Research Article

INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230-8407 [LINKING]

Characterization and speciation of fungal isolates of different clinical samples from a teritiary care centre, Hyderabad.

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DOI: http://doi.org/10.56802/irjp.2025.v16.i09.pp92-103

ABSTRACT

Fungal infections are major health concern, especially in critically ill and immunocompromised patients. This study was conducted to understand the pattern and seasonal trends of fungal infections at tertiary care Hospital, Telangana, over a period of one year from January to

December 2023. A total of 1032 clinical samples were collected from OPD and IPD patients. All samples were examined using KOH microscopy, followed by fungal culture on SDA and other differential media.

Results: Out of 1032 samples, 152 (14.72%) were positive on KOH microscopy, and 118 (77.63%) were culture positive. Fungal infections were more common in males (n=72) than females (n=46), with highest number of cases in 21–40-year age group. Most infections occurred during winter, November to February. Skin samples had highest positivity (22.88%), followed by nail (19.49%), nasal cavity (13.56%), sinus (12.71%), biopsy (11.02%), polypoidal mass (5.93%), lung tissue (3.39%), hair and ear canal (2.54% each), BAL and sputum (1.69% each), and FESS, oral swabs, and tonsils (0.85% each). A total of 25 different fungal species were isolated. The most common were *Aspergillus flavus* and *A. niger* (11.86% each), followed by *A. fumigatus* (10.17%). Among dermatophytes, *Trichophyton rubrum* (9.32%) was most common, followed by *T. tonsurans*, *T. mentagrophytes*, and *Microsporum gypseum*. Other fungi included *Candida albicans*, *Candida tropicalis*, *Penicillium*, *Fusarium*, *Mucor*, *Malassezia*, and others in smaller numbers.

Conclusion: This study highlights the wide range and seasonal pattern of fungal infections. Early detection through microscopy and culture helps in starting appropriate antifungal treatment and reducing unnecessary antibiotic use.

Keywords: ***fungal speciation, KOH, culture, samples, seasonal variation.***

INTRODUCTION

Fungal infections are emerging as an important cause of morbidity and mortality especially in critically ill patients. Indiscriminate and inappropriately used broad spectrum antimicrobial agents as well as use of immunosuppressive drugs in various diseases has contributed to the increased propensity for fungal infections caused by both yeasts and moulds.[1] Fungal infections of human beings originate mostly from exogenous sources and are acquired mainly by inhalation and traumatic implantation. Some infections particularly of Candida are mostly derived from

endogenous sources. Last few decades have witnessed an alarming rise in the incidence of fungal infections particularly in cases of AIDS, malignancy, transplant recipients, patients in ICU, and patients on steroid therapy. Indiscriminate use of antibiotics also has led to the suppression of the local micro flora, which has helped in the growing incidence of fungal infections.[10,11,12] Epidemiologic studies have identified intravenous catheters, broad spectrum antibiotics, mucosal colonization, neutropenia, previous surgical procedures, total parenteral nutrition, extremes of age, and concomitant bacteraemia as significant risk factors for fungal infections. [5-7] In past fungi were considered to be merely non pathogenic agents or simply lab contaminants but are now proved to be significant pathogens and are encountered as emerging agents of significant fungal diseases e.g. apophysomyceselegans and Saksenaeavasiformis which may lead to fatal consequences even in immunocompetent individuals.[4] fungal profile reports are available from different parts of country. The local patterns of fungal isolates from clinical specimen may change with time and geographical area. One needs to be familiar with recent local trends in order to improve diagnosis. The present study was undertaken with a view to find local patterns of fungal isolates in Telangana, southern India at our tertiary care hospital between period of January 2023-December 2023

AIM & OBJECTIVE

- To assess fungal pathogenic spectrum from various clinical samples.
- To assess current trend of fungal profile related to our geographical area

MATERIALS AND METHODS

A prospective study was done on various clinical samples collected from OPD and IPD patients consulting Osmania General hospital, Telangana,India.

All clinical specimens were collected under appropriate clinical guidelines and proper criteria were maintained during the transportation of specimen.

All samples were analyzed by direct microscopy and culture as per standard microbiological procedures. For direct microscopy, 10-40% potassium hydroxide (KOH) stain was used to visualize presence of any fungal element. For yeasts, Gram's staining was done. For fungal culture, all samples were inoculated on two isolation media: one in Sabouraud's dextrose agar (SDA) and the other in SDA with chloramphenicol in duplicate. The culture tubes were incubated at 25 ºC and 37 ºC for four weeks.

The identification of fungi was done by macroscopic and microscopic evaluation of the fungal morphology. The fungus were identified by observing texture, colour, growth rates, mycelium and conidium types, Micro culture on slides technique was used for observation of filamentous fungi by Lacto-phenol cotton blue (LPCB) mount.The yeast isolates were identified by standard tests like Gram stain, periodic acid-Schiff(PAS),Germtube test, Dalmau method and urease production. Differential media such as Czapeck's media(CZA), Malt extract agar(MEA), Potato dextrose agar(PDA), Corn meal agar(CMA) was also used for isolation and further speciation of fungi.

RESULTS

A total of 1032 suspected fungal samples were received which included skin, hair, nail, ear canal, tissue, biopsy, BAL, FESS, sinus, nasal cavity, polyp, swab and tonsil.

Out of 1032 samples received 152 (14.72%) were positive for fungal elements in KOH. These positive samples were cultured on various differential media and the species was identified.

Among the 152 KOH positive samples, 118 (77.63%) were positive on culture. Males(n=72) had higher incidence of fungal infections than females (n=46). **Figure-I**

Highest incidence was seen in 21-40 age group both in males and females as shown in **figure- II**

According to the seasonal distribution, more cases were seen in November to February i.e., in winter season. **Figure III**

FIGURE I

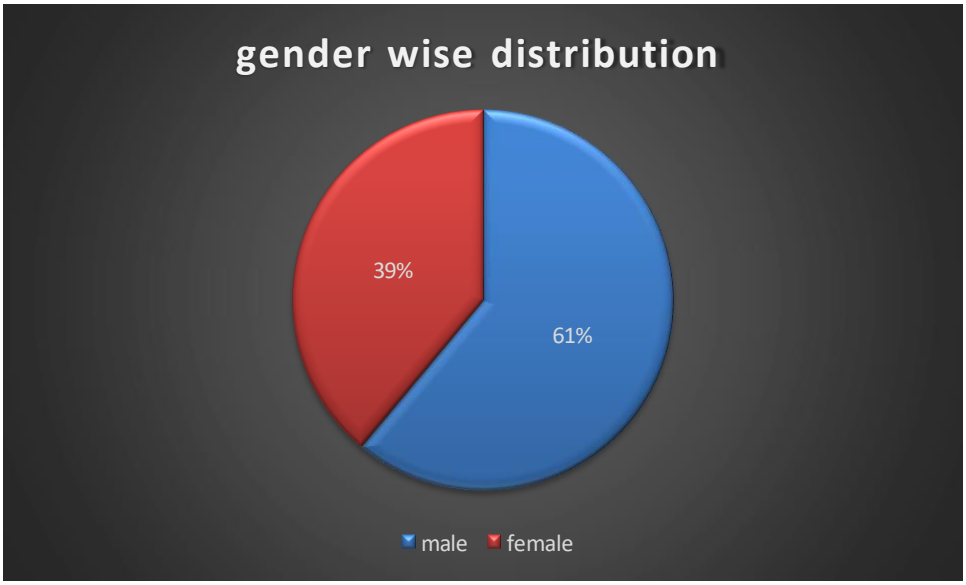


FIGURE II

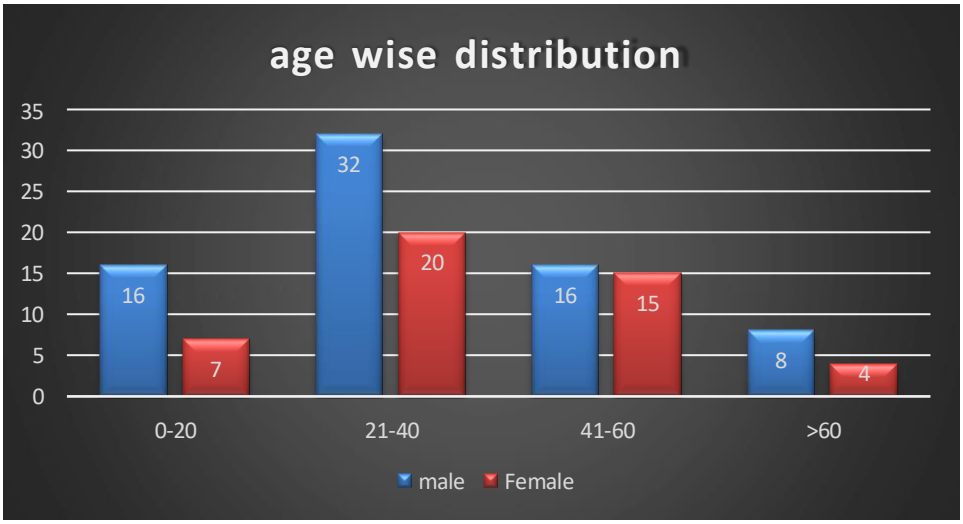
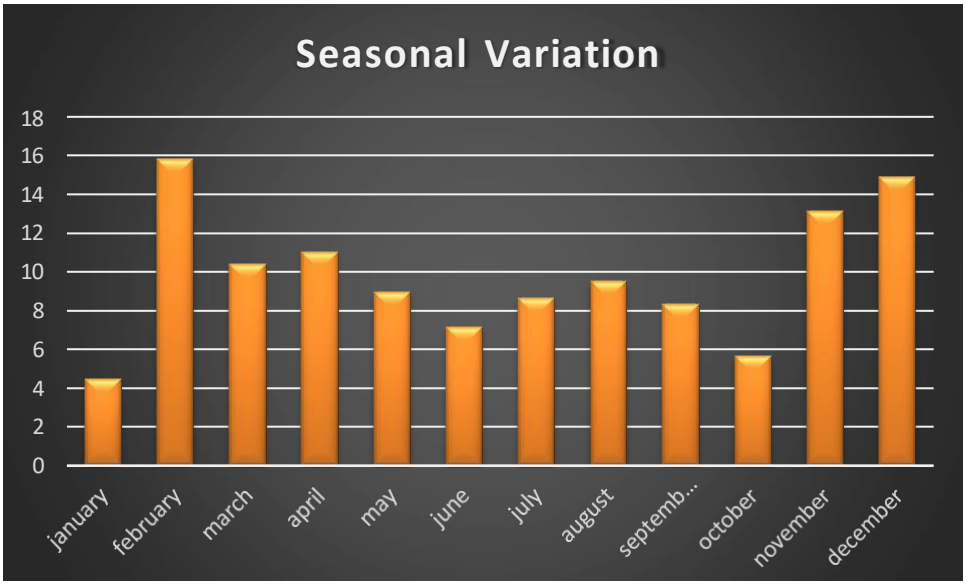


FIGURE III



A total of 25 different fungal species were isolated from various clinical samples as shown in

table-I

TABLE: I (a)

	Bal	Biopsy	Nasal	FESS	Hair	Lung Tissue	Nail	ear canal	polypoidal mass
Aspergillus flavus		4	2	1				1	1
Aspergillus fumigatus		3	1			2			2
Aspergillus nidulans	1								
Aspergillus niger		2	8						2
Candida albicans		1					1		
Candida pseudotropicalis		1						1	
Candida tropicalis			1					1	
Cladosporium									
Curvularia									
Fusarium			1				2		
Malassezia furfur					2				
Microsporum canis							2		

Microsporum gypseum							3		
Mucor	1		1			1			
Penicillium			1			1			2
Rhizopus			1						
Tinea rubrum		1							
Tinea versicolor									
Trichophyton mentogrophytus							2		
Trichophyton rubrum		1					9		
Trichophyton tonsurans					1		4		
Trichophyton verucossum									
Trichophyton violaceum									
TOTAL	2	13	16	1	3	4	23	3	7

TABLE: I (b)

	Sinus	Skin	Sputum	Swab oral cavity	tonsils	TOTAL
Aspergillus flavus	5					14
Aspergillus fumigatus	4					12
Aspergillus nidulans	1					2
Aspergillus niger	2					14
Candida albicans		1		1	1	5
Candida pseudotropicalis			1			3
Candida tropicalis	1		1			4
Cladosporium	1					1
Curvularia	1					1
Fusarium						3
Malassezia furfur						2

Microsporum canis						2
Microsporum gypseum		4				7
Mucor						3
Penicillium						4
Rhizopus						1
Tinea rubrum						1
Tinea versicolor		4				4
Trichophyton mentogrophytus		6				8
Trichophyton rubrum		1				11
Trichophyton tonsurans		5				10
Trichophyton verucossum		3				3
Trichophyton violaceum		3				3
TOTAL	15	27	2	1	1	118

Highest positive samples were collected from skin (22.88%), followed by nail (19.49%), nasal cavity (13.56%), sinus (12.71%), biopsy (11.02%), polypoidal mass (5.93%), lung tissue (3.39%), hair and ear canal (2.54%). BAL and sputum showed positivity of 1.69% each. FESS, swab oral cavity and tonsils showed positivity of 0.85% each.

Among skin samples dermatophytes were isolated in most numbers i.e; trichopyton mentagrophytes (n=6), followed by trichophyton tonsurans (n=5), microsporum gypseum and tinea versicolor (n=4) each. Next most isolated fungi in skin samples were trichophyton verucossum and trichophyton violaceum(n=3) each. trichophyton rubrum and candida albicans were isolated in least amount from skin samples i.e; n=1.

Among nail samples the most isolated fungi was a dermatophyte, trichophyton rubrum (n=9), followed by trichohyton tonsurans (n=4), microsporun gypseum (n=3). Next organism isolated in

highest number is trichophyton mentagrophytes and microsporum canis (n=3) each. Fusarium (n=2) and Candida albicans (n=1) were isolated in least amounts.

Nasal cavity showed growth of A.niger in highest number i.e; n=8 followed by A. flavus (n=2) followed by A. fumigatus, candida tropicalis, fusarium, mucor, penicillium and Rhizopus (n=1) each.

Sinuses showed growth of A. flavus(n=5), A. fumigatus(n=4), A.niger (n=2) followed by A. nidulans, candida tropicalis, Cladosporium, curvularis (n=1) each.

Biopsy showed growth of A. flavus(n=4), A.fumigatus (n=3), A. niger(n=2) followed by candia albicans, C. pseudotropicalis, tinea rubrum and trichophyton rubrum (n=1) each.

Most isolated organism from polyps were A. fumigatus, A. niger and penicillium (n=2) followed A. flavus (n=1)

Lung tissue showed growth of A. fumigatus (n=2) followed by Mucor and penicillim (n=1) each

Hair sample showed growth of Malassezia furfur (n=2) followed by trichophyton tonsurans (n=1). Ear canal had growth of A. flavus, candida tropicalis and candida pseudotropicalis (n=1) each. BAL showed growth of A. nidulans and mucor (n=1) each.

Candida tropicalis and candida pseudotropicalis were isolated from sputum (n=1) each. FESS showed growth of A. flavus (n=1), oral cavity and tonsils specimens had candida albicans (n=1)

DISCUSSION

Fungal infections, also known as mycoses, can affect various parts of the body and can range from mild to severe.Our study showed that out of 1032 samples received 152 (14.72%) were positive for fungal elements in KOH this is in consistence with the study done by HardikGuriaya.et.al [12]. Males had higher incidence than females and age group 21-40 yrs, this

is in consistence with madhu Chauhan.et.al [15] and R.Jayaprada, M.nagaraja, G.L.SSamanth et.al [13]. Highest positivity was seen in skin samples 22.8% and dermatophytes were more commonly isolated. This is in consistence with the study of madhu Chauhan.et.al [15].

In our study on fungal specimens collected for analysis, a diverse array of species was identified. The most prevalent fungi included Aspergillus flavus and Aspergillus niger, each comprising 11.86% of the total specimens, followed closely by Aspergillus fumigatus at 10.17%. this is in consistence with study of R.Jayaprada, M.nagaraja, G.L.SSamanth et.al [13].Among dermatophytes, Trichophyton rubrum led with 9.32%, followed by Trichophyton tonsurans (8.47%) and Trichophyton mentogrophytus (6.78%). Other notable findings included Microsporum gypseum (5.93%), Candida albicans (4.24%), and several species each representing around 3% of the samples: Candida tropicalis, Penicillium, and Tinea versicolor. This is in consistence with the study of madhu Chauhan.et.al [15]. Less frequently encountered fungi included Candida pseudotropicalis, Fusarium, Mucor, and various Trichophyton and Aspergillus species, each accounting for 2.54% or less of the total. Rarer findings, such as Malassezia furfur, Microsporum canis, and others like Cladosporium, Curvularia, Rhizopus, and Tinea rubrum, each constituted less than 1% of the specimens. This distribution highlights the diversity of fungal species present in the study sample, reflecting both common pathogens and less frequently encountered environmental fungi.

These findings highlight the clinical relevance of the identified fungal species, as they have the potential to cause a wide range of infections, from localized skin and nail infections to more serious and potentially life-threatening systemic mycoses, especially in immunocompromised individuals.

CONCLUSION

Our study highlights the spectrum of mycotic infections and sesonal variation. Microscopic examination using KOH and fungal culture are reliable diagnostic methods for detecting fungal infections. Early identification of fungus through microscopic analysis can serve as an important screening test to provide a presumptive diagnosis of fungal infection. This information can assist clinicians in discontinuing antibiotic treatment and instead initiating appropriate empirical antifungal therapy, which can lead to improved clinical outcomes for such patients.

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