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Research Article

A Study of Invasive Fungal Infections at A Tertiary Level Hospital: Prospective Study

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ABSTRACT

Background: Invasive fungal infections primarily affect immunocompromised hosts, leading to severe disease and elevated morbidity and mortality rates. Invasive fungal infections are predominantly caused by species of *Candida*, *Mucorales*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis*. The major risk factors predisposing individuals to invasive fungal infections include uncontrolled diabetes mellitus with resultant hyperglycemia, neutropenia, immunosuppressive therapies such as corticosteroid use, and primary or secondary immunodeficiency disorders, particularly acquired immunodeficiency syndrome (AIDS). Prompt diagnosis of invasive fungal infections is crucial, as immunosuppression markedly increases the risk of mortality; therefore, timely initiation of therapy based on antifungal susceptibility and established guidelines is essential.

Aim: to study the prevalence and etiology of invasive fungal infections.

OBJECTIVES

1. To study the etiological agents causing invasive fungal infections.
2. Identify the fungi up to the species level.
3. Correlate findings of direct microscopic examinations with culture.
4. To study the risk factors associated with invasive fungal diseases.
5. To study antifungal sensitivity in candida species by conventional and automated methods.

Method: Patients clinically suspected of having fungal infections based on signs and symptoms, and who did not respond to broad-spectrum antibiotics, were included in the study. Clinical assessment was performed, and samples were collected for the identification of fungal isolates. Antifungal susceptibility testing was carried out on the recovered isolates.

Results: This study notes the occurrence of disease with predominance of male patients (61%), with male to female ratio of 1.57:1. Fever was the commonest (75%) symptom followed by cough (40.25%).The overall prevalence of invasive fungal infections was 34.12% with candidiasis as the commonest (32.08%) followed by aspergillosis (1.36%) and cryptococcosis (0.68%). Candidemia (Candida bloodstream infections) was the commonest (54.25%) form of infection, mainly caused by *C. albicans.* The non albicans species isolated were *C. tropicalis, C. parapsilosis, C. auris, C. guillermondii, and C. duobushaemulonii.* The prevalence of cryptococcosis observed was (0.68%). No case of Histoplasma capsulatum was observed. In vitro antifungal susceptibility was performed by the Disc diffusion method according to CLSI guidelines (2018) and VITEK 2 Compact automated system.

Conclusion: Invasive fungal infections (IFIs) represent a major cause of increased morbidity and mortality in critically ill patients, Early clinical suspicion, prompt laboratory identification, and timely initiation of appropriate antifungal therapy are crucial for improving patient outcomes.

Keywords*: Invasive fungal infections, Aspergillosis, Candidiasis, Cryptococcosis, Immunosuppression, , Pneumocystosis Mucormycosis.*

INTRODUCTION

Invasive fungal infections (IFIs) represent a major cause of increased morbidity and mortality in critically ill patients.[1] IFDs are an emerging problem worldwide, are generally very difficult to cure and the associated mortality remains very high depending on the pathogen and patient population. Fungal species are approximately 7 percent (6, 11,000 species) and they are distributed in soil, plant debris, and other organic substrates[2], approximately 600 species are humanpathogens.[3]Invasive fungal infection prevalence rose from 2.2% (1987) to 5.1% in last 12-year period.[4]According to recent data, 3 million people worldwide are thought to be affected by chronic severe fungal infections, whereas approximately 1.9 million patients get acute invasive fungal infections (IFI) each year. An estimated 1.6 million fatalities per year are linked to all fungal illnesses, many of which are fatal infections.[5] Nearly 70% of all IFIs in the world are caused by invasive candidiasis (IC), followed by cryptococcosis (20%) and aspergillosis (10%).[6] The identification of candida species is important in the diagnostic laboratory .There is a prognostic and therapeutical significance, in the identification of candida species and thus early and correct antifungal therapy can be initiated.[7]Antifungal resistance is a serious issue in both time and space because fungi belonging to the species Candida, Aspergillus, Cryptococcus, and Pneumocystis have been exhibiting considerable rates of antifungal resistance worldwide.[8,9]Several new antifungals have expanded prophylaxis and treatment options for invasive fungal infections Overview of treatment options for invasive fungal infections.[10]

METHODS

Aims/Objectives:

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OBJECTIVES

1. To study the etiological agents causing invasive fungal infections.
2. Identify the fungi up to the species level.
3. Correlate findings of direct microscopic examinations with culture.
4. To study the risk factors associated with invasive fungal diseases.
5. To study antifungal sensitivity in candida species by conventional and automated methods.

This is a prospective descriptive study of total 293 patients admitted with signs and symtoms suggestive of invasive fungal infections(IFI) and satisfying the inclusion criteria were included in the study during a study period of 18 month in the department of Microbiology of an urban tertiary care teaching hospital in Westarn India.

Inclusion criteria-

Clinically suspected of fungal infections on the basis of signs and symptoms and non-responsive to broad spectrum antibiotics.

Exclusion criteria-

All the patients of cutaneous fungal infections.

Source of samples: Specimens collected were blood, body fluids, pleural fluid, cerebrospinal fluid, bronchoalveolar lavage, urine, pus, fine needle aspiration cytology, and surgical drain fluid.

Clinical assessment:

The detailed relevant clinical history was taken of each patient with regard to name, age, sex, clinical diagnosis, H/o antibiotic therapy, H/o clinical immune status and H/o clinical interventions.

Sample processing:

Samples were processed for microscopic examination, fungal cultures and antifungal sensitivity as follows:

Microscopic examination:

The following preparation was made:

1) Potassium hydroxide mount[11]

For all specimens besides CSF, 10% KOH preparations were made.

2) **Gram Stain[12]**

3) **India ink preparation 11**

This was done for demonstrating the capsule of Cryptococcus.

Culture

The samples were inoculated aseptically on 2 sets of Sabouraud's Dextrose Agar (SDA) and incubated at 25°C and 37 °C respectively till the growth was obtained or for a minimum of 1 month.

IDENTIFICATION OF ISOLATES:

A) Identification of yeast and yeast-like fungi –

When the growth was observed, the colony morphology was noted, a smear was made, and the gram was stained. The isolate was further processed for species identification based on microscopic & colony morphology.

a) Identification of candida

1) Germtube test[13]
2) CornmealTween80agar(Dalmauplatetechnique)[11]
3) SugarFermentation[11]
   CarbohydrateAssimilationTest[13]

b) Identification of cryptococcus

The isolates were identified as Cryptococcus based on the following.

1) Colony characteristics mucoid cream to buff colored colony which Changed to brown color on prolonged incubation.
2) Microscopic appearance of the suspected colony on Gram stain and India ink preparation.
3) Growth at 37°C

Microscopic morphology showing gram-positive, round yeast cells with single narrow-based budding denoted Cryptococcus

4) Hydrolysisofurea.[11]

In vitro antifungal susceptibility test by disc diffusion method

The strains of Candida species were subjected to susceptibility testing against Fluconazole, Caspofungin, and Voriconazole by disc diffusion test as per CLSI guidelines (2018).

Interpretation:

The zone of inhibition was measured and interpreted as follows.[15]

Antimicrobial Agents	Sensitive	Intermediate	Resistance
Fluconazole	>17	14-16	<13
Caspofungin	>17	15-16	<14
Voriconazole	>17	15-16	<14

A) Identification of molds(11)

This was done based on the following-

1) **Colony morphology** (16)-color, texture (granular, velvety, cottony, etc), pigment, the surface on obverse, and pigment on reverse was noted. 2) Lactophenol Cotton Blue (LPCB) Teasemount.

2) Lactophenol Cotton Blue(LPCB) Tease mount(74)

Slide culture [11]

RESULTS

A total of 293 patients admitted with signsandsymptoms suggestive ofinvasivefungalinfections (IFI)and satisfying the inclusion criteria wereincludedin thestudy. A total of 100 (34%) specimens from suspected cases yielded fungi out of 293.

Cases were analysed as follow:

Sex wise Distribution of cases:

Out of 293 patients, 179 (61%) were males and 114 (39%) were females. Males predominated in the current study, with a male-to-female ratio of 1.57:1.

Table 1: Frequency of clinical manifestations in study populations.

Sr. No	Clinical finding	Total
1	Fever	219 (75%)
2	Cough	117 (40.25)
3	Headache	86 (30.03%)
4	Altered sensorium	80 (27.30%)
5	Breathlessness	65 (22.18%)
6	Convulsions	59 (20.13%)
7	Weakness	88 (30.03%)
8	Loose motion	39 (13.31%)
9	Neck stiffness	21 (7.16%)
10	Others*	89 (30.37%)

- Difficulty in eating, mouth ulcer, vomiting, giddiness, pain in abdomen, loss of weight, haemoptysis.

The distribution of various specimens collected from suspected invasive fungal infections.

Blood	81
Bal	59
CSF	52
Tissue	31
Pus	24
Urine	20
TRS	20
Pleural fluid	06
Total	293

Microscopic examination showed presence of fungal elements in 94 samples including blood, BAL, Tissue, pus, Urine. Two CSF specimens showed presence of capsulated, budding yeast cells morphologically resembling Cryptococcus.

One specimen of BAL and pleural fluid showed the presence of fungal elements (septate hyphae with acute angle branching)

Two specimen of CSF showed capsulated yeast cells of *Cr. neoformans* in india ink preparation

Figure 1 : Specimen of BAL showing the presence of fungal elements (septate hyphae with acute angle branching.
Culture confirmation was seen in 100 samples

Figure 2: showing growth of Candida albicans on Sabouraud's Dextrose Agar

Figure 03: Showing growth of *Aspergillus flavus* onSabouraud's Dextrose Agar

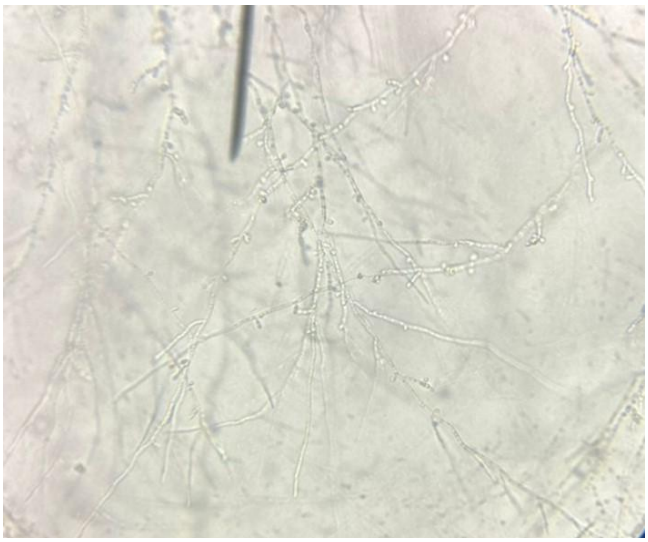

Figure 04: Showing morphology of *C.tropicalis* on Cornmeal Agar

Candidemia (Candida bloodstream infections) was the commonest form of infection observed. 51 Candia species were isolated from blood, 16 from BAL, 08 from urine, 09 from pus, 05 from tissue, and 05 from TRS.

Two isolates of *cryptococcus neoformans* were grown from CSF samples.

Three strains of Aspergillus were grown from BAL and one strain from pleural fluid. Out of three strains from BAL, Two were of *Aspergillus flavus* and One was of *Aspergillus glaucus.*

One strain of pleural fluid was of *Aspergillus flavus.*

Candida was the most frequently fungus (94%) in the present study.

In the present study the various Candida species isolated were *C. albicans, C. tropicalis, C. guillermondii and C.parapsilosis. C.auris*.*C.albicans was* the predominant isolate, *C. tropicalis* was the next common isolate followed by *C. parapsilosis, C.auris, C.guilliermondii* and *C.duobushaemulonii*

1. **Antifungal Susceptibility patternin Invasive Candidiasis**

Out of the 94 candida species, 68 species were tested for antifungal susceptibility by conventional Kirby Bauer disc diffusion method, whereas 26 were tested by VITEK 2Compact.

In vitro susceptibilities of the candida species to the antifungal agents studied by conventional Kirby Bauer Disc Diffusion

Table 2: *C. albicans n=27*

Antifungals	S	I/SDD	R
Fluconazole	26 (96.29%)	01 (3.70%)	00 (00%)
Voriconazole	24 (88.88%)	02 (7.40%)	01 (3.70%)
Caspofungin	23 (85.18%)	01 (3.70%)	03 (11.11%)

Table 03: *C. tropicalis n=23*

Antifungals	S	I/SDD	R
Fluconazole	17 (73.91%)	01 (4.34%)	05 (21.73%)
Voriconazole	16 (69.56%)	02 (8.69%)	05 (21.73%)
Caspofungin	21 (91.30%)	01 (4.34%)	01 (4.34%)

Table 04: *C. parapsilosis n=16*

Antifungals	S	I/SDD	R
Fluconazole	11 (68.75%)	02 (11.76%)	03 (17.64%)
Voriconazole	10 (62.50%)	03 (17.64%)	03 (17.64%)
Caspofungin	13 (81.25%)	02 (11.76%)	01 (05.88%)

Identifications andantifungals susceptibilitybyVITEK2Compactautomatedsystem

Table 06: *C. albicans n= 05*

Antifungals	S	I/SDD	R
Fluconazole	05 (100%)	00 (00%)	00 (00%)
Voriconazole	04 (80.00%)	00 (00%)	01 (20.00%)
Capsofungin	04 (80%)	00 (00%)	01 (20.00%)
Micafungin	04 (80%)	00 (00%)	01 (20.00%)
Amhotericin B	04 (80%)	00 (00%)	01 (20.00%)
Flucytosin	04 (80%)	00 (00%)	01 (20.00%)

Table 07: *C. tropicalis n=06*

Antifungals	S	I/SDD	R
Fluconazole	05 (83.33%)	01 (16.66%)	00 (00%)
Voriconazole	05 (83.33%)	00 (00%)	01 (16.66%)
Capsofungin	06 (100%)	00 (00%)	00 (00%)
Micafungin	06 (100%)	00 (00%)	00 (00%)
Amhotericin B	06 (100%)	00 (00%)	00 (00%)
Flucytosin	06 (100%))	00 (00%)	00 (00%)

Table 08: *C.parapsilosis =07*

Antifungals	S	I/SDD	R
Fluconazole	05 (71.42%)	01 (14.28%)	01 (14.28%)
Voriconazole	06 (85.71%)	00 (00%)	01 (14.28%)
Capsofungin	07 (100%)	00 (00%)	00 (00%)
Micafungin	07 (100%)	00 (00%)	00 (00%)
Amhotericin B	06 (85.71%)	00 (00%)	01 (14.28%)
Flucytosin	07 (100%)	00 (00%)	00 (00%)

Table 09: *C. auris n=05*

Antifungals	S	I/SDD	R
Fluconazole	02 (40%)	00 (00%)	03 (60%)
Voriconazole	02 (40%)	01 (20%)	02 (40%)
Capsofungin	04 (80%)	00 (00%)	01 (20%)
Micafungin	04 (80%)	00 (00%)	01 (20%)
Amhotericin B	02 (40%)	00 (00%)	03 (60%)
Flucytosin	02 (40%)	00 (00%)	03 (60%)

The increase in immunocompromised patients suffering from various diseases has led to an increase in the global burden of invasive fungal infections (IFIs).[17]

DISCUSSION

The present study was carried out in the department of microbiology attached to a tertiary care hospital. During this study, all the patients admitted with signs and symptoms suggestive of invasive fungal infections (IFI) and satisfying the inclusion criteria were screened and the respective samples were taken for the confirmation of diagnosis.

In the present study majority of the patients were in the age group of 41-60 with a male predominance and a male-to-female ratio of 1.54:1. YubhishaDabas etal reported a male predominance (66%)[18];Nicole Harrison et al also reported a male predominance (53.7%).[19]

In the present study majority of the patients were in the age group of 41-60. YubhishaDabasetal reported a male predominance (66%)[18];Nicole Harrison et al also reported a male predominance (53.7%).[19]

In the present study majority of the patients were in the age group of 41-60. Yaling Li et al reported the majority of patients were above the ageof 65 years (40%).[20]

In the present study, common predisposing factors were prolonged stay in intensive care (>7 days) (15.62%) followed by prolonged exposure to corticosteroids,antibiotics(13.54%), surgical intervention(12.5%), sepsis(12.5%), AIDS(10.41%), Diabetes mellitus(8.33%), Tuberculosis(7.29%), COPD(5.20%), malignancy(4.16%), LBW with prematurity(4.16%) Yaling Li et alreported the most common Predisposing factor prolonged hospitalization (96.1%), total parenteral nutrition (79.4%), and the presence of intravenous catheters (78.8%)[21]

In the present study, among the 100 fungal isolates, majorityof them were Candida non-albicans (62%) followed *by Candida albicans(32%), Aspergillus species* (4%), and *cryptococcus neoformans* (2%).

Chakrabarti et al showed invasive candidiasis as the most common mycotic infection across India.[22]

Kauffman et al also showed that the most common IFI were invasive candidiasis followed by Aspergillosis.[23]

In this study, the most common Candidaspecies isolates in blood samples were *Candida albicans* (31.37%) followed by *Candida tropicalis* (27.45%), *Candida parapsilosis* (25.49%), *Candida auris* (7.84%),*Candida duboshimulonii* (3.92%), *Candida guillermondii*(3.92%).

Peter G. Pappas et al reported *C. albicans (46%),* C. *glabrata* (20%), *C. parapsilosis* (14%), *C. tropicalis* (12%), *C. guilliermondii* (3%) *C. krusei* (2%) in blood samples.[24]

In this study the most common Candidaspecies isolates in BALwere*Candida albicans* (31.25%) followed by Candida tropicalis (37.5%), Candida parapsilosis (31.25%),

Sahar Kianipour et al reported *C. albicans*/*dubliniensis* complex (58.6%) and nonalbicans isolates (41.4%) as common isolates in BAL.[25]In this study the most common Candida species isolates in urine samples were *Candida albicans* (50%), *candida tropicalis* (25%) and *Candida parapsilosis* (25%).

Umamaheshwari S et al reported, C. tropicalis (46.2%), followed by C. albicans (19.58%), C. glabrata (16.06%), and C. parapsilosis (4.62%) in urine samples.[26]

In the present study, *candida albicans* showed 96.29% sensitivity to Fluconazole, 88.88% sensitivity to Voriconazole, and 85.18% sensitivity to Caspofunginby disc diffusion.*C. tropicalis* showed 73.91% sensitivity to Fluconazole, 69.56% sensitivity to Voriconazole, and 91.30% sensitivity to Caspofungin. Whereas C. *parapsilosis* showed 68.75% to Fluconazole, 62.50% sensitivity to Voriconazole and 81.25% sensitivity to Caspofungin.

Maria Noni et al reported[27]Among *C. albicans* isolates, fluconazole and voriconazole resistance was not detected. Regarding caspofungin, 97.7% of isolates were found to be susceptible, *Candida parapsilosis* showed 98.1% sensitivity to Caspofungin, 92.2% sensitivity to Fluconazole and 98.1% sensitivity to Voriconazole from tertiary Greek pediatric hospital

Ajitha Reddy Edula e al repored*C. albicans had* (97.91%) sensitivity to voriconazole, (95.83%) to fluconazole, *C. tropicalis* showed (94.11%) sensitivity to voriconazole, (82.35%) to fluconazole, *C. parapsilosis had* (87.5%) sensitivity to voriconazole, (75%) to fluconazole, *C. dublineneisis*had (100%) sensitivity to voriconazole, (100%) to fluconazole.[28]
In the present study, among the 100 fungal isolates, 4 (4%) were of Aspergillus species, 3 species of Aspergillus were grown from BAL and 1 from pleural fluid.

Out of 3 species from BAL, two were of *Aspergillus flavus* and one was of *Aspergillus glaucus.*

One isolate of pleural fluid was of *Aspergillus flavus.*

Brandon J Webb et al reported 8.9% of Aspergillus species.[29]

In our study among 100 fungal isolates 2 (2%) were of *Cryptococcus neoformans* from CSF samples.

Yaling Li et[30] alreported Cryptococcus neoformans (2.8%) from CSF sample.

In our study, there was a predominance of candida species isolation, followed by Aspergillus species and *cryptococcus neoformans.*

Although histoplasmosis is the most common opportunistic infection in endemic areas the disease is not frequently reported from India except for the north-eastern Indian states like West Bengal which is considered as endemic region for histoplasmosis.[31]

In the present study no evidence of histoplasmosis reported.

SUMMARY AND CONCLUSIONS

The study was conducted in the Department of Microbiology of a Government medical college attached to a tertiary care hospital with the aim to study the prevalence and etiology of invasive fungal infections in patients with signs and symptoms suggestive of invasive fungal infections.

A total of 293 patients presenting with signs and symptoms suggestive of invasive fungal infections were studied.
Various specimens were collected aseptically including blood, body fluids, pleural fluids, cerebrospinal fluid, Bronchoalveolar lavage, urine, pus, fine needle aspiration cytology and surgical drain fluid.

The specimens were subjected to microscopic examination by KOH, Gram, and India Ink preparations.
The samples were inoculated on Sabouraud's Dextrose Agar.
The yeast identification was made by colony morphology, microscopic morphology of the growth, germ tube test, Dalmau technique on cornmeal agar, Assimilation and fermentation tests, and hydrolysis of urea.

Molds were identified by Macroscopic and microscopic morphology of growth.
In vitro antifungal susceptibility was performed against Fluconazole, Voriconazole and Caspofungin by disc diffusion method according to CLSI guidelines (2018).

There was a predominance of male patients (61%), with male to female ratio of 1.57:1.
Fever was the commonest (75%) symptom followed by cough (40.25%).

The overall prevalence of invasive fungal infections was 34.12% with candidiasis as the commonest (32.08%) followed by aspergillosis (1.36%) and cryptococcosis (0.68%).
Candidemia (Candida bloodstream infections) was the commonest (54.25%) form of infection, mainly caused by *C. albicans.*

The non albicans species isolated were *C. tropicalis, C. parapsilosis, C. auris, C. guillermondii, and C. duobushaemulonii.*
The prevalence of cryptococcosis observed was (0.68%).
No case of Histoplasma capsulatum was observed.

In vitro antifungal susceptibility was performed by the Disc diffusion method according to CLSI guidelines (2018) and VITEK 2 Compact automated system.

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