Section: Microbiology

Original Research Article

FUNGAL CULTURE OUTCOME OF VARIOUS SPECIMENS: ONE YEAR RETROSPECTIVE STUDY IN RURAL TERTIARY CARE CENTRE

Anagha S. Vaidya[1], Vaibhav Rajhans[2], Anita Nair[3], Deepika Bhalerao[4], S. Roushani[5]

[1]Professor, Department of Microbiology, DR. B.V.P RMC, PIMS (DU) Loni, Maharashtra, India.
[2]Associate Professor, Department of Microbiology, DR. B.V.P RMC, PIMS (DU) Loni, Maharashtra, India.
[3]Assistant Professor, Department of Microbiology, DR. B.V.P RMC, PIMS (DU) Loni, Maharashtra, India.
[4] Professor, Department of Microbiology, DR. B.V.P RMC, PIMS (DU) Loni, Maharashtra, India.
[5] Professor & HOD, Department of Microbiology, DR. B.V.P RMC, PIMS (DU) Loni, Maharashtra India.

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Corresponding Author:
Dr. Tadvi Khan Saleha Kauser
Email: khansalehambbs@gmail.com

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Abstract

Background: To know the specimen wise distribution of various fungal isolates received in last one year 2. To know the fungal culture positivity in received specimens in last one year 3. To know commonest fungal isolate in received specimens in last one year. **Materials and Methods:** All the samples of patients received in microbiology department for fungal culture were processed for microscopic examination and culture. **Result:** A total of 143 specimens were included in the present study for fungal culture. Out of 143 samples 55 (38.46%) were culture positive. Non albicans candida spps. were predominant fungal isolate. Common isolates among moulds was mucor spps, followed by A.flavus. Cryptococcus laurentii was one of the rare fungal isolate from the present study. **Conclusion:** Non albicans-Candida spp. emerged as an important cause of infections. COVID-19 pandemic has resulted in widespread unprecedented magnitude of fungal infection in community. In COVID 19 or any immunocompromised conditions the incidence of rhino-orbito-cerebral Mucormycosis is likely to rise. Early diagnosis and management with appropriate antifungals and surgical debridement can improve survival. The clinician & microbiologist collaboration will help in improving patient care.

INTRODUCTION

Fungal isolates are now emerging as major pathogens. Reasons for this are the emergence of acquired immunodeficiency syndrome, misuse/overuse of antimicrobials, increasing the incidence of diabetes mellitus, organ transplantation and chemotherapy. These organisms are capable of affecting not only immunocompromised patients but also healthy immune-competent individuals. Some species of non-albicans Candida are more common and which was the predominant species earlier.[1,2] Candida and Aspergillus species are the most common causes of fungal infection but other yeasts and filamentous fungi are emerging as pathogens. Among the filamentous fungi, apart from Aspergillus spp, others like Fusarium spp., Scedosporium spp., Penicillium spp. and Zygomycetes are becoming increasingly common.[3,4] The incidence of infections due to non-albicans species is increasing particularly in patients treated in the intensive care unit.[5] Mucormycosis, a serious angioinvasive infection caused by common filamentous fungi, that is, mucormycetes, constitutes the third most common invasive fungal infection following aspergillosis and candidiasis.[2] The disease can be transmitted by direct inoculation of the spores into disrupted skin or mucosa or inhalation of spores. The etiologic agents can cause infections with high mortality in immunocompromised, mainly diabetic patients.[6] Mucorales includes several species involved in rhinocerebral, pulmonary, gastrointestinal, cutaneous and other less frequent infections in immunocompetent and immunocompromised individuals, and all are characterized by tendency to disseminate.[7-10] Risk factors for fungal infections include prolonged neutropenia and use of corticosteroids, hematological malignancies (leukemia, lymphoma, and multiple myeloma) aplastic anaemia, myelodysplastic syndromes, solid organ or hematopoietic stem cell transplantation, human immunodeficiency virus (HIV) infection, diabetic and metabolic acidosis, intravenous drug

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abuse, prematurity, and advanced age.[10,11] So the study was planned

1. To know the specimen wise distribution of various fungal isolates received in last one year.
2. To know the fungal culture positivity in received specimens in last one year
3. To know commonest fungal isolate in in received specimens in last one year.

MATERIALS AND METHODS

Laboratory Based Study

This is a Retrospective study of one year duration (January 2021- December 2021).

All specimens sent to the Department of Microbiology DR.B.V.P RMC, PIMS(DU) LONI for fungal culture during this period were included in the study. Nail clippings obtained after cleaning the area with 70% alcohol and collected in sterile petri dishes were sent to the laboratory. Sterile body fluids, tissues, pus, pus swabs, and urine were collected aseptically and transported in sterile containers or syringe. Sputum was collected and transported in wide mouth, clean, dry containers. Blood was collected directly in brain heart infusion broth bottles. Specimens were examined by preparing wet mounts or by potassium hydroxide preparation. India ink stain and Gram stain were used for examination of cerebrospinal fluid (CSF).

Direct microscopy: Small amount of sample was placed on a clean slide, a drop of 10% KOH for soft tissue and 40 % KOH for nail was added and a cover slip was placed on it. The wet mount kept aside in a wet chamber for 20 – 30 minutes.[1] Examination of the slide was performed microscopically using 10x and 40x objective lens. On KOH preparation, the samples which showed a characteristic yeast cell, septate / non-septate, acute angled /wide angled branching hyphae was considered as KOH positive for fungal elements and subjected to fungal culture.

Fungal Culture: Culture was done on two Sabouraud's dextrose agar (SDA) plates with & without cycloheximide and one plate was incubated at 37°C and second plate at room temperature 25°C. The culture plates were examined on alternate days for the first 2 weeks and then twice weekly for the next 2 weeks. Based on the morphological details, the rate of growth, colour, texture and pigmentation of obverse and reverse isolates were identified

LPCB Mount: Microscopy of the mould's growth was done using lactophenol cotton blue mount. Microscopy of the yeast growth were identified by Gram stain and germ tube test.

Slide culture preparation was done to see the sporulation.

Vitek 2 System: Yeast and yeast like growth further subjected for speciation.

RESULTS

143 clinical specimens were received in the department of microbiology in our hospital for fungal culture, between January 2020- December 2021. Out of 143 samples 55 were culture positive (38.46%).

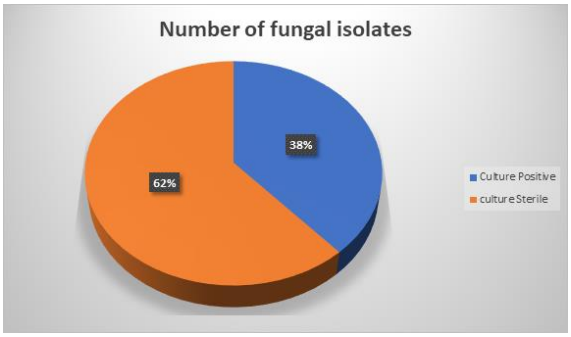

Figure 1: Fungal culture positivity in present study

The highest rate of isolation was from body fluids/pus/tissue (89.09%). This was followed by sputum (7.27%), urine (3.63%) [Table 1].

Table 1: Distribution of fungal culture isolates from various specimens

Type of Specimens (143)	*Tissue (84)	Sputum (5)	Pus (7)	Corneal scraping (19)	Urine (6)	CVP tip (1)	**Body fluids (12)	Skin/ Hair/Nails (1)	Conjunctival swab (4)	Throat swab (4)
No. of Fungal isolates (55)	42	4	3	2	2	1	1	0	0	0

*Tissue includes- maxillary sinus, deep from middle meatus, paranasal tissue, palatal tissue, sinus polyp, nasal specimen, mandibular tissue, corneal button.

** Body fluid includes anterior chamber tap, vitreous fluid, aqueous fluid, ascitic tap, endotracheal secretion.

Among the isolates, 31 (56.36%) were mould, 23 (41.81%) were yeast-like fungi, and 1 (1.81%) was yeast (Cryptococcus laurentii). Dimorphic fungi were not isolated in this study [Table 2].

Table 2: Number of fungal isolates

Type of fungal Isolates	Number (%)
Moulds	31 (56.36%)
Yeast like	23 (41.81%)
yeast	1 (1.81%)
Total	55

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Most of the fungal isolates were from tissue/body fluid/pus [Table 3]

Table 3: Spectrum of isolates among various Specimens

Sr. No.	Various Specimens	Candida	Mucor spps	A.flavus	Aspergillus fumigatus	Fusarium spps	A.niger	Penicillium spps	Cryptococcus laurentii
1	Maxillary sinus (53)	10	11	4	2	0	0	1	1
2	Corneal scraping (19)	0	0	0	0	2	0	0	0
3	Deep from middle meatus (9)	0	1	0	1	0	0	0	0
4	Nasal secretion (9)	0	3	0	0	0	1	0	0
5	Pus(7)	3	0	0	0	0	0	0	0
6	Urine (6)	2	0	0	0	0	0	0	0
7	Paranasal tissue (6)	0	3	0	1	0	0	0	0
8	Ant. Chamber tap (5)	0	0	0	0	0	0	0	0
9	Sputum (5)	4	0	0	0	0	0	0	0
10	Conjunctival Swab (4)	0	0	0	0	0	0	0	0
11	Throat swab (4)	0	0	0	0	0	0	0	0
12	Vitrous fluid (3)	0	0	0	0	0	0	0	0
13	Palatal perforation (3)	2	0	0	0	0	0	0	0
14	Aqueous fluid (2)	0	0	0	0	0	0	0	0
15	Sinous polyp (2)	0	0	0	0	0	0	0	0
16	Nail (1)	0	0	0	0	0	0	0	0
17	CVP tip (1)	1	0	0	0	0	0	0	0
18	Mandibular tissue (1)	0	0	0	0	0	0	0	0
19	Ascitic tap (1)	0	0	0	0	0	0	0	0
20	Corneal Button (1)	0	0	1	0	0	0	0	0
21	Endotracheal secretion (1)	1	0	0	0	0	0	0	0
Total	143	23	18	5	4	2	1	1	1

SR.NO. PARAMETERS GROUP 1 (n=35) GROUP 2 (n=35) GROUP 3 (n=35) GROUP 4 (n=35)

1. Urine retention and re-catheterization 3(8.57%) 1(2.85%) 00
2. Symptomatic UTI 1(2.85%) 4(11.42%) 8(22.85) 10(28.57)
3. Post-Operative positive urine culture 00 2(5.71%) 7(20%)- NOT INCLUDED IN MY STUDY

   Out of 55 specimens 23 were candida spps (41.81%), among 23 isolates of candida spps, Candida albicans were 3 (13.04%) and non-albicans Candida were 20 (89.95%) followed by Mucor spps 18 (32.71%), Aspergillus Flavus 5(9.90%), Aspergillus fumigatus 4(7.27%), Fusarium 2(3.63%), Aspergillus niger 1(1.81%), Penicillium spps 1(1.81%), Cryptococcus Laurentii 1(1.81%). [Figure No.2]

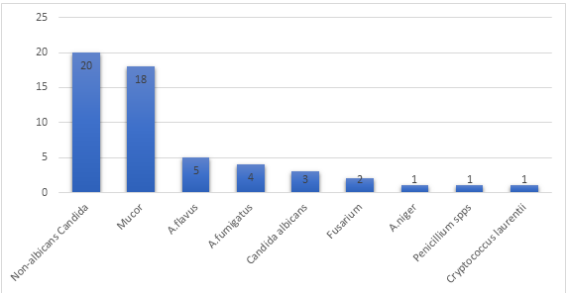

Figure 2: Various fungal species isolated in present study

Out of 20 Non-albicans candida 9 (45%) were C. parapsilosis followed by C.trpoicalis 8 (40%), C. glabrata 2(10%), C. krusei 1(5%). [Figure 3]

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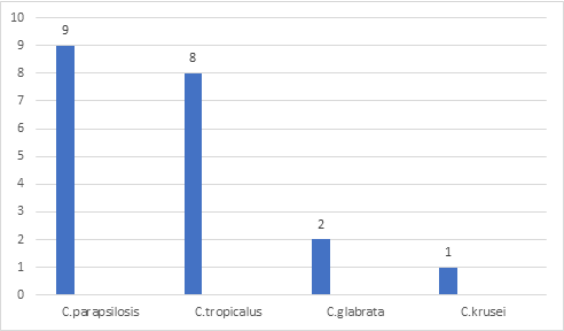

Figure 3: Non albicans candida species

DISCUSSION

In our study, we observed that isolates of non-albicans Candida had predominance over C.albicans similar to various other studies from different parts of the world.[12,13] Among the yeast-like isolates 23 most of the isolates of candida albicans 3(13.03%) and non-albicans Candida 20 (89.95%) were from tissue specimens. S.Bhama et al study shows 57.69% of non albicans isolates from tissue.[14] A study conducted in the United States reported non-albicans Candida as an emerging pathogen causing fungemia.[15] M.A Pfaller et al shows 46.73% of non albicans candida isolates. M.A Pfaller et al shows the predominance of C. parapsilosis 15.0% in non albicans candida.[16] Cryptococcus laurentii 1 (1.81%) was isolated from maxillary sinus. Among moulds the most common was mucor spps 18 (56.06%) Farah Yasmin et al shows Mucormycosis is an emerging problem in individuals with COVID-19 patients,[17] Chakrabarti et al. showed an increasing trend of Mucormycosis from a single centre at successive periods, with an annual incidence of 12.9 cases per year during 1990-1999 35 cases per year during 2000-2004,[18,19] and 50 cases per year during 2006– 2007.[20] The overall numbers increased from 25 cases per year (1990–2007) to 89 cases per year (2013–2015).[21] S.Bhama et al study shows Aspergillus flavus (20.98%) followed by A. niger (14.81%) and A. fumigatus (12.34%).[14] Fusarium spps 1(3.22%), penicillium spps 1(3.22%). In our study increased mucor isolates were associated with COVID-19 infection. Due to steroid treatment for COVID infection, showed in many studies. The isolates of A. flavus, A. niger and A. fumigatus isolates in this study were obtained from body fluids/pus/tissue. A. flavus has been reported to be the most common fungal isolate in studies on FRS from India.[14,22-25]

CONCLUSION

Till now non albicans-Candida has been considered non-pathogenic, however trends are changing with time. In our study, Non Albicans Candida spp. were common isolate associated with various clinical types of candidiasis. Therefore, it can be concluded that non albicans-Candida spp. emerged as an important cause of infections. Its isolation from clinical specimens can no longer be ignored as non-pathogenic isolate nor can it be dismissed as a contaminant. Pandemic COVID-19 has resulted in widespread mortality, morbidity and economic upheavals of an unprecedented magnitude. The incidence of Mucormycosis is likely to rise, both as a co-infection and as a sequela of COVID-19. Early diagnosis of fungal infections and management with appropriate and aggressive antifungals and surgical debridement can improve survival. The microbiologist & clinician collaboration will help in improving patient care.

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