ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

ORIGINAL RESEARCH

Spectrum of fungal isolates from various clinical specimens

[1]Hardik Guriaya, [2]Shipra Galhotra, [3]Nitika Dhuria, [4]Harleen Kaur Gill, [5]Vishal Sharma

[1]Msc Microbiology, [2,3]Assistant Professor, [4]Post Graduate Junior Resident, [5]Associate Professor, Department of Microbiology, G.G.S. Medical College and Hospital, Faridkot, Punjab, India

Correspondence:
Shipra Galhotra
Assistant Professor, Department of Microbiology, G.G.S. Medical College and Hospital, Faridkot, Punjab, India
Email: shipragalhotra@gmail.com

Abstract

Background: In the recent years, the pathogenic mycoses and fungi have emerged as important infectious agents. The fungus infections can be mild and only superficial or cutaneous or may cause life-threatening systemic illnesses.They have an ever increasing global disease burden and regional estimates for specific fungal diseases are often unavailable or dispersed.This study was carried out to study the spectrum of fungal isolates from various clinical specimens in a tertiary care hospital.

Material and methods: The study was conducted at department of Microbiology, Guru Gobind Singh Medical College and Hospital, Faridkot.Total 610 clinical specimens suspected of fungal infection were processed by KOH direct microscopy and culture.

Results: Total positivity for fungal infection was 17.8% (109/610). On KOH direct microscopy, 92 were positive while on culture 86 were positive. On culture, various isolates obtained were*Non albicans candida* (35%),*Candida albicans* (28%), *Cryptococcus spp.* (2%), *A.fumigatus* (12%), *A.flavus*(5%), *Rhizopus spp.*(7%), *Mucor spp.*(3%), *Trichophyton spp.*(8%). In this study, younger male population was found to be more susceptible to fungal infection.

Conclusion: This study brings to light the spectrum of common fungal isolates from a tertiary care hospital.Early detection of fungus by microscopic examination can be used as an important screening test for presumptive diagnosis of fungal infection and it would help the clinician in stoppage of antibiotic therapy and in initiating empirical antifungal therapy earlier, which will help better clinical outcome in such category of patient.

Keywords: mycoses, direct microscopy, culture.

Introduction

Fungal infections are not notifiable infections like viral, bacterial or parasitic diseases. Hence these are not given much attention. But in recent years, the pathogenic mycoses and fungi have emerged as important infectious agents. Although fungi are widespread in distribution, only a few species are pathogenic to humans. The fungus infections can be mild and only superficial or cutaneous or may cause life-threatening systemic illnesses (1).

In the earlier years, yeasts were the most common causative agents of invasive mycoses. But nowadays, moulds especially the *Aspergillus spp.* have become frequent in certain groups of patients suffering from cancer or prolonged neutropenia and in patients with solid organ transplantation (2). *Aspergillus* which is transmitted through inhalation is present in the

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

environment ubiquitously. Patients with underlying lung disease as well as immune compromised patients are usually affected with chronic pulmonary aspergillosis (CPA). In all pulmonary syndromes *Aspergillus fumigatus* is the most common species, but *Aspergillus flavus* is also causing various forms of allergic rhinosinusitis, postoperative aspergillosis and fungal keratitis (3).

Candiduria, the presence of *Candida* species in urine is an asymptomatic condition that results from contamination during urine collection in patients with bladder colonization or upper urinary tractinfection and haematogenous spread from other sites (4). Predisposing factors which are responsible for candiduria includes long stays at hospitals, urinary indwelling catheters, immunosuppressive therapy in immunocompromised patients, abnormality in urinary tract, renal transplantation, broad spectrum antibacterial therapy, and hemodialysis(5-9). However, it was commonly found in elderly people aged 85 years and older (10).

Recently, particular from India, several cases of mucormycosis in people with COVID-19 have increased. Mucormycosis (also called zygomycosis) is a serious fungal diseases caused by a group of moulds called mucoromycetes (11).The primary reason is that spores of mucorales germinate in people affecting with COVID-19 due to the presence of ideal environment i.e. low oxygen, high glucose, acidic medium levels, decreased phagocytic activity of white blood cells (WBC) due to immunosuppression (SARS-CoV-2 mediated, steroid-mediated or background comorbidities) and high iron levels coupled with several other shared risk factors including prolonged hospitalization with or without mechanical ventilators (12). Mucormycosis, an angioinvasive disease that is characterised by tissue infarction and necrosis. The types of fungi that mostly cause mucormycosis include *Rhizopus spp., Mucorspp., Rhizomucor spp., Syncephalastrum spp., Cunninghamella bertholletia, Apophysomycesspp.,* and *Lichtheimia spp.*(13). The *Rhizopus oryzae* is most common species of zygomycetes which is responsible for 60% cases of mucormycosis in humans and most common presentation is Rhino-orbital-cerebral mucormycosis form (14). Another pandemic threat of COVID-19 disease is invasivepulmonary aspergillosis (IPA) that could be due to immune paralysiscaused by viral induced ARDS and hypoxia which is compromising the innate host defence. It usually occurs in immunosuppressed patients such as those receiving chronic corticosteroid treatment or with prolonged neutropenia from malignancy (15,16).

One more fungal disease called dermatophytosis, is a public health problem in many parts of the world particularly in developing countries (17,18).Species of *Epidermophyton*, *Microsporum*, and *Trichophyton* are the major causes of superficial mycosis (17,19).

The fungal isolates are playing a significant role in the pathogenicity of the most infections which are previously used to be considered as laboratory contaminants. But now, these organisms arecapable of affecting not only the immunocompromised patients but also healthy immunocompetent individuals (20). From the clinical specimens, the isolation of these agents may indicate colonisation, infection or disease, hence it is posing a challenge to the clinicians while interpreting the reports.

There is no clear data on the prevalence of fungal infections in India. They have an ever increasing global disease burden and regional estimates for specific fungal diseases are often unavailable or dispersed. But, the climate of our country is suitable for growth of wide varieties of fungus and hence its infections. The present study was carried out for the spectrum of fungal isolates from various clinical specimens in our hospital.

Material and methods

The study was conducted at department of Microbiology Guru Gobind Singh Medical College and Hospital, Faridkot. The samples were collected from the patients suspected to have fungal infections during period of 6 months. These fungal isolates were from various

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

clinical specimens, which included plucked hair samples, nail clippings, skin and scalp scrapings, swabs (like throat, vaginal and conjunctival swabs etc), tissues, pus, sterile body fluids (gastric aspirates, peritoneal fluid, synovial fluid, vitreous fluid etc), sputum and corneal scrapings. Total 610 samples were included in this study. The study was carried out with the consent of the Institutional Ethics Committee.

All samples were processed by direct microscopy and culture. The samples were analyzed by using 40% KOH for nail specimens and 10% KOH for other samples except CSF. For the CSF specimens, India ink preparation and wet mount was done. Gram Staining was done to identify the yeast infection. For fungal culture, all samples were inoculated on two different culture media: one in Sabouraud's dextrose agar (SDA) without antibiotics and another on Sabouraud's dextrose agar with antibiotics (chloramphenicol and cycloheximide). The culture tubes were incubated at 25°C and 37°C for four weeks. These were examined daily for the first week and twice a week for subsequent period.

The fungal species isolated were identified by macroscopic and microscopic evaluation of the fungal morphology. Macroscopically, fungus was identified by observing texture, colour, growth rates,mycelium and conidium types. Identification of fungi was based on various methods including LCB (Lactophenol Cotton Blue) preparation, Gram staining and germ tube tests. The results thus obtained were complied, tabulated and analyzed statistically to obtain valid conclusions.

Observations and results

Table 1: Age wise distribution of patients suspected of fungal infection (n=610)

S. No.	Age (years)	No. of patients(n=610)
1	0-20	141 (23%)
2	21-40	197 (32%)
3	41-60	169 (28%)
4	>60	103 (17%)

Table 2: Gender wise distribution of patients suspected of fungal infection (n=610)

S. No	Gender	No. of patients (n=610)
1	Male	369 (61%)
2	Female	241 (39%)
3	Total	610

Table 3: Distribution of various specimens (n=610)

Sample type	Number	Percentage (%)
CSF	232	38
Urine	92	15
Buccal Mucosa	67	11
Nasal Mucosa	39	6
Skin Scraping	35	6
PUS	34	5
Sputum	28	5
Others(Tissue, Swab, Bal, Vaginal Discharge, etc.)	83	14

Table-4: Results/outcome of fungal infections from clinical specimens (n=610)

S. No.	Results	Number	Percentage(%)
1	Positive	109	17.9%
2	Negative	501	82.1%

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

Table 5: Comparison of KOH direct microscopy and Culture from clinical specimens (n=610)

Direct Microscopy	Gold Standard Culture	
	Culture Positive	Culture Negative
KOH Positive	69	23
KOH Negative	17	501

Table 6: Fungal profile of agents from various specimens (n=86)

Fungal Isolates	Urine	Buccal Mucosa	Tissue	Nasal Mucosa	Skin	CSF	Pus	Nail cliping	Hair	Vaginal	Swab	Bal	Sputum	Others
C.albicans	23	0	0	0	0	0	0	0	0	1	0	0	0	0
Non albicans candida	29	0	0	0	0	0	0	0	0	0	0	0	0	1
Cryptococcus spp	0	0	0	0	0	2	0	0	0	0	0	0	0	0
A.flavus	0	1	0	3	0	0	0	0	0	0	0	0	0	0
A.fumigatus	0	3	2	2	0	0	0	0	0	0	0	0	1	2
Rhizopus spp	0	4	0	2	0	0	0	0	0	0	0	0	0	0
Mucor spp	0	1	2	0	0	0	0	0	0	0	0	0	0	0
Trichophyton spp	0	0	0	0	7	0	0	0	0	0	0	0	0	0

Figure 1: Distribution of fungal infection from various clinical specimens (n=109)

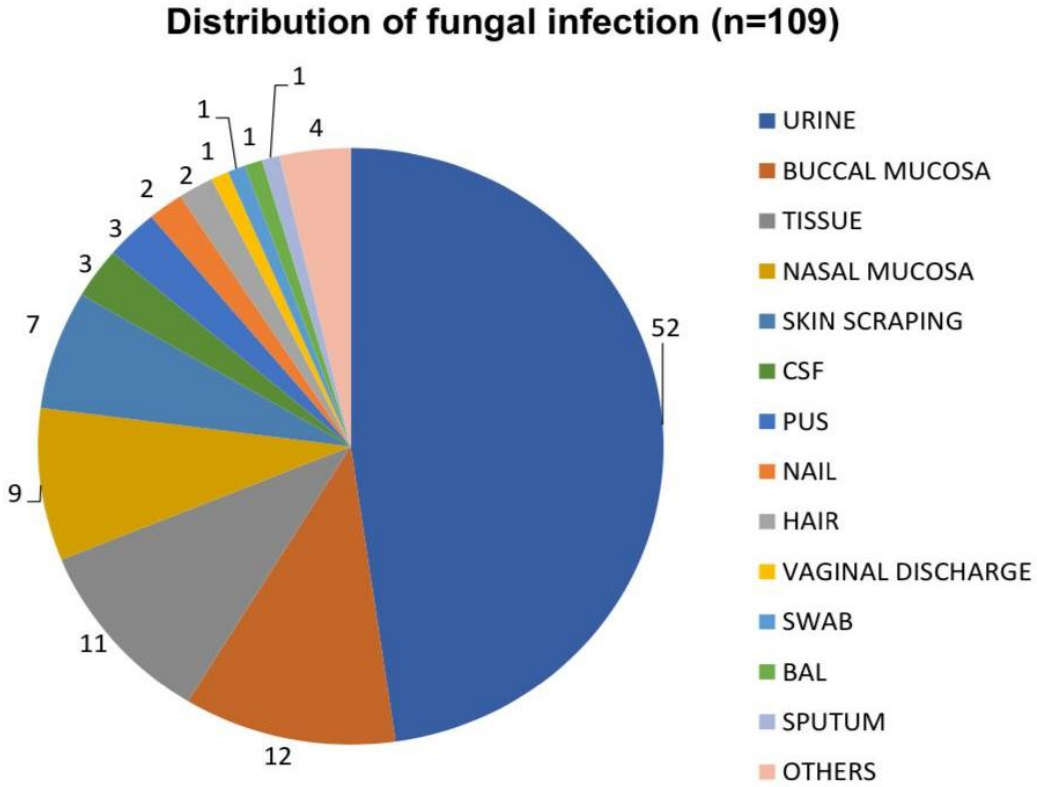

Figure 2: Distribution of Fungal Isolates (n=86)

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

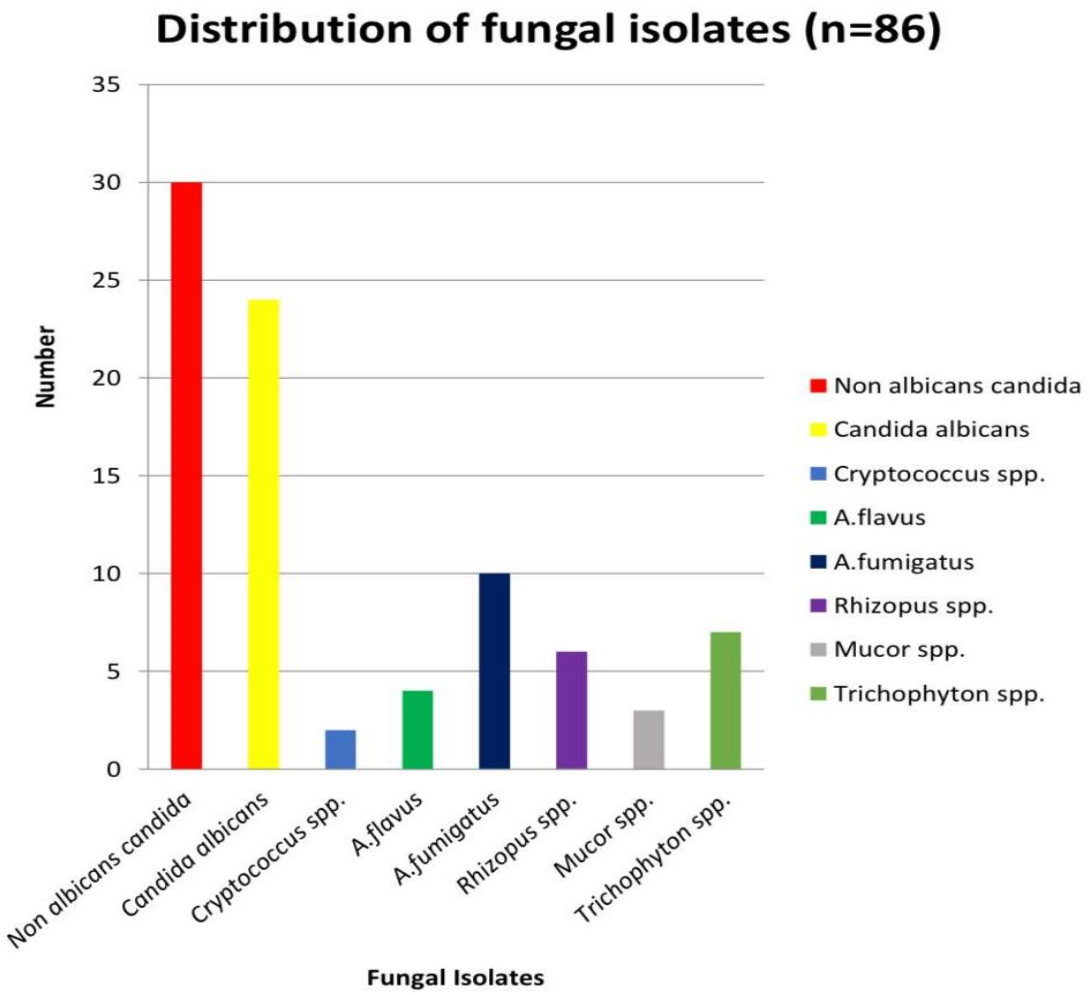

Image 1: Sabouraud's dextrose agar (SDA) showing white, creamy, yeast like colonies.

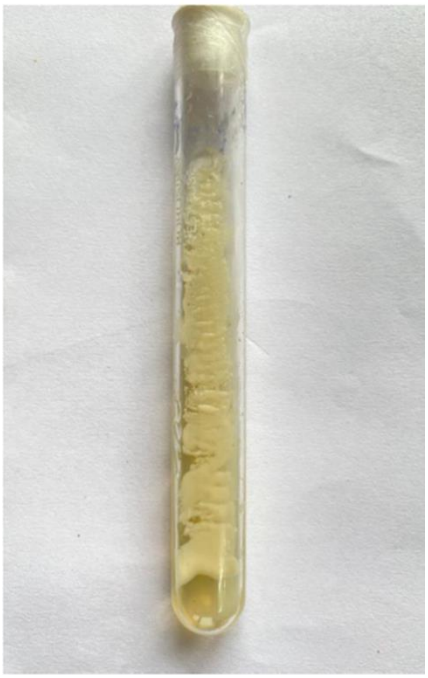

Image 2: Lactophenol cotton blue preparation (LCB) showing ribbon like hyphae with nodal rhizoids of Rhizopus spp. under high power (400x).

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

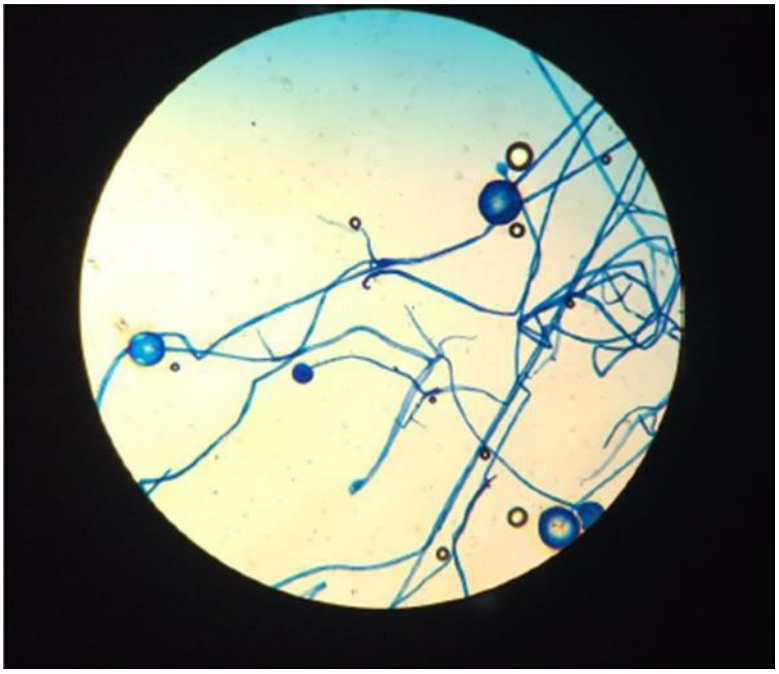

Image 3: **Lactophenol cotton blue preparation (LCB) showing septate hyphae with conidiophores, vesicles and conidia covering the vesicle under high power (400x).**

Discussion

The incidence of mycotic infections is on rising trend for the past two decades due to its ubiquity and wide distribution. But the diagnosis and their treatment are often delayed. The reason could be insidious origin of fungal infection and their association with many co-existing illness.

In thisstudy, various clinical specimens suspected of having fungal infections received in the Microbiology Department were processed as per the standard microbiological procedures. A total of610 consecutive specimens were included in the study.

In this study, most of the patients suspected of fungal infection were 21-40 years followed by age group of 41-60. This is in accordance to many other studies (21,22).This could be due to increasedphysical activity and sweating in the young population especially in hot and humid

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

environment.In this study males were more commonly suspected of having fungal infection with male to female ratio being 1.5:1.Similar findings were also observed by Sridevi and Vishal et al., (23,1). This might be because of increased involvement of males in the outdoor activities.

Most common specimen received in the microbiology laboratory was CSF followed by urine.Total fungal positivity in our study was found to be 17.8% which is almost similar to studies done by Shujat et al., and Tyagi et al., in the past (24,25).

In the present study, KOH was able to detect fungal infection in 92 cases while culture detected fungal infection in 69 of these cases. False negative results on culture might be because of improper sample collection or it may be because of the reason that some samples may not have been crushed before subjecting to the test.

Fungal infections were detected most commonly in urine samples (48%) and all isolates were yeast like organisms which grew on culture. Our results are similar to studies done by Jayaprada et al., (26) Among these isolates, NAC were predominant. The increased incidence of fungal infection in urine samples might be because of increase in catheter associated urinary tract infections. Also, prophylactic treatment with antifungal drugs against Candidiasis has also led to emergence of Candida species.In our study, Candida spp. were most commonly isolated followed by Aspergillus and Mucor Similar findings have been reported by Gandham et al., and Tyagiet al., in previous studies (25,27).Aspergillus species constituted 16% of the positive samples and *A.fumigatus* was the most common isolate followed by *A.flavus*. In Zygomycetes, Rhizopus spp. was the most common isolates. Similar findings were observed by a study done in Northern Pakistan (24).

All skin scrapings were positive for KOH and culture. Trichophyton spp. (100%) were isolated on culture in all skin scrapings. Similar findings were observed by Pathan et al., (2). In our study three CSF specimens were positive for Cryptococcus on examination by India ink preparation and two of them further grew on culture. All these patients were positive for HIV. Mucormycosis was found maximally in specimen of buccal mucosa andnasal mucosa. Sino nasal site has been found to be the most commonsite of involvement in mucormycosis.

This study was conducted to find spectrum of fungal isolates from variousclinical specimens. Our study had shown prevalence of fungal infectionin a tertiary care hospital. We also found that wet mount preparation ofKOH for detection of fungal infection could be use as importantscreening test for presumptive diagnosis of fungal infection.

Conclusion

KOH examination and fungal culture are good diagnostic methods for the detection of fungal infection. Early detection of fungus by microscopic examination can be used an important screening test for presumptive diagnosis of fungal infection and it would help clinician in stoppage of antibiotic therapy and in initiating empirical antifungal therapy earlier, which will help better clinical outcome in such category of patient.In the present study NAC infections especially in UTI cases are increasing globally which is a cause of concern in our country and making its speciation necessary for the microbiologists.

References

1. Mittal M, Sharma V, Gupta V. Isolation of fungus from various clinical specimens in patients of a tertiary care hospital of North India Lit. 2019;250:96.
2. Pathan SS, Patel DM. A microbiological study of fungal etiology invarious clinical specimens of patients attending the tertiary care hospital. Int J Pharm Pharm Sci. 2013; 5(3):333-5.
3. Kosmidis C, Denning DW. The clinical spectrum of pulmonary Aspergillosis. Thorax. 2015;70(3):270-7.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

4. Kauffman CA. Candiduria. Clinical Infectious Diseases. 2005 15;41:371-6.
5. Seifi Z, Azish M, Salehi Z, Mahmoudabadi AZ, Shamsizadeh A. Candiduria in children and susceptibility patterns of recovered Candida species to antifungal drugs in Ahvaz J Nephropathol.2013;2(2):122.
6. Zarei-Mahmoudabadi A, Zarrin M, Ghanatir F, Vazirianzadeh B. Candiduria in hospitalized patients in teaching hospitals of Ahvaz. Iran. J Microbiol. 2012;4(4):198-203.
7. Sobel JD, Fisher JF, Kauffman CA, Newman CA. Candida urinary tract infections—epidemiology. Clin Infect Dis. 2011;52:433-36.
8. Bukhary ZA. Candiduria: a review of clinical significance and management. Saudi J Kidney Dis Transpl. 2008;19(3):350-60.
9. Jain M, Dogra V, Mishra B, Thakur A, Loomba PS, Bhargava A. Candiduria in catheterized intensive care unit patients: emerging microbiological trends. Indian J Pathol Microbiol. 2011;54(3):552-55.
10. Fraisse T, Crouzet J, Lachaud L, Durand A, Charachon S, Lavigne JP, Sotto A. Candiduria in those over 85 years old: a retrospective study of 73 patients. Int Med. 2011;50(18):1935-40.
11. Richardson M. The ecology of the Zygomycetes and its impact on environmental exposure. Clin Microbiol Infect. 2009;15:2-9.
12. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. Diabetes & Metabolic Syndrome: Med Clin Res Rev. 2021;15(4):102146.
13. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, Sein M, Sein T, Chiou CC, Chu JH, Kontoyiannis DP. Epidemiology and outcome of zygomycosis: a review of 929reported cases. Clin Infect Dis. 2005;41(5):634-53.
14. John E, Bennett RD, Blaser MJ. Elsevier Health Sciences: Amsterdam. The Netherlands. 2014.
15. Falcone M, Massetti AP, Russo A, Vullo V, Venditti M. Invasive aspergillosis in patients with liver disease. Med Mycolo. 2011;49(4):406-13.
16. Cicek N, Yildiz N, Kadayifci EK, Gokce I, Alpay H. Invasive aspergillosis in a patient with end stage renal disease. Medical Mycol Case Rep. 2017;18:12-4.
17. Nweze EI. Dermatophytosis in Western Africa: a review. Pak J Biol ScI. 2010;13(13):649-56.
18. Nweze EI, Eke I. Dermatophytosis in northern Africa. Myco. 2016;59(3):137-44.
19. Adefemi SA, Odeigah LO, Alabi KM. Prevalence of dermatophytosis among primary school children in Oke-Oyi community of Kwara state. Niger J Clin Pract. 2011;14(1):23-28.
20. Shrihari N, Kumudini T.SD, Mariraj J, Krishna S. Mycological profile of fungal infections from various clinical samples in a tertiary care hospital. J Pharm Biomed Sci. 2012;17(03):1-5.
21. 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 Pac J Trop Biomed. 2012;2(1):S401-5.
22. Chauhan M, Mahajan S, AbroiRK, Abroi A. Spectrum of fungal isolates of clinical specimens from a tertiary care hospital of North India. Int J Sci Res. 2019;8(4):1199-1202.
23. Sridevi M, Vimala C, Chitra S. Clinicopathological spectrum of fungal infections in a Tertiary Care Centre. Indian J Pathol Oncol. 2018(1):130-5.
24. Shujat U, Ikram A, Abbasi SA, Ayyub M, Mirza IA, Fayyaz M. Spectrum of superficial and deep fungal isolates in Northern Pakistan. Virol Mycol. 2014; 3:131.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

25. Tyagi S, Oberoi A, Alexander VS, Mohan S. Changing trends: anoverview of increasing burden of fungal infections from a tertiarycare hospital in Punjab (north India). Int J Basic Appl Sci.2014;3(1):26-29.
26. Jayaprada R, Nagaraja M, Kumar GLS, Venkataramana B, Kalawat U. Common fungal isolates from routine clinical specimens: a two years' study from a tertiary care hospital in South India. J Clin Sci Res. 2017;6:2-9.
27. Gandham NR, Jadhav SV, Sardar M, Vyawahare C, Misra RR. Thespectrum and aetiology of mycotic infections from a tertiary carehospital from western part of India. J Clin Diagn Res.2013(10):2157-2159.