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BURDEN OF FUNGAL INFECTIONS AND COMMON FUNGAL ISOLATES RECOVERED FROM ROUTINE CLINICAL SAMPLES AT A TERTIARY CARE HOSPITAL OF NORTH INDIA - A TWO-YEAR RETROSPECTIVE STUDY.

Microbiology

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ABSTRACT

Introduction: Fungi have emerged in the past two decades as major causes of human disease, especially among those individuals who are immunocompromised or hospitalized with serious underlying diseases. The increasing burden of fungal infections in hospital settings is a cause of concern. **Aims & objectives:**This study was undertaken to determine: 1) The actual burden of fungal infections at a tertiary care hospital. 2) The common fungal isolates responsible for such infections. 3) To distribution of mycological agents across different clinical samples. **Materials and methods:**A total of 1669 samples were received in the lab during April 2018 to October 2020. All the relevant clinical, demographic, and epidemiological details were noted. Samples were then subjected to direct microscopy and culture. **Results:**The most frequent sample received in the laboratory was Nail (n=340, 20.4%), followed by CSF (n=324, 19.4%), bronchoalveolar lavage (BAL) (n=294, 17.6%), skin scrapping (266, 15.9%), sputum (n=64, 3.8%) and corneal scraping (53, 3.2%). The highest number of samples received from Dermatology department (671, 40.2%), followed by General medicine (328, 19.7%) and Respiratory medicine (290, 17.4%). Direct microscopy was positive in 372 (22.3%), while, Culture was positive in 574 (34.4%) samples. Candida species was the most common isolate followed by Dermatophytes, Scopulariopsis species, Penicillium species etc. Males were more commonly affected. **Conclusion:** Fungi are opportunistic organisms which are ubiquitous in nature. The incidence of life-threatening fungal infections caused by true pathogenic or opportunistic fungi has been increasing since the past two decades. Fungal isolates were recovered from almost all body sites, most common being the skin and appendages. Superficial fungal mycosis, although not life threatening can be persistent, intractable, and lead to severe morbidity besides causing considerable disfigurement. Early diagnosis and aggressive treatment remain the key in reducing the overall burden.

KEYWORDS

INTRODUCTION

Fungi have emerged in the past two decades as major causes of human disease, especially among those individuals who are immuno compromised or hospitalized with serious underlying diseases. Among these patient groups, fungi serve as opportunistic pathogens, causing considerable morbidity and mortality. This increase in fungal infections can be attributed to the ever-growing number of immuno compromised patients, including transplant patients, individuals with acquired immunodeficiency syndrome (AIDS), patients with cancer and undergoing chemotherapy, and those individuals who are hospitalized with other serious underlying conditions and who undergo a variety of invasive procedures [1].

The spectrum of these fungal infections range from those caused by Candida, Cryptococcus, and Aspergillus to Zygomycetes. Newer fungal pathogens like non-albicans Candida, various species of Zygomycetes, and Penicillium species are also being increasingly reported over the last 10 years [2]. The normally intact skin and mucous membrane serve as the first line of defense against most of the infections, but any break in this continuity makes the individual vulnerable to various fungal infections [3,4].

Aims & objectives:

This study was undertaken to determine:

1) The actual burden of fungal infections at a tertiary care hospital.
2) The common fungal isolates responsible for such infections.
3) To distribution of mycological agents across different clinical samples.

Materials and methods:

This retrospective study was done in the Postgraduate department of Microbiology, Government Medical College, Srinagar J&K. Samples were collected from patients suspected of having fungal infections from SMHS and associated hospitals during April 2018 to October 2020. A total of 1669 samples were received in the lab during this period as shown in Table 1. It was emphasized that samples were i) Appropriately collected ii) Promptly transported iii) Properly received iv) Correctly processed, inoculated on appropriate culture media, and incubated at a suitable temperature.

The clinical samples were subjected to direct microscopy with 40 % potassium hydroxide (KOH) for nails and 10% KOH for other samples (skin scrapings, hair, tissue, and other fluids) to visualize the presence of any fungal element and Gram stain to demonstrate budding/yeast-like cells, hyphae and pseudohyphae. The KOH wet mounts were screened under low power (10x) and then under high power (40x) for visualization of fungal elements. All CSF samples and other samples with requisition were additionally subjected to staining with Indian ink (Nigrosin) for identification of Cryptococcus species. All samples were inoculated on two sets of media: (1) Sabouraud dextrose agar (SDA, HiMedia Laboratories) with 5% chloramphenicol and (2) SDA with 5% chloramphenicol and cycloheximide. The culture tubes were incubated at 25°C and 37°C. The cultures were examined twice a week for 6 weeks and if no growth was obtained till 4 weeks, they were declared negative.

The identification of fungi was based on macroscopic and microscopic examination of the culture isolates. The macroscopic examination of dermatophytes & filamentous growth was characterized by duration of growth, surface morphology, and pigment production on the reverse. The microscopic examination of filamentous fungal growth was observed with lactophenol cotton blue stain. Oval budding yeast-like cells resembling Candida on Gram staining were speciated by germ tube test, production of chlamydospores on corn meal agar & colour of colonies on CHROM agar. Spherical & budding yeast forms resembling Cryptococcus species were identified by standard procedures [5]. The results were considered positive when smear results were consistent with culture, or growth of the organism was demonstrated on two or more occasions with negative smear results or repeated appearance in smear with negative culture results.

Table 1; Different types of specimens received

SPECIMES	N (%)
Nail	340 (20.4)
Skin Scrapping	266 (15.9)
Skin Biopsy	17 (1)
Tissue	98 (5.9)
BAL Fluid	294 (17.6)
Pus	32 (1.9)

CSF	324 (19.4)
Nasal Polyp	26 (1.7)
Corneal Scrapping	53 (3.2)
Aqueous Tap	1 (0.05)
Vitreous Tap	15 (0.9)
Blood	35 (2.1)
Urine	20 (1.2)
Sputum	64 (3.8)
Pleural Fluid	55 (3.3)
Peritoneal Fluid	6 (0.3)
Hair	18 (1.1)
Swab	4 (0.2)
Bone Marrow	1 (0.05)
Total	1669 (100)

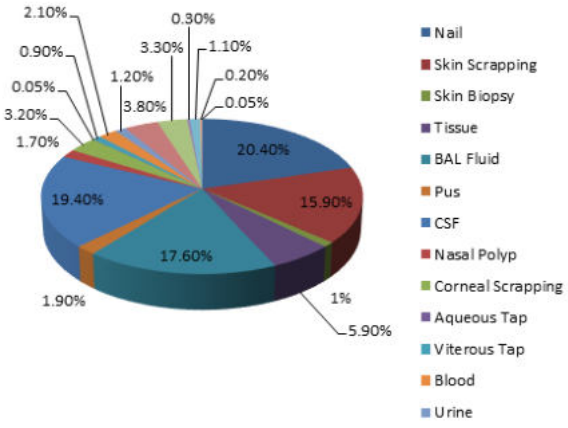

Fig 1; Percentage distribution of specimens received.

RESULTS

The frequency distribution of samples from clinically suspected fungal infections from various clinical departments is given in Fig 1. The most frequent sample received in the laboratory was Nail (n=340, 20.4%), followed by CSF (n=324, 19.4%), bronchoalveolar lavage (BAL) (n=294, 17.6%), skin scrapping (266, 15.9%), sputum (n=64, 3.8%) and corneal scraping (53, 3.2%). From the total 1669 samples received, suspected male cases (946, 56.7%) were in predominance than females (723, 43.3%). Table 2 shows Age and gender-wise distribution of samples from suspected cases, as seen that most samples were from suspected cases in the age groups of 20-39 (n=512, 30.7%) and 40-59 (n=569, 34%). The breakdown of 1669 samples received from different departments is depicted in Fig 2, with the highest number of samples received from Dermatology department (671, 40.2%), followed by General medicine (328, 19.7%) and Respiratory medicine (290, 17.4%). Direct microscopy (KOH & Gram stain) was performed on 1632 samples with 372 (22.3%) giving positive results while 1260 (75.5%) gave negative results as seen in Fig 3. There were 574 (34.4%) culture positive isolates, while 593 (35.5) were culture negative. The fungal isolates obtained after culture from various clinical samples are extensively depicted in Table 3. From a total of 574 isolates, Candida species was the most common isolate accounting for 181 (31.5%), followed by Dermatophytes 158 (27.5%), Aspergillus species 133 (23.1%), Scopulariopsis species 23 (4%), Penicillium species 19 (3.3%), Rhizopus species 13 (2.3%), Non Albicans Candida 11 (1.9%), Mucor species 4 (0.7%) and Fusarium species 3 (0.5%) as shown in Fig 4. The relative proportion of fungal isolates obtained from various clinical samples is shown in Table 4, as seen Skin & appendages (Skin scrapping, skin biopsy, nails, hair, and tissue) yielded the maximum isolates (n=343) with the majority (n=155) belonging to Dermatophytes group, (n=77) to Aspergillus species and (n=67) to Candida species. Lower respiratory tract samples (BAL & Sputum) yielded (n=162) with Candida species (n=94) being the predominant isolate followed by Aspergillus (n=43). The comparison of microscopy and culture of various clinical samples is shown in Table 5; both positive 222 (13.3%), only microscopy positive 372 (22.2%), and only culture positive 574 (34.4%). Indian ink (Nigrosin) staining was performed on 319 samples of which 03 (Two CSF and one Nasal polyp tissue) were positive for irregular sized, encapsulated, spherical yeast cells of Cryptococcus species. Details of the different diagnostic modalities used in this study are summarized in Table 6.

DISCUSSION

Fungi are opportunistic organisms which are ubiquitous in nature. The incidence of life-threatening fungal infections caused by true pathogenic or opportunistic fungi has been increasing since the past two decades. The emergence of these infections has created a challenge in their diagnosis and management [6]. These infections are often insidious and their diagnosis is usually delayed because of the coexisting illnesses [7]. Numerous studies have identified common risk factors for patients developing fungal infections. Most of these risk factors are very common in all hospitalized patients, and it is therefore difficult to determine which patients are at greatest risk for developing fungal infections. In general, two populations have been at risk for acquiring invasive fungal infections. The first are persons with increased susceptibility to infection because of their geographic location. The second population includes persons with increased host susceptibility (i.e., severely ill, immunocompromised, or malnourished individuals) who develop opportunistic infections. Although the diagnostic and therapeutic modalities for some fungi are improving, there is still much to learn about many of the other fungi that are diagnosed in our hospitals nowadays. Fungi are eukaryotic cells; they are more complex than bacteria. A thorough appreciation and understanding of fungal infections, including diagnostic and therapeutic modalities, are needed among clinicians and microbiologists to provide better patient care [6].

In our study, 1669 samples from various infections suspected of fungal etiology were received in the mycology division of our department during the period of analysis, out of which nail was the most frequent (n=340, 20.4%). Previous studies [9] have reported that onychomycosis is the commonest nail disorder encountered in clinical practice, constituting 20-40 % of all diseases of the nails [10] and 30 % of superficial mycotic infections [11]. In our study, nail infections amounted to 19.8% (n=330) of total fungal infections. Various Indian workers have reported the incidence to be 0.5 to 5% in the general Indian population [12]. This has been related to a variety of etiological factors, including a rise in immunocompromised patients, an aging worldwide population, and a rise in environmental risk factors secondary to life style changes. Dermatophytes, especially Trichophyton rubrum, are the most frequently implicated causative agents in onychomycosis. Previously regarded as contaminants, yeasts are now increasingly recognized as pathogens in finger nail infections, as are some moulds [13]. Another recent study from north India reported that the most common fungal isolates in onychomycosis were dermatophytes (49.5%), followed by Candida spp. (40.4%) and nondermatophyte molds (10.1%) [14]. In our study, dermatophytes accounted for 73/340 (21.5%) of fungal nail infections followed by Candida species in 41/340 (12%) cases (candida spp. 37, non-albicans candida 04), Aspergillus species accounted for 40/340 (11.8%) cases. When compared to other superficial mycoses, this condition is persistent, intractable and poses serious concern to treating clinicians as it often becomes a chronic source of recurrent superficial mycotic skin infections, besides causing considerable disfigurement.

Lower respiratory tract infections in patients with co-morbid conditions and who are hospitalized are associated with significant mortality and higher health care costs. Opportunistic fungal infections, in comparison to endemic mycoses, are the common causes of fungal pneumonia in developing countries such as India [15]. In a previous study [16], 43% of lower respiratory tract samples were positive for fungi in patients with chronic nonresolving respiratory infections. In our study, patients with high suspicion for fungal infections, whose lower respiratory specimens (BAL & sputum) were received in our lab [n=358 (BAL 294 & sputum 64)] had Community acquired pneumonia CAP (n= 112) which was the most common clinical diagnosis, followed by Lung consolidation (n=106), Pulmonary TB (n= 65), Lung abscess (n=25), Bronchiectasis (n=20), Lung carcinoma (n=15), Aspergilloma (n=08), Lung collapse (n=05) and Miliary TB (n=02). Candida species (n=94) and Non albicans candida (n=04) accounted for 98/358 (27.4%) fungal LRT infections, followed by Aspergillus species in 43/358 (12%) and Scopulariopsis species in 09/358 (2.5%) cases.

Fungal bloodstream infections are associated with significant patient mortality and health care costs. The inability to diagnose many invasive fungal infections in a timely manner continues to be a significant problem, and improved diagnostic methods are needed to permit early detection of infection. In our analysis, out of the total 35 blood culture samples submitted with a suspected fungal etiology,

candida grew in 7 samples (C. Krusei 04, C. albicans 02, and C. tropicalis 01), while Aspegillus niger grew in 01 sample. Candida bloodstream infections are a common cause of late-onset sepsis in the NICU and are associated with significant mortality and neuro developmental impairment [17].

Scarring of the cornea as a result of suppurative keratitis (Corneal ulceration) is an important cause of preventable blindness after unoperated cataract in some developing countries in the tropics [18]. Fungi are identified as the main etiological agent of corneal ulceration in as many as 2/3rd of these cases: a recent study from Ghana identified a 44% fungal etiology of suppurative keratitis in 800 patients of south India [19]. Reported incidences of fungal keratitis from different regions of India vary from 5% to 40% [20]. More than 105 species of fungi, classified in 56 genera, have been reported to cause oculomycosis [21]. In our study, 69 samples (Corneal scrapping 53, aqueous tap 01, and vitreous tap 15) were received from the department of Ophthalmology. Direct microscopy was positive in 17/69 (24.6) cases, while only 2 samples were culture positive that grew one each Candida albicans and non-albicans Candida.

In this study, 35.5% of fungal infections could have been diagnosed based on the findings from direct microscopy, but by culturing we were able to successfully identify 47.7% of the causative agents. Indicating that although difficult and time consuming, culture still remains the gold standard in regard to identifying the fungal isolate up to the species level, which is also pivotal in selecting a suitable antifungal therapy. However, in resource poor facilities where fungal culture may not be possible, direct microscopy of clinical samples is still an inexpensive yet effective method for the diagnosis of fungal infections.

The mycologist is responsible for implementing laboratory techniques for optimal detection and recovery of fungi in culture, for making an accurate identification, and, if necessary, for performing antifungal susceptibility testing [8]. Given the geographical location of Kashmir valley and its temperate climate with extremely cold winters (November to March) and a relatively milder summer with moderate humidity levels, when compared to the rest of northern Indian states doesn't make it a suitable environment for fungal infections, more so the superficial mycosis. That said, people at risk are always vulnerable to develop fungal infections.

Table 2; Age and gender-wise distribution of samples

Age group (years)	Male N (%)	Female N (%)	Total N (%)
0 - 19	71(4.3)	81(4.9)	152 (9.2)
20 - 39	251(15.1)	261(15.6)	512 (30.7)
40 – 59	357(21.3)	212(12.7)	569 (34)
60 & above	267(16)	169(10.1)	436 (26.1)
Total	946(56.7)	723(43.3)	1669 (100)

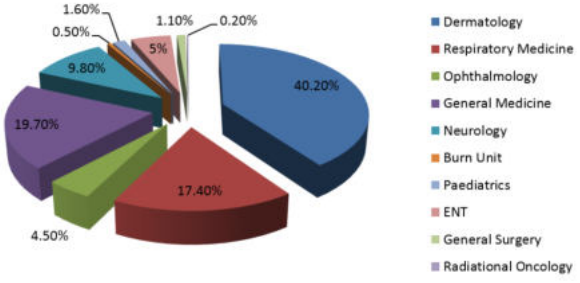

Fig 2; Frequency of samples received from different departments.

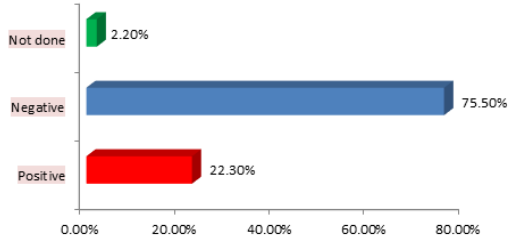

Fig 3; Direct microscopy results (KOH & Gram stain).

Table 3; Fungal isolates from different clinical specimens.

Not done	502	30%
No growth / Culture negative	593	35.50%
Aspergillus niger	44	2.60%
Aspergillus fumigatus	28	1.70%
Aspergillus nidulans	1	0.05%
Penicillium spp	19	1.10%
Trichophyton spp	39	2.30%
Epidermophyton spp	16	0.90%
Trichophyton mentagrophytes	29	1.70%
Trichophyton rubrum	19	1.10%
Candida glabrata	17	1%
Cladosporium spp	1	0.05%
Mucor spp	4	0.20%
Aspergillus spp	34	2%
Bipolaris spp	2	0.10%
Alternaria spp	8	0.50%
Trichophyton tonsurans	36	2.20%
Scopulariopsis brumpti	1	0.05%
Trichophyton verrucosum	8	0.50%
Aspergillus flavus	26	1.60%
Candida albicans	57	3.40%
Cryptococcus neoformans	1	0.05%
Candida spp	52	3.10%
Scopulariopsis spp	23	1.40%
Zygomycetes spp	1	0.05%
Non albicans Candida	11	0.70%
Candida tropicalis	30	1.80%
Malassezia spp	1	0.05%
Candida guilliermondii	4	0.20%
Paecilomyces spp	6	0.40%
Trichosporon spp	2	0.10%
Candida parapsilosis	9	0.50%
Candida krusei	12	0.70%
Fusarium spp	3	0.20%
Epidermophyton floccosum	7	0.40%
Microsporum spp	4	0.20%
Lecythophora spp	1	0.05%
Exserohilum spp	1	0.05%
Rhodotorula spp	4	0.20%
Rhizopus spp	13	0.80%
Rhizopus spp	13	0.80%
Total	(1669)	(100%)

Total Culture positive samples = 574 (34.4%)
Total Culture negative samples = 593 (35.5%)
Culture not done / Not requested = 502 (30%)

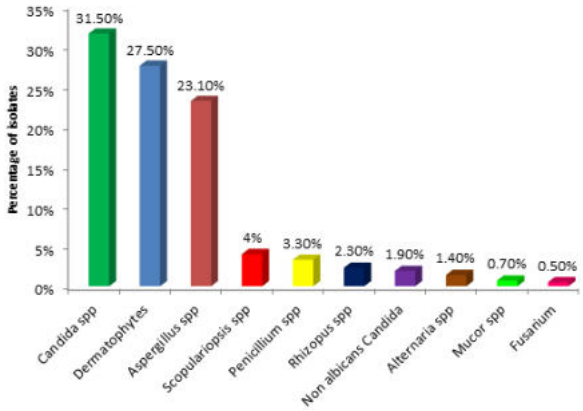

Fig 4; Distribution of different species of fungi from different clinical specimens.

Table 4; Relative proportion of Fungal isolates recovered from various clinical samples.

Fungal isolate	Skin & appendages* (n=346)	LRT Samples# (n=162)	Body fluids^ (n=05)	Blood & Bone marrow (n=07)	Urine (n=05)	Corneal scrappings (n=01)	Others (n=19)
Candida spp.	67	94	03	06	04	0	07
Dermatophytes spp.	155	03	0	0	0	0	0
Aspergillus spp.	77	43	02	01	0	0	10
Scopulariopsis spp.	14	09	0	0	0	0	0
Penicillium spp.	11	07	0	0	0	0	01
Rhizopus spp.	11	01	0	0	0	0	01
Non Albicans Candida	05	04	0	0	01	1	0
Mucor spp.	03	01	0	0	0	0	0
Fusarium	03	0	0	0	0	0	0

*Skin & appendages (Skin scrapping, skin biopsy, nails, hair, tissue)
#LRT (Lower respiratory tract – BAL Fluid & Sputum)
^Body fluids (CSF, aqueous tap, vitreous tap, pleural fluid, peritoneal fluid)
Others (Pus, swabs, nasal polyp tissue)

Table 5; Specimen wise correlation between direct microscopy and culture in clinical samples.

Specimen	Direct Microscopy	Culture Positive	Direct microscopy + Culture positive
Nail	118	182	80
Skin Scrapping	94	118	64
Skin Biopsy	09	07	05
Tissue	40	51	17
BAL Fluid	33	136	21
Pus	06	08	03
CSF	03	03	0
Nasal Polyp	17	15	10
Corneal Scrapping	13	01	01
Aqueous Tap	0	0	0
Vitreous Tap	04	01	01
Blood	01	08	01
Urine	04	06	03
Sputum	18	31	14
Pleural Fluid	03	01	0
Peritoneal Fluid	0	0	0
Hair	09	04	02
Swabs	0	01	0
Bone Marrow	0	0	0
Total (%)	372 (22.2%)	574 (34.4%)	222 (13.3%)

Total samples received = 1669 (From April 2018 to Oct 2020)
Total Direct microscopy positives = 372 (22.2%)
Total Culture positive samples = 574 (34.4%)
Total DM + Culture positives = 222 (13.3)

Table 6; Different diagnostic modalities used.

Method	No. of samples Tested	No. of positive samples
Direct microscopy (KOH, Gram stain)	1632	372
Culture	1167	574
Indian ink stain	319	03

Photo 1; Gram stain showing Budding yeast cells with pseudohyphae formation

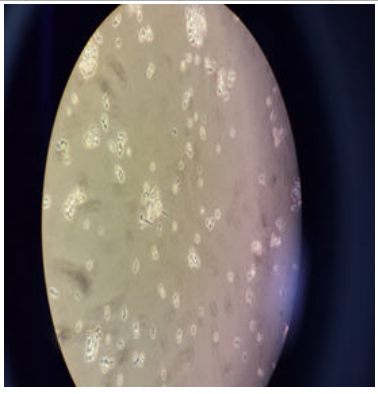

Photo 2; Yeast cells showing germ tube formation.

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CONFLICT OF INTEREST
There is no conflict of interest.

AUTHOR'S CONTRIBUTION
All authors have made a substantial, direct, and intellectual contribution to the research work, and approved it for publication.

FUNDING
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DATA AVAILABILITY
All data sets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
This study was approved by the ethical clearance committee.

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