Spectrum of Fungal Isolates of Clinical Specimens from a Tertiary Care Hospital

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***Abstract*: Background In the past few years, there has been an increase in infections caused by fungii which may be due to increased size of the population which is at risk, which includes transplant recipients, cancer patients, those who are on immunosuppressives, those who undergo prolonged hospitalization etc. Moreover, which were previously considered as non-pathogenic have been increasingly implicated. Hence, this study was taken up to study the profile of mycological infections.**

Methods: A total of 2060 clinical specimens were studied. Case history of patients was recorded. All samples were studied by direct microscopy and culture as per standard microbiological procedures.

Results: In the study, growth was observed in 620 specimens (30.09%). Maximum number 272 (43.87%) of isolates were obtained feom BAL specimens followed by sputum 189(30.48%).Candida albicans was isolated from 225(36.29%) cases, while Candida non albicans were 165 (26.61%) . Aspergillus fumigatus was the most common species 97(15.64%). Among dermatophytes, T. rubrum was the commonest etiological agent followed by T. mentagrophytes .

Conclusion: Culture and identification of mycotic infections is essential for suitable antifungal treatment.The role of diagnostic mycology laboratory is important in the identification of these infections which help in management of fungal infections.

Keywords: Yeasts, Candida, Aspergillus, Dermatophytes

Introduction

Fungal infections are worldwide in distribution of which superficial infections are the most common human infections.[1] Invasive fungal infections are a significant health problem in immunocompromised patients. The incidence of opportunistic fungal infections have been increased in immunodeficient individuals and hospitalized patients.[2] Early initiation of antifungal therapy is critical in deducing the high mortality rate in these patients. Rapid diagnosis of systemic fungal infections remains limited and culture detection of fungal isolates is often delayed due to slow or absent growth of fungal isolates from clinical samples. [3]

A correct diagnosis is important to initiate appropriate treatment and essential for epidemiological purposes. In the background of immunosuppression, detection of these agents becomes mandatory for the effective management of mycoses to prevent further invasions.[4]

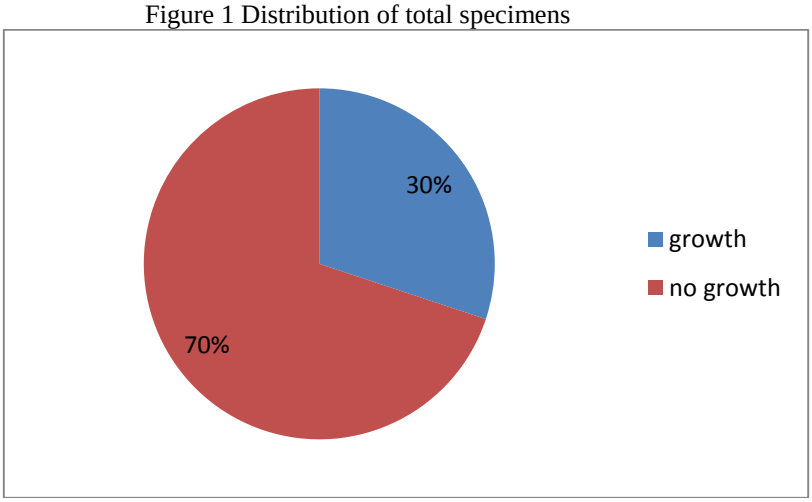
Use of broad spectrum antibiotics in last few decades for controlling bacterial infections has led to immunosuppression and introduction of fungal infections.Hence, it is important to know the spectrum of fungal infections with improved diagnostic methods and proper routine laboratory practices. So, this study was carried out to find out the spectrum of fungal pathogens at our tertiary care hospital.

Material and methods

The prospective study was conducted in the Department of Microbiology of a tertiary care hospital for a period of 1 year (January 2017 to June 2018). Various clinical specimens like BAL, sputum, Skin/hair/nail, Urine, Pus/ Swab, Blood, Corneal scrapping received at Mycology laboratory were included in study. We have confirmed fungal etiology in all samples by repeated isolation except in samples like BAL and Corneal scraping A total of 2060 clinical specimens were studied. Case history of patients was recorded. All samples were studied by direct microscopy and culture as per standard microbiological procedures as follows:[5,6,7]

Samples were received from patients of all age groups presenting with clinically suspected fungal infections. Samples were analysed by direct microscopy [KOH and Gram stain] and culture. Direct microscopic examination was done to visualize the presence of fungal elements or any budding yeast cells was done using wet mount preparation and 40% KOH for nail clipping and 10% KOH for rest other samples (corneal scraping, skin scraping, hair, pus etc). Gram staining was done to look for gram positive yeast cells. Culture of all samples were done by inoculating in duplicate on Sabouraud's dextrose agar (SDA) with antibiotics (chloramphenicol and cycloheximide) and without antibiotics. Culture tubes were incubated at 25°C and 37°C and examined for six weeks for the growth of any fungus. Identification of fungi was done by macroscopic examination of fungal growth. Lactophenol cotton blue mount was made to observe characteristics such as conidium types and hyphae. The yeast isolates were identified by gram stain and germ tube test. Chrome agar and cornmeal agar were used for identification of Candida species.

Figure 1 Distribution of total specimens



Results

Table 1. Age and gender wise distribution of the samples

Age range	Male	Female	Total
<15	38	19	57
15-30	44	10	54
31-45	66	25	91
46-60	123	107	230
>60	100	88	188
Total	392	228	620

Table 2. Spectrum of isolates among various samples

Organism	BAL	sputum	Skin/hair/nail	Urine	Pus/ Swab	Blood	Corneal scrapping	Tissue biopsy	Fluids	Total(%)
Yeasts										
C. albicans	112	27	37	28	10	10		1		225
C.tropicalis	31	13	3			5				52
C. glabrata	12	27	1							40
C. parapsilosis	15	23								38
C.krusei	12	19				4				35
Moulds										
A. fumigatus	53	24	9		4		3		2	96
A. flavus	22	15	5		3		3	1		49
A. niger	5	22	6							33
A. nidulans	6	10	1					1		17

A.versicolor	3									3
Dermatophytes										
Trichophyton rubrum			7							7
Trichophyton mentagrophtye			2							2
Zygomycetes										
Rhizopus species		5			2		1			8
Mucor species					3					3
Others										
Fusarium species		2					4			6
Penicillium species	1									1
Curvularia species							2			2
Exophiala wernickii								1		1
Cladosporium species		2								2
Total	272	189	71	28	22	19	13	4	2	620

Discussion

Fungal infections has been increasing since the past two decades. Fungal infections are often insidious and their diagnosis is often delayed due to coexisting illnesses. The emergence of these infections has created a challenge in their diagnosis and management.[3] So, this study was carried out to find out the spectrum of fungal pathogens at our tertiary care hospital.

A total of 2060 clinical specimens were received during the study period of one and a half year. Out of these, fungal growth was observed in 620 specimens (30.09%). (Figure 1) Narayan et al[8] found 690 fungal isolates in their study.

In the present study, maximum number of isolates (37.09%) were observed in age group of 46-60 years followed by age group of > 60 years 188(30.32%). The reason might be due to the more outdoor activities, and greater physical exertion in this group and comparatively low immunity in this age group. In the study of Narayan et al [8], majority of the patients belonged to the adult, of age group of 21-50 years. Male preponderance was also seen in the present study with male to female ratio of 1.71. Narayan et al [8] found predominance of males over females [M:F=1.56]. Other Indian studies that showed male preponderance were done by Nawal et al. [9], Surendran et al.[10] who also had similar observation. (Table 1)

From BAL, maximum number 272(43.87%) of isolates were obtained followed by sputum 189(30.48%). In the study by Narayan et al, Majority of the samples received were sputum samples 409 (58.09%). Most of the patients showed underlying lung conditions and were immunocompromised patients and this might be the reason from maximum positivity from BAL specimen. Hence, proper history and clinical correlation are important tools that guide towards proper diagnosis. (Table 2)

Candidal infections have steadily increased. Candidia spp. has been reported as the fourth leading cause and most common among fungal pathogens in critical care settings. [11] In the present study, *Candida albicans* was isolated from 225(36.29%) cases, while *Candida non albicans* were 165 (26.61%) i.e *C.tropicalis* (8.38%), *C. glabrata* (6.45%), *C. parapsilosis* (6.12%) and *C.krusei* (5.64%) was isolated from 40(39.22%) cases. This also correlates with the study of Nageshwari et al who also isolated majority of *Candida albicans* (51.4%) and non albicans Candida was isolated from 68(20.23%) cases. [11] Maximum Candida isolates were obtained from BAL specimen followed by skin and urine specimens .Nageshwari et al [11] obtained maximum isolates from sputum whereas Naryan et al [8] obtained maximum Candida isolates from skin specimens.

Among the *Aspergillus species*, *A. fumigatus* was the most common species 97(15.64%). Narayan et al [8] found *A. fumigatus* from 20 (7.87%) cases These were more commonly obtained from BAL specimens followed by sputum specimens. These patients were reported to have chronic lung diseases.

In the present study, 13 corneal scrapings were found to be culture positive. These were also positive in direct microscopy. In the present study, *Fusarium species* was the most common isolates with four isolates of *Fusarium species* . While processing corneal scrapings, the importance of direct microscopy as a useful diagnostic tool needs to be highlighted. Narayan et al [8] found A. fumigatus *species* to be the commonest isolate in keratomycosis cases.

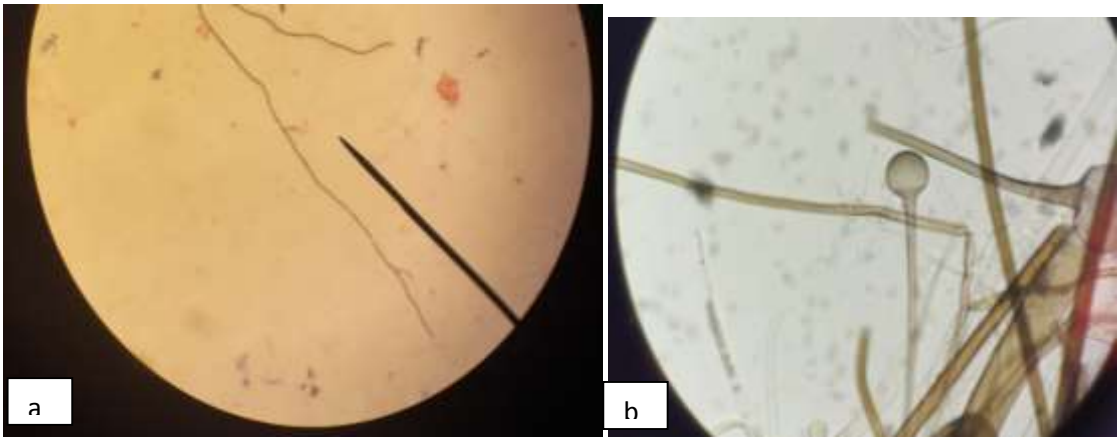
Dermatophytoses are superficial infections of keratinised tissue, the skin, hair and nails, caused by dermatophytes. The prevalence of dermatophytosis is determined by environmental conditions, personal hygiene and individuals‟ susceptibility. The variation in clinical presentation is related to the species of the fungus, size of the inoculum, the involved sites, and the immune status of the host. [12] Among dermatophytes, *T. rubrum* was the commonest etiological agent (7 cases) followed by *T. mentagrophytes* (2 cases) . *Trichophyton rubrum* was isolated from 7 samples and *T mentagrophtye* was isolated from 2 samples. This finding is comparable with the study of Anup Kainthola & Puneet Gaur et al who observed *T. rubrum* from 9 cases and *Trichophyton mentagrophtye* from 3 cases out of total 20 cases.[13]

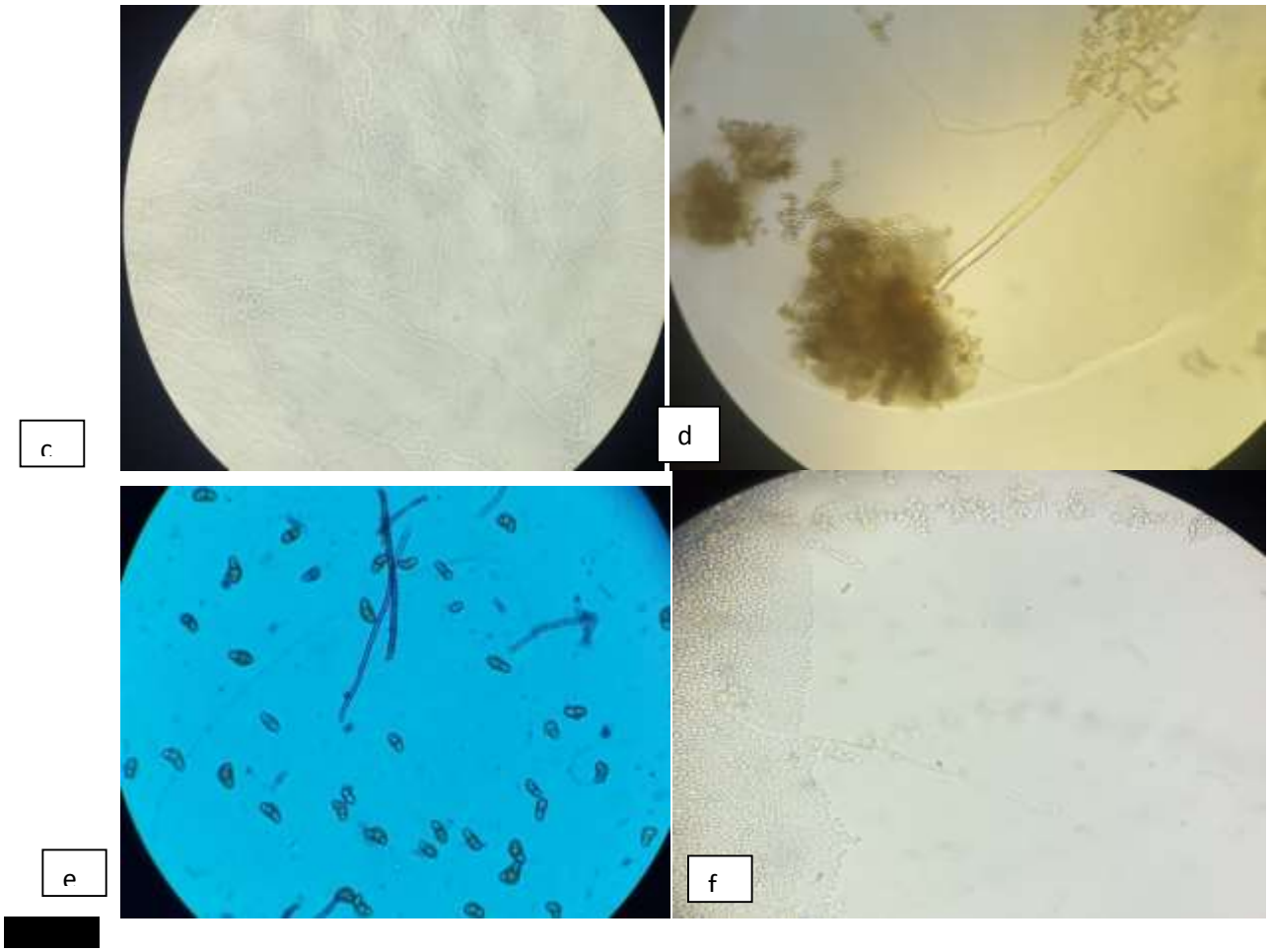
In the present study, one isolate of *Penicillium species* was isolated from BAL specimen.
Other fungi isolated in the study were *Rhizopus species*, *Curvularia species* , *Exophiala wernickii*, *Cladosporium species*. All these were isolated from patients having underlying lung conditions,

This study depicts various isolates obtained from the various specimens to know the spectrum of fungal infections. This would help clinicians to know the pattern of fungal infections and can help in management of the cases.

Conclusion

Fungal infections are increasing globally making early diagnosis necessary. An understanding of fungal infections in each set-up will help greatly in improving diagnostic and therapeutic approaches. Resistance to antifungal agents is alarming. So culture and identification of fungal infections to the genus and species level is essential for commencement of suitable antifungal therapy. The clinician-microbiologist collaboration will help in improving patient care.





a)Gram stain showing hyphae b) slide culture on SDA showing Mucor c) slide culture on SDA showing T.mentagrphyte d) slide culture on SDA showing A.flavus e) slide culture on SDA showing Curvularia f) Cornmeal agar showing chlamydospore of Candida albicans

REFERENCES:

1. BrownGD, DenningDW, GowNAR., LevitzSM, NeteaMG, WhiteTC, Hidden killers: Human fungal infections. Sci. Transl. Med. (2012)4,165rv13.
2. Nucci M, Marr KA.Emerging fungal diseases. Clin Inf Dis 2005;41(4):521-526
3. Kashyap B, Das S, Kaur IR, Jhamb R, Jain S, Singal A, Gupta N, Fungal profile of clinical specimens from a tertiary care Hospital, Asian Pacific Journal of Tropical Biomedicine, 2012;1-5.
4. Kannan P, Janaki C, Selvi G S. Prevalence of dermatophytes and other fungal agents isolated from clinical samples. Indian J Med Microbiol 2006; 24: 212-215.
5. Collee JG, FraserAG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology 14thed. Churchill Livingstone Publication, New York.1996;695-717
6. Chander J. 2017. Text Book ofMedical Mycology. 4th Edn. Mehta Publishers, New Delhi, India.
7. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. Koneman's Colour Atlas and Textbook of Diagnostic Microbiology. 7th ed. Lippincott Williams and Wilkin's, Philadelphia. 2016;1156-246.
8. Narayan S, Kumudini TS, Mariraj j, Krishna S.The Prevalence of Keratomycosis, Dermatophytosis and Onychomycosis in a tertiary care hospital. Int J Med Health Sci 2012;1(3):25-30.
9. Nawal P, Patel S, Patel M, Soni S, Khandelwal N. A Study of Superficial Mycosis in Tertiary Care Hospital.NJIRM 2012; 3(1): 95-99.
10. Surendran KAK, Bhat RM, Boloor R, Nandakishore B and Sukumar D. A Clinical and Mycological Study of Dermatophytic Infections. Indian J Dermatol. 2014 May-June; 59(3):262–267.
11. Gandham NR, Jadhav SV, Sardar M, Vyawahare C, Misra RR. The spectrum and aetiology of mycotic infections from a tertiary care hospital from western part of India. Journal of clinical and diagnostic research: JCDR. 2013 Oct;7(10):2157.
12. Bhavsar Hitendra K, Modi Dhara J, Sood Nidhi K, Shah Hetal S, A Study of Superficial Mycoses With Clinical Mycological Profile In Tertiary Care Hospital in Ahmedabad, Gujarat; National Journal Of Medical Research.
13. Anup Kainthola, Puneet Gaur, Alkesh Dobhal and Shailendra Sundriya; Prevalence of Dermatophytoses in Rural Population of Garhwal Himalayan Region, Uttarakhand, International Research Journal of Medical Sciences _ ISSN 2320 –7353 Vol. 2(8), 9-12, August (2014)