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ORIGINAL RESEARCH

Fungal Disease Profile in a tertiary care hospital in New Delhi

[1]Anusha Sharma, [2]Ravinder Kaur, [3]Deepti Rawat

[1]Department of Microbiology, Lady Hardinge Medical College, New Delhi, India
[2]Director Professor, [3]Professor, Department of Microbiology, Lady Hardinge Medical College, New Delhi, India

Corresponding author
Anusha Sharma
Department of Microbiology, Lady Hardinge Medical College, New Delhi, India
Email– anusha00s4@gmail.com

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ABSTRACT

Background: Fungi are eukaryotic organisms which possess a rigid cell wall containing chitin, mannose, and other polysaccharides with cytoplasmic membranes containing sterols. They possess true nuclei with a nucleolar membrane and paired chromosomes and divide asexually, sexually, or by both processes. **Objective:** to study Fungal Disease Profile in a tertiary care hospital New Delhi. **Methods:** The present study was a prospective comparative study conducted in the Department of Microbiology, Lady Hardinge Medical College, New Delhi from November 2022 to March 2024. A total of 100 patients, clinically and microbiology diagnosed with fungal infections were included in this study. **Result:** Maximum number of patients belonged to 20-49 years (44%) age group, with slight male preponderance (male: female:1.04:1).The most common presenting complaint in the study population was fever present in 27(27%) followed by discoloration in 22%. There was no underlying disease in 81 % patients. Most of the patients had complaints related to respiratory system (11 %) followed by Taenia infection (3%). There were no apparent risk factors in 28 % patients, Catheterisation (15%) was the major risk factor followed by presence of comorbidities, intubation, low birth weight, increased age, malnutrition, Neutropenia, Post-operative history, prior antifungal, History of blood transfusion, immunosuppressive therapy (chemotherapy), Antitubercular drug intake, oral ulcers and hyperalimentation and positive HIV status. Most patients had involvement of skin, appendages and soft tissues (36%) followed by respiratory system (26%), genitoirunary system (7%), gastrointestinal system (5%),CNS and ENT and PNS system (4% each). Nail clippings comprised 27% of the samples followed by blood (17%), urine (20 %) respiratory samples (19%) which included sputum (11%), Tracheal aspirate (4%) and BAL (3%) followed by skin scrapings (7%), while hair clipping, peritoneal drain fluid, and pus were 1% each.

Conclusion: The findings advocate for heightened clinical suspicion, improved diagnostic capabilities, and judicious use of antifungal therapies to address the challenges posed by these infections.

Keywords: Fungal Diseases, clinical suspicion, diagnostic capabilities, antifungal therapies, tertiary care hospital

INTRODUCTION

Infections caused by fungi are called mycoses. There are four main groups of fungal infections-superficial, subcutaneous, systemic and opportunistic mycoses. Superficial mycosis is common worldwide with an expected acquisition risk of 10%-20% in a lifetime(1).The global cases of superficial mycosis stand at 1,000,000 cases Subcutaneous mycoses, is majorly confined to the subcutaneous tissue and dermis, mainly seen in the tropics and subtropics. While the systemic mycoses like Invasive Aspergillosis (IA),systemic candidemia etc. are caused by fungi which invade deep structures such as the lungs, liver, and spleen as well as spreading, usually through hematogenous route, to the skin and mucosal surfaces to produce generalized or localized disseminated infections(2) .In most climates, opportunistic infections are common in patients with underlying conditions that make a person more susceptible to infection—such as severe neutropenia, HIV/AIDS, or the use of a subcutaneous line which determines the progress of infections clinically. Systemic opportunistic fungal infections are seen in all countries and the use of medicinal or surgical procedures as well as the existence of conditions that favor infection are the key factors defining their incidence(2).

There are expected to be around a billion cases of fungal infections of the skin, nails, and hair, tens of millions of cases of mucosal candidiasis, and over 150 million cases of serious fungal diseases leading to a significant morbidity or mortality. The clinical

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presentation varies from minor mucocutaneous infections that cause no symptoms to potentially fatal systemic infections. Furthermore, the death rate linked to fungal disease, at over 1.6 million globally, is roughly three times higher than that of malaria and comparable to tuberculosis (3).There is an estimation of 1749 cases of recurrent vulvovaginal candidiasis (RVVC) per lakh population, 18 cases of IA per 100000 population, total cases of CPA stands at 1738913 annually, candidemia and mucormycosis accounts for 13.5 and 14.0 cases respectively per lakh population per year in India (4).

There has been an increase in incidence of fungal infections due to, increased length of ICU stay (in turn leading to increased incidences of prolonged antibiotic use, hyper alimentation, catheterization) and hospital stay, and colonization is difficult to distinguish from invasive disease, HIV epidemic, growing number of immunocompromised individuals , indiscriminate use of easily available over-the-counter antifungals and antibiotics, increased travel, and heightened identification of fungi add to the problem. Furthermore, these infections are difficult to diagnose as there is common symptomatology, are exceedingly difficult to treat, and even with the use of proven antifungal medications, the fatality rate is still very high

Hence, our aim was to study Fungal Disease Profile in a tertiary care hospital New Delhi

MATERIAL AND METHODS

This prospective comparative study was conducted from November 2022 to March 2024 in Microbiology department, Lady Hardinge Medical College.

Sample Size

Sample size formula =n = $(Z\alpha/2+Z\beta)$ $(p1(1-p_1)+p_2(1-p2))/(p_1-p2)^2$ $\alpha = 0.05$ (for a confidence level of 95%)

$Z\alpha/2 = 1.96$

$Z\beta = 0.84$ (For a power of 80%, β is 0.2) $p1=90.2$ [18]

$p2=78$ [19]

Calculated sample size=138

We took a convenient sample size of 100 isolates for this study.

A total of 100 patients, clinically and microbiology diagnosed were included in this study.

Study Methodology

The demographic details of the patients were noted as per the protocol before taking detailed history of the presenting complaints and underlying disease if any. Past history, birth history, immunization history, family and treatment history were also elicited. General and systemic examination were done and recorded. All relevant investigations including radiological investigations of the patients were done and noted apart from microbiological investigations.

Collection of samples and transport

Different clinical samples were collected as per the site of involvement and clinical suspicion.

Processing of the samples in Microbiology Department was done as follows: Sample processing

- Urine- Sample was centrifuged and inoculated in media containing antibacterial agents.
- Tissue- Sample was minced not grounded and the tissue was pressed into culture media so that it was partially embedded in it .
- CSF- The specimen was centrifuged at 2000 X g for 10 min and the concentrated sediment was used to inoculate the culture medium and for microscopy and the supernatant was used for antigen detection.

1. DIRECT MICROSCOPIC EXAMINATION

2. CULTURE: BLOOD CULTURE

Culture of Other samples (Nail,Skin,Hair clippings)

VITEK 2 FOR IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING

The VITEK®-2 compact system (bioMérieux, France) is a fully automated system that performs fungal identification by biochemical analysis using colorimetry.(105)

Results were checked after 18-20 hours using the Vitek-2 program on the computer (103)

MALDI-TOF MS

It was performed using MALDI-TOF VITEK MS® 3.0 system (bioMerieux, France). system following the steps in the user manual. It analysis the spectrum of protein which is unique for each organism with a reference database available to identify the organism.

IDENTIFICATION BY MALDI TOF MS

MALDI-TOF VITEK MS® 3.0 system
(bioMerieux, France).

Filamentous fungi extraction and procedure:

1. The hyphae/conidia were collected with a moistened sterile cotton swab.
2. It was inoculated into 900 uL of 70% ethanol in a microcentrifuge tube.
3. The suspension was vortexed for 3-5 seconds and then centrifuged at 10,000 X g for 2 min.
4. The supernatant was discarded and the pellet was resuspended with 40 uL of 70% formic acid followed by 40 uL of acetonitrile.
5. The suspension was vortexed for 3 -5 sec and recentrifuged at 10,000 X g for 2 min.
6. 1uL of the supernatant was transferred to the target slide.
7. Once air dried, 1uL of matrix(CHCA) was applied and allowed to dry.
8. For each isolate a single extraction was performed and spotted in duplicate.
9. And was directly spotted and overlaid with matrix on each aquisition group (16 each) on each slide as a calibrant and quality control as per manufacturer's protocols.

The results are obtained after the software matches the spectra obtained for the test isolates with the data

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available in the reference library of the manufacturer after approximately 30-40 min.

RESULTS

The study was conducted at the Department of Microbiology, Lady Hardinge Medical College and associated hospitals. 100 patients, microbiologically confirmed for fungal infection were included in the study. Demographic information, history, clinical signs, risk factors, investigational data etc. were recorded, microbiological investigations were performed and the following observations were made.

Table -1 Age and sex distribution of patients(n=100)

Age (in years)	Male		Female		Total	
	No.	%	No.	%	No.	%
0-28DAYS	7	7	2	2	9	9
28days-<1yr	5	5	3	3	8	8
1-9	7	7	6	6	13	13
10-19	3	3	3	3	6	6
20-29	7	7	11	11	18	18
30-39	6	6	6	6	12	12
40-49	4	4	10	10	14	14
50-59	6	6	4	4	8	8
=/>60	6	6	4	4	10	10
total	51	51	49	49	100	100

Maximum number of patients belonged to 20-49 years(44%)age group ,18%belonging to 20- 29 years (18%) and 12% in 30-39 years age group, followed by 10 % in >60 years age group, 9% in 0-28 days ,8 % each in 50-99 years and 28days- <1 year and 6% in 10-19 years of age group.
51 patients (51%) were male and 49 (49%) were female.

Table 2- Distribution of patients(n=100)

Patient distribution	Number	%
OPD	46	46
IPD	38	38
ICU	14	14
OT	2	2
TOTAL	100	100

Most of the patients 46(46.00%) were from OPD followed by IPD (38%), ICU (14.00%). Only 2 patients (2.00%) were from OT (ENT OT).

Table 3: Presenting complaints of patients(n=100)

Presenting Complaint	Number	Percentage (%)
Fever	27	27
Discoloration of nail	22	22
cough	10	10
Fever not responding to antibiotics	10	10
Generalised swelling	9	9
Wheeze	5	5
Difficulty in breathing	5	5
Abdominal distention	5	5
Chest indrawing	5	5
Skin discoloration	3	3
Burning micturition	3	3
Coryza	3	3
Scalp lesion	3	3
Nasal blockage	2	2
Hoarseness of voice	1	1
Foul smelling discharge	1	1
Pain abdomen	1	1
Jaundice	1	1
Oral thrush	1	1

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Chest pain	1	1
Eczema	1	1
Swelling	1	1
Drowsiness	1	1
Tremors	1	1
Seizure	1	1
Oral ulcer	1	1
Diminition of vision	1	1
Hairfall patches	1	1
Ingrown nail	1	1
Discharge	1	1
Trauma	1	1

The most common presenting complaint in the study population was fever present in 27(27%) of patients followed by discoloration of nails in 22.00%, cough and fever not responding to antibiotics in 10.00%, generalized swelling in 9.00%, difficulty in breathing , abdominal distention and wheezing in 5.00% each , coryza, skin changes and scalp lesions in 3.00% each and nasal blockage in 2.00%.Other presenting complaints were hoarseness of voice, foul smelling discharge, pain abdomen ,jaundice ,oral thrush, trauma eczema, swelling,drowsiness, tremors,seizure ,oral ulcer,diminution of vision,hairfall patch,ingrown nails and discharge in 1.00% each.

Table 4– Underlying disease profile of patients (n=100)

Disease profile	Number	Percentage (%)
Respiratory Distress Syndrome - RDS(paediatric)	4	4
COPD	2	2
Asthma	2	2
ARDS	2	2
Pulmonary TB	1	1
Existing Taenia infection	3	3
Coronary Artery Disease	2	2
Malignancy	1	1
Diabetic Ketoacidosis	1	1
Chronic Liver Diseases	1	1
No underlying disease	81	81

There was no underlying disease in 81 % patients. Most patients had complaints relating to respiratory system (11 %) with Respiratory distress syndrome (RDS)in 4%, followed by COPD, Asthma and Acute Respiratory Distress Syndrome in 2% each and Pulmonary tuberculosis (TB) in one patient each. Taenia infection was seen in 3%, coronary artery disease in 2% and malignancy, diabetic ketoacidosis and chronic liver diseases were seen in 1% patients each.

Table 5: Associated risk factor profile of the patients(n=100)

Risk Factors	Number	Percentage
Catheterisation	15	15
Comorbidity (DM/HTN)	12	12
Intubation	8	8
Low birth weight	7	7
Malnutrition	6	6
Age>60	6	6
Prolonged hospital stay (>7 days)	5	5
Neutropenia	4	4
Prior antifungal use	4	4
Post operative	4	4
Profession(florist)	2	2
Corticosteroid therapy	2	2
Transfusion	1	1
Malignancy	1	1
Chemotherapy	1	1

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Positive HIV status	1	1
Hyperalimentation	1	1
History of ATT intake	1	1
Oral ulcer	1	1
No apparent risk factor	28	28

There were no apparent risk factors in 28 % patients ,Catheterisation (15%) was the major risk factor. The presence of comorbidities (majorly diabetes) was seen in 12%,followed by intubation in 8%,low birth weight(<1500gm) in 7%, increased age (>60years) and malnutrition were seen in 6% each Neutropenia ,Post operative history and prior antifungal use accounted for 4% each .History of blood transfusion, immunosuppressive therapy (chemotherapy), Antitubercular drug intake, oral ulcers and hyperalimentation and positive HIV status were present in 1% each.

Table 6 -Profile of involvement of various organ systems as per the clinical presentation (n=100)

Organ system involved	Number	Percentage (%)
Skin ,appendages ,soft tissue	36	36
Respiratory system	26	26
Genitourinary system	7	7
GI system	5	5
CNS	4	4
ENT& PNS	4	4
CVS	2	2
Ophthalmology system	1	1

ENT PNS- Ear nose throat and paranasal sinuses

Most patients had involvement of skin, appendages and soft tissues (36%) followed by Respiratory system in 26%. Genitourinary system was involved in 7 % cases followed by gastrointestinal system in 5%. Nervous system and ENT & PNS system accounted for 4% cases each with Ophthalmology cases accounting for 1% of cases.

Table 7 : Sample profile of patients(n=100)

Sample type	Number	Percentage
Nail clippings	27	27
Blood	17	17
Catheterized urine	15	15
Sputum	11	11
Skin scrapings	7	7
Urine(Mid Stream Urine)	5	5
Tracheal Aspirate	5	5
BAL	3	3
C.S.F	3	3
Tissue	3	3
Corneal scraping	1	1
Hair	1	1
Peritoneal drain fluid	1	1
Pus swab	1	1

Depending on the symptomatology and the organ system involved, a total of 100 samples were collected from the patients from different sites. Nail clippings were collected from 27 patients, Blood was collected from 17 patients and urine in 20 patients (15 catheterised samples and 5 mid-stream urine samples). Respiratory samples included sputum, which was collected in 11 patients, Tracheal aspirate in 4 patients and BAL in 3 patients. Skin scrapings were collected from 7 patients, while hair clipping, peritoneal drain fluid, swab, tracheal aspirate were collected from 1 patient each.

DISCUSSION

There has been a rise in the cases of fungal infections in India and around the world primarily due to a rise in comorbid states, the immunocompromised population and increased advancement of medical healthcare and subsequent increased length of ICU and hospital stay. The presentation of fungal

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infections ranges from mild superficial infection to life threatening disseminated infections in many. There is a difficulty in diagnosing fungal infections due to overlapping symptomatology and presentation with bacterial infections along with an overall low level of clinical suspicion and awareness. The antifungal drugs available for the treatment of fungal infections are limited, many fungi being inherently resistant to many antifungals, making it important for their judicious use, for optimal treatment and to prevent the development of resistance. Hence it is imperative to identify the causative fungi and do their antifungal susceptibility testing at the earliest for possible early targeted therapy.

A total of 100 clinically and microbiologically confirmed patients with fungal infections were included in the present study. Most of the patients belonged to the age group 20-49 years (44%) followed by 1-9 years (13%) and >60 years (10%). Male preponderance was seen in our study (51%), females (49%),which was similar to different studies conducted in India and abroad with Table 1 showing various trends in various studies.

Table 8- Comparison of demographic profile of patients .

Study	No. of isolates	Area of study	Time of study	Major Age group(years)	Gender (M/F)	Remarks
Present study	100	Delhi, India	2022-24	20-49 (44%)	M:F::1.02:1	
Kashyap et al(5)	2228	Delhi, India	2009-10	14-60(64%)	M >F	Coherent to our study
Parikh et al(6)	78	Gujarat, India	2013-16	Mean age 46.9 years	M:F::1.7:1	Coherent
Chauhan et al(7)	212	Kangra, India	2014	21-50(66%)	M:F::1.27:1	Coherent
Colak et al(8)	996	Turkey	2019-20	>70(60%)	M:F::1.1:1	Not coherent

Most of the studies showed a male preponderance which could be due to a higher rate of outdoor physical activity in males, inducing increased sweating especially in the hot and subtropical climate of India, ideal for fungal growth (9). Also, in many cases, the limited exposure and access available to medical care in women, might be responsible for the relatively higher number of fungal infections witnessed in males.

In our study most of the patients of fungal infections were Out Patients (OPD)(46%) followed by inpatients (38%) and ICU patients (14%).This is coherent with the study of Chauhan et al., in Kangra, in 2016-2017, where 64% samples were received from OPD. However, Colak et al, in Turkey, in 2020, reported a majority of patients (69.4%) belonging to ICU followed by IPD (5.5%).(6-8)

In our study, the most common presenting complaint in the study population was fever, present in 27(27%) of patients with cough and fever not responding to antibiotics in 10% followed by discoloration of nails in 22%, generalized swelling in 9%, difficulty in breathing, abdominal distention and wheezing in 5% each, among others.

Similar to our study, P Rotjanapan et al. (10) in 2017, in a multicentre study in Asia, found fever (69%) to be the most frequent presenting complaint of patients ,followed by cough, breathlessness and chest pain. Singh et al in Mumbai in 2015(11), also reported fever (36.3%) to be the most commonly associated risk factor followed by altered sensorium, cough and breathlessness, among others.While Zhang et al., 2018,(12) in their study conducted in a hospital in China, reported cough (66.2%) to be the most common presenting complaint followed by expectoration, fever and dyspnoea.

In our study, most patients were found to have involvement of skin, appendages and soft tissues (36%) followed by Respiratory system in 26%. Genitourinary system was involved in 7 % cases followed by gastrointestinal system in 5%. Nervous system and ENT & PNS system accounted for 4% cases each with Ophthalmology cases accounting for 1% of cases. However, Singh et al., in 2015, in Mumbai, had central nervous system to be the most commonly involved system followed by respiratory system and GIT.

In our study, there were no apparent risk factors in 28 % patients. Catheterisation (14%) was the major risk factor. The presence of comorbidities (majorly diabetes) was seen in 12%, followed by intubation in 8%, low birth weight(<1500gm) in 7%, increased age (>60years) and malnutrition in 6% each. Neutropenia, post operative history and prior antifungal use accounted for 4% each. History of blood transfusion, immunosuppressive therapy (chemotherapy), Antitubercular drug intake, oral ulcers and hyperalimentation and positive HIV status were present in 1% each.

Rafi et al., 2016 in Puducherry, (13)in their study had observed catheterization to be the most commonly associated risk factor with urinary candidiasis followed by diabetes mellitus, surgical procedures, long term use of broad-spectrum antibiotics among others. Jain N et al.(14) and Paul et al.(15) also reported that an indwelling urinary catheter was the most common risk factor associated with candiduria. This higher association can be explained by the simple fact that catheterization can lead to the migration of the organisms from periurethral surface into the bladder, which increases the chances of acquiring UTI.

Quite in concordance with our study, P Rotjanapan et

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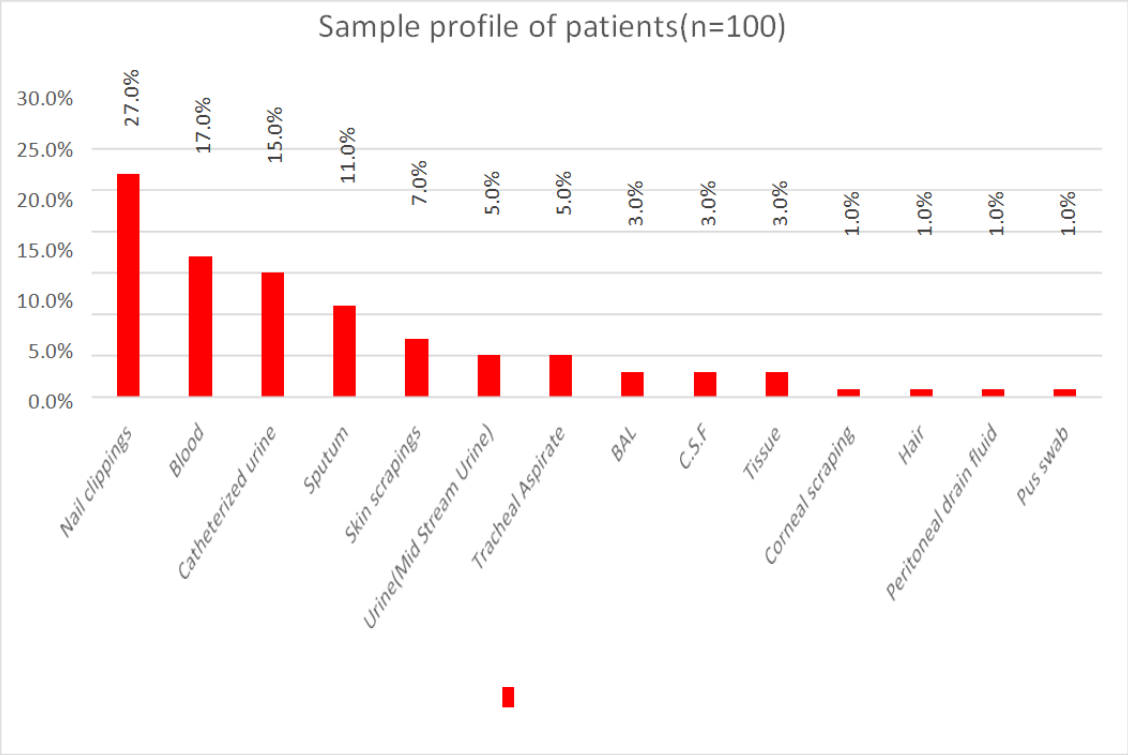
al., 2017, in a multicentre study in Asia, found diabetes mellitus as an important associated risk factor with almost one third patients having DM, although the most commonly associated risk factor was the prolonged use of corticosteroids (39.4%) followed by neutropenia (38.7%)(10). Alsalman et al., 2017 in Bahrain, also reported that 23% patients in their study suffered from DM and 23% had a history of corticosteroid use, followed by COPD (17%), malignancy (15%) with neutropenia in 9% cases. Again, Vasquez et al., 2016 in USA, found DM to be the most frequently associated risk factor followed by mechanical ventilation, COPD and sarcoidosis. (16,17)

Poissy et al. in their study found the presence of central venous catheter, total parenteral nutrition, previous septic shock and exposure to antibiotics to be the risk factors associated in ICU as well as non-ICU patients diagnosed with candidemia (18). Chowta et al., in their study also found the common risk factors to be the use of intravenous cannula followed by long term use of antibiotics and HIV infection among others. (19)

Jinjian et al., 2016 in China, found that the incidence of candidemia was inversely proportional to the birth weight especially in premature infants indicating that low birth weight and prematurity are both risk factors for the development of candidemia. (20,21). Extremely Low Birth Weight (ELBW) infants have been found to be at a 10 times greater risk of acquiring candidemia. (22). We had 7% patients with low birth weight in our study.

The different association of various risk factors for the development of fungal infections is also evidenced in different studies as under: (133)

Reference	Place of study	Important risk factors
Hernández et al. 2020	Mexico	Corticosteroid use
Goel et al., 2009	Rohtak	Use of broad-spectrum antibiotics
Xess et al., 2007	New Delhi	Antibiotics
Chowta et al. 2007(123)	Mangalore	Prolonged use of antimicrobials
Kumar et al., 2005	Chennai	Chemotherapy, steroid therapy, use of broad-spectrum antimicrobials
Sahni et al., 2005	New Delhi	Use of broad-spectrum antibiotics
Jautova et al. 2001	Slovakia	Corticosteroid use



We collected a total of 100 samples out of which 27 were nail clippings, 20 urine, 17 blood samples. Respiratory samples included sputum, which was collected in 11 patients, tracheal aspirate in 4 patients and BAL in 3 patients. Skin scrapings were collected from 7 patients, while hair clipping, peritoneal drain fluid, swab, tracheal aspirate were collected from 1 patient each.

Various studies (23-25) on infections by *Candida* species, found urine to be the most frequently received sample as follows:

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Reference	Place of study	Sample distribution
Chawdhary et al., 2019(23)	Jaipur	Urine > Blood > Swabs > ET secretion > Vaginal swabs > Others
Montes et al., 2019(24)	Central America	Urine > Sputum > Vaginal swabs
Rathore et al., 2018(25)	Udaipur	Urine > Sputum > ET secretion > Throat swab > CVC tip > Pus > Blood > Others

Unlike our study, in studies on dermatophytosis by Kumar et al., 2022, in Chandigarh and Mani et al., 2019, in Rajasthan, skin scraping was the most common samples followed by nails and hair, while nail clippings were the most common sample in our study followed by skin scrapings and hair. This may be attributed to the random sample availability during the period of our study (26,27).

Singh at el in 2015 in Navi Mumbai in their study found Aspergillus species to be isolated most commonly from sputum samples (20.67%) followed by nasal and paranasal sinuses (15.9%), pus (15.88%),BAL (13.54%) among others (114).Xess et al in 2004 in New Delhi had reported that maximum Aspergillus species were isolated from respiratory samples (32.8%) followed by blood (24.7%) and nasal polyp (24.56%).(28)

Most fungi can be detected without staining, especially in specimens such as sputum, urine,exudates, and cerecrospinal fluid using reduced light-brightfield microscopy or phase contrast microscopy. However over the years staining methods such as India ink, calcofluor white , methamine silver ,periodic acid -Schiff (PAS) and others have been developed to detect fungal elements more easily .

CONCLUSION

This study highlights the significant burden of fungal infections in a tertiary care setting in New Delhi, affecting a wide range of organ systems and patient demographics. The findings advocate for heightened clinical suspicion, improved diagnostic capabilities, and judicious use of antifungal therapies to address the challenges posed by these infections. Given their high morbidity and mortality—comparable to major infectious diseases like tuberculosis—fungal infections warrant increased awareness and resource allocation in healthcare settings to mitigate their impact effectively.

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