (40.5%).

Most common presentation in our study was fever (65.3%) followed by shortness of breath (41.2%) and pain abdomen (25%). Fever without specific signs and symptoms was seen. Similarly Noorifard M et al[17] reported fever(100%) the most common clinical symptom.

In our study, most common fungal infection observed was *candidiasis* (61.4%) followed by aspergillosis (28.7%) and mucormycosis (6.2%). Similarly, Ahir HR et al[9] in 2018 reported *candidiasis* (97.4 %) as the most common fungal infection.

Among all *candida* isolates, majority of the isolates were non-albicans *candida* (NAC) (87.6%) followed by *Candida albicans* (12.4%). Among the 120 NAC, most common species obtained was *Candida tropicalis* (62.7%) followed by *Candida gulliermondi* (8.7%) and *Candida* parapsilosis (5.8%). These findings establish the great importance of NAC as pathogen in clinical samples. In contrast to our study, few studies reported *C. albicans* as the predominant pathogen (39.42%), followed by *C. parapsilosis* (34.02%).[18] The distribution of the species are different in various regions and studies. For management of patients, determination of changes in the distribution of *candida species* is important

Pulmonary aspergillosis (71.9%) was most common manifestation among all the cases of aspergillosis, followed by sino-nasal aspergillosis (18.8%), cutaneous aspergillosis (7.8%) and ocular aspergillosis (1.6%). Similarly in a study by Swu-Jane Lin et al,[19] 70% of the infections were pulmonary and 9% of patients had disseminated and/or CNS aspergillosis.

Aspergillus flavus is prevalent in tropical countries like India, Pakistan, Sudan and leading to majority of cases of aspergillosis. Also in our study, out of 64 cases, *Aspergillus flavus* (78.13%) was the most common species obtained followed by *Aspergillus* fumigatus (21.88%). Similarly, JishnuBTet al[20] observed A. flavus was the most predominant species and was identified in 46 (63.80%) cases of Aspergillosis. Unlike to this, Tashiro T et al[21] observed A. fumigatus (41%) and *A. niger* (32%), as the predominant species. In 1 respiratory sample 2 isolates were obtained, 1 was A. flavus and other was *Mucor*.

In the present study, suspicion of Invasive pulmonary Aspergillosis (IA) was categorized. Cases of IA were further classified as probable IA-32 and possible IA-45 and no case was as proven as per European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria. Whereas, a study categorize 81 patients as proven IA (14.8%), probable IA (48.2%), possible IA (27.2%), or no IA (8, 9.9%) as per EORTC/MSG criteria.[22] Out of total 13 cases of mucormycosis, most common infection was sino-nasal mucormycosis (61.5 %) followed by cutaneous mucormycosis(30.8%). *Mucorales* are present in air community and hospital settings. Most of the infections are nosocomial in origin. Cutaneous mucormycosis was seen after trauma, burns and nosocomial infection after surgery. Out of 13 cases, histopathology was done in 9 cases, which all were co-insiding with the culture reports.

Rhizopus arrhizus is the most common agent causing mucormycosis in India and globally. In the present study, among the 13 isolates of mucormycosis, most common species was Rhizopus (57.2%) followed by Mucor (42.8%). Parkash H et al[23] observed Rhizopus the most common cause of mucormycosis.

The antifungal susceptibility was studied for azoles (fluconazole, voriconazole) amphotericin-b and echinocandins (caspofungin and micafungin). In our study, in *C. albicans* 100% susceptibility was seen to Fluconazole and Echinocandins followed by 94.15% to amphotericin-b and least susceptibility was seen to voriconazole (88.2%). Senstivity to fluconazole and micafungin was statistically significant in *C. albicans* as compared to NAC.

In NAC isolates, maximum susceptibility was seen to Amphotericin-B (89.2%) followed by Voriconazole (82.5%). Hazrat Bilal et al[24] observed 100% sensitivity of C.glabrata to amphotericin-b. In candidemia, higher susceptibility of voriconazole (84.4%) and amphotericin-b (93.8%) observed, as compared to 78.4% and 81.1%in body fluids respectively. Whereas few studies showed decreased susceptibility to azole agents and resistance to amphotericin-b in candidemia patients.[25] Long term prophylaxis is associated with development of resistant to these drugs.

Outcome was observed in terms of discharge and death. Out of 216 patients, 94.1% patients discharged and 5.9% patients had fatal outcome. In a study conducted by TakVet al, very hi 68(43.31%) out of 157 patients had fatal outcome.[26] Some other studies also showed high mortality rate of 30% to 70%In our study, higher mortality (66.7%) was seen in elderly age group (>60 years) than younger age group(<60 years). Maximum patients who were discharged were of younger age group.[27]

5. Conclusions

Fungal infections are associated with significant morbidity and mortality. *Candidiasis* was the most common infection followed by aspergillosis. Candidemia is the major factor associated with fatal outcome with *C.tropicalis*, *C.gulliermondii* and *C. parapsilosis* being predominant pathogens. The growing rate of non-albicans *candida* resistance to azole confirms the monitoring of changes in distribution of pathogenic *candida species*. Until rapid susceptibility testing is available, empiric therapy should be used based on patients clinical condition, risk factors, site of infection and local anti fungal microbiological patterns. In many countries, very effective anti fungal agents are

available. But, in developing countries like ours these are very expensive and not available to all the patients. So, early diagnosis and continuous surveillance of fungal infections is important for better outcome of patients.

6. Conflicts of Interest

There was no conflicts of interest associated with this publication.

7. Source of Funding

None.

Acknowledgment

None.

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Cite this article: Singh M, Gupta V, Mahajan R, Chaudhary J, Aggarwal M. Clinico-mycological profile of fungal infections in a tertiary care hospital. *IP Indian J Immunol Respir Med* 2023;8(4):133-139.