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Spectrum of Superficial and Deep Fungal Isolates in Northern Pakistan

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Abstract

Fungi are an important cause of superficial and deep infections in our population. Lack of expertise in proper identification and inadequate diagnostic facilities often lead to underreporting of these infections and thus underestimation of true disease burden. This study was conducted at Department of Microbiology, Armed Forces Institute of Pathology Rawalpindi, Pakistan, from January 2011 through December 2013. Samples included specimen collected from superficial and deep tissues, respiratory tract specimen, blood, bone marrow and other body fluids. Skin (35.1%) and nail (10.2%) samples were the most common specimens from superficial body sites. Tissue specimens from various body organs and bronchoalveolar lavage fluid were the predominant specimens received for investigation of deep seated fungal infections, contributing 34.9% and 5.9% of the total specimens respectively. Yeasts were isolated from 75(22.6%) samples; different species of Candida accounted for majority of the isolates. Growth of molds was detected in 257(77.4%) samples with *Aspergillus* spp. accounting for 149 (44.9%) of the isolates. Among dermatophytes, *Trichophyton interdigitale* 13(3.9%) was the most common isolate. Moulds other than dermatophytes were also isolated from skin, hair and nail samples and *Alternaria alternata* (4.8%) was the most common non-dermatophyte isolated from these sites. Fungal infections and their spectrum varies considerably in different geographical locations and in all cases not responding to antibiotics and high risk groups, a possibility of fungal cause should be sought.

Keywords: *Aspergillus* species; Dermatophytes; Fungi; Moulds; Yeasts

Introduction

Fungal infections constitute a significant health problem in our country. Due to inadequate mycological diagnostic services a major percentage of these infections remain undiagnosed. Invasive fungal infections are a leading cause of mortality and morbidity in critically ill patients with or without malignancy and despite recent development of more active and less toxic antifungal agents, death rates from invasive fungal infections remain unacceptably high. [1,2]

The prevalence and pattern of fungal isolates is clearly dependent upon geographic location and climate. The hot and humid climate in our region favors fungal growth in the environments. [3] Same is true for the hospital set-ups where air-flow systems are mostly inadequate thus exposing the patients to a variety of fungi. Additionally, lack of awareness about the fungal illnesses or their existing pattern adds to the clinicians' problems.

The continuous surveillance of fungal isolates in a setup leads to better understanding among medical health professionals about fungi and their pathogenic potential which in turn helps in improving infection control practices. This study was conducted to find out latest pattern of fungal isolates in our set-ups as a continuum of updating the clinicians for guidance. [3,4]

Material and methods

The study was carried out at Microbiology Department, Armed Forces Institute of Pathology from January 2010 through December 2012. Permission was sought from Institutional Bioethical Committee. Sampling technique was non-probability convenience. All clinical samples for fungal culture received at AFIP were included in the study. No discrimination was made on the basis of age or gender.

Urine, stool, vaginal swabs and samples from the same patient were excluded from the study. A total of 1758 clinical specimens were included in this study. Skin, hair, nails were collected from patients suspected of superficial mycoses while samples like respiratory samples, cerebrospinal fluid and tissues from patients suspected of having deep seated fungal infections.

Scrapings from nail and hair samples were immersed overnight in a drop of 10% KOH over a slide and were kept inside humidified boxes. Non-dermatological samples like fluids were first centrifuged and sediments were used for microscopic examination to detect any fungal hyphae. The samples were simultaneously inoculated on Sabouraud's dextrose agar (SDA) (Oxoid, UK), SDA containing chloramphenicol, and SDA containing cycloheximide and chloramphenicol. The inoculated plates were incubated in cold incubator at 22°C after placing in polythene zipper bags to prevent contamination. [3,4] The culture plates were examined twice weekly for any growth.

Species identification was done through colony morphology and microscopic examination of fungal isolates by cellophane tape mount method. The origin of the colony was specially ensured to be from inoculation site in the medium plate. For rapid identification of fungal colonies, sticky side of cellophane tape was gently touched to the colony and placed on a drop of lactophenol blue on a glass slide and tape adhered to the slide. The slide was then examined by the consultant.

Yeast identification was done through Candida CHROMagar (DIFCO, USA) and API 20C Aux (bioMerieux, France). ATCC 10231

Candida albicans, ATCC 15126 *Candida glabrata*, ATCC 1022 *Aspergillus fumigatus* and ATCC *6663 Alternaria alternata* were used as control strains.

Results

A total of 1758 specimens were received for fungal culture, out of which 341 (19.3%) yielded fungal growth while 1417 (80.6%) were negative. Mean age of the patients was 39.8 ± 10 years; range 2 months to 85 years. Maximum cases were reported in 2010-11 most probably due to the heavy rains and inundation during the monsoon season with high humidity promoting fungal growth.

The specimen diversity was vast ranging from skin, hair and nails to CSF, tissue from different body sites, vitreous fluid, pleural fluid, joint fluid and fluid samples from other sterile sites. Specimen from respiratory tract sputum, bronchoalveolar lavage fluid and endobronchial washings, pus, eye swabs, ear swabs, blood and bone marrow were also received for fungal culture (Table 1).

Specimen	n(%)
Nail	618(35.1)
Tissue	614(34.9)
Skin	180(10.2)
EB washings/BAL	101(5.7)
Sputum	81(4.6)
Hair	49(2.8)
Blood	47(2.7)
Ocular specimen	38(2.1)
CSF	21(1.2)
Bone marrow	9(0.5)

Table 1: Specimen received (n=1758)

Significant growth of moulds was yielded in 266 samples accounting for 78% of the total isolates; *Aspergillus* species was predominant 148 (43.4%). Other major moulds isolated included *Alternaria alternata* 27 (7.9%), *Penicillium* spp 16 (4.7%), *Rhizopus arrhizus* 8 (2.3%), *Bipolaris hawaiiensis* 7 (2%), *Scytalidium dimidiatum* 6 (1.7%), *Fusarium dimerum* 6 (1.7%), *Cladophiapophora carrionii* 5 (1.4%) Yeasts were isolated from 75 samples constituting 22% of the total yield; *Candida* spp. accounted for majority of the isolates (Table 2).

Isolate	n(%)
Moulds (n=232)	
Aspergillus fumigatus	65(19)
Aspergillus flavus	43(12.6)
Aspergillus niger	33(9.6)
Alternaria alternata	27(7.9)
Penicillium spp	16(4.7)
Rhizopus arrhizus	8(2.3)
Bipolaris hawaiiensis	7(2)
Aspergillus tereus	7(2)
Scytalidium dimidiatum	6(1.7)
Fusarium dimerum	6(1.7)
Ulocladium chartarum	6(1.7)
Cladophialophora carrionii	5(1.4)
Paecilomyces lilacinus	1(0.3)
Paecilomyces variotti	1(0.3)
Scedosporium apiospermum	1(0.3)
Yeasts (n=75)	
Candida albicans	26(7.8)
Candida glabrata	24(7.2)
Candida krusei	11(3.3)
Candida tropicalis	10(3)
Candida parapsilosis	2(0.6)
Cryptococcus neoformans	2(0.6)

Table 2: Significant fungal isolates (n=307)

Among dermatophytes, *Trichophyton interdigitale* 13 (3.8%) was the most common isolate followed by *Trichophyton mentagrophytes* 10 (2.9%), *Trichophyton violaceum* 5 (1.4%), *Trichophyton rubrum* 2 (0.6%), *Trichophyton tonsurans* 2 (0.6%); details are given in Table 3.

Isolate	n(%)
T. interdigitale	13(3.9)
T. mentagrophytes	10(3)
T. violaceun	5(1.5)
T. rubrum	2(0.6)
T. tonsurans	2(0.6)
T. verrucosum	1(0.3)
E. floccosum	1(0.3)

Table 3: Dermatophytic isolates (n=34)

Moulds other than dermatophytes were also isolated from skin, hair and nail samples like *Alternaria alternata* 16 (4.7%). Site-wise distribution of important fungal isolates is given in Table 4.

	Dermatophytes	Aspergillus spp	Candida spp	Alternaria alternata	Rhizopus arrhizus	Scytalidium dimidiatum	Fusarium

							dimerum
Skin	10	-	-	5	1	-	-
Hair	4	-	-	-	-	-	-
Nail	20	-	15	11	-	2	-
Tissue	-	41	17	11	5	3	2
BAL	-	63	7	-	2	-	-
Sputum	-	35	26	-	-	-	-
CSF	-	-	-	-	-	-	-
Pus	-	7	-	-	-	-	-
Blood	-	-	8	-	-	-	-
Ocular	-	2	-	-	-	1	4

Table 4: Site-wise distribution of important fungal isolates (n=302)

Discussion

Fungi are rampant in our country due to warm and humid climate and so are the fungal infections. The lack of requisite diagnostic infrastructure and expertise leads to underreporting of actual disease burden from fungal infections. Both healthy and immunocompromised populations are prone to various forms of fungal ailments ranging from superficial skin infections to life threatening invasive fungal infections respectively. [1,5] The successful management of infections caused by fungi depends on early diagnosis, control or reversal of any underlying disease and effective antifungal therapy.

Aspergillus spp are an important cause of mortality in hematopoietic cell transplant, solid organ transplant, patients of hematologic malignancies and in intensive care units. [7] *Aspergillus fumigatus* is the most frequent species of *Aspergillus* causing clinical disease however, other species, most commonly *A. flavus*, *A. terreus*, and *A. niger*, are also implicated in invasive infections in humans. A. terreus has been associated with amphotercin B resistance. [7] In immunocompromised hosts, *Aspergillus* most commonly presents as invasive pulmonary aspergillosis leading to subsequent dissemination.

In our study *Aspergillus* species constituted 43.4% of positive samples out of which *Aspergillus fumigatus* was the most frequent isolate followed by *Aspergillus flavus* and *Aspergillus niger*. Most of these samples were received from Armed forces Bone Marrow Transplant Centre Rawalpindi, Oncology Department and intensive care units. Invasive Aspergillosis was the most common life threatening infection encountered in the hematopoietic stem cell transplant population accounting for 43% of invasive fungal infections according to the Transplant Associated Infections Surveillance Program (TRANSNET), a network of 23 transplant centers in the United States, *Aspergillus* spp were the most common fungal pathogens in lung transplant recipients as well. [6] *Aspergillus niger* is an important causative agent of otomycosis and was also the predominant pathogen in our study from otorhinology clinic mainly patients of otitis externa; similar observation have been reported previously. [7,8] *Aspergillus* spp. was the most common non-dermatophyte mould isolated from study conducted by Vyas et al from Jaipur, India followed by *Fusarium* spp. [9]

Candida spp can cause opportunistic infections after long antibiotic therapy and immunosupression when the normal flora is disturbed. [10] Among solid organ transplant (SOT) recipients, Candida infections are the commonest cause of invasive fungal infection. [10] Use of azoles in prophylaxis and first line drugs against candidiasis has led to emergence of candida species with resistance to azoles like *Candida glabrata* and *Candida krusei*. [11] In our isolates, only 26 out of 75 (34.6%) candida isolates were *Candida albicans* and others belong to other candida species predominantly *Candida glabrata*, *Candida krusei* and *Candida tropicalis*. Candida spp. was also reported as an important cause of onychomycosis by Ahmed et al. [12] In our study Candida albicans was the most frequent isolate followed by *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. parapsilosis*. There were 8 blood cultures positive for growth of *Candida* spp; 4 isolates were *C. albicans*, and 2 *C. glabrata*. One isolate each was identified as *C. krusei* and *C. parapsilosis* were isolated from blood culture specimen. Candida spp were predominant isolates from BAL/EB washings, sputum and tissue specimens in our study.

Most cutaneous infections are the work of the homogeneous group of keratinophilic fungi known as dermatophytes. In our study *Trichophyton interdigitale* was the most predominant pathogen in skin, nail and hair specimens. Previous data published from our institute had a similar pattern. [4,13] However, in recent years non-dermatophytes have emerged as an important cause of onychomycosis in Pakistan. [13,14] Reports from different parts of the world including India, Sri Lanka, Brazil and Argentina have also described non-dermatophytes filamentous fungi as an important cause of onychomycosis. [15-18] Among non dermatophytes *Alternaria alternata*, *Aspergillus glaucus*, *Aspergillus tereus* and *Fusarium* species were the predominant isolates in our setup. *Ulocladium chartarum*, Scedosporium apiospermum, Scedosporium prolificans and *Cladophialophora carrionii* were also isolated from a few cases highlighting the role in superficial infections.

Zygomycetes are quite prevalent in the surrounding. Zygomycetes, *Rhizopus* spp, *Mucor* spp and *Absidia* spp can can opportunistic infections. The range of these infections range from skin and

subcutaneous infections to life threatening rhinocerebral zygomycosis and rhino-orbital zygomycosis which occur in diabetes mellitus and other immunocompromised states [19-22]. In our study *Rhizopus arrhizus* was the most common isolate among Zygomycetes. The isolates were recovered from Bronchoalveolar lavage, sputum and tissue samples.

Hyaline hyphomycetes are responsible for invasive fungal infections in immunocompromised individuals. *Fusarium* spp, *Scedosporium* spp and dematiaceous fungi are emerging pathogens in solid organ transplant recipients. [23] *Fusarium* spp, *Scedosporium apiospermum* are important cause of and fungal keratitis after trauma and corticosteroid use. [24] *Scytalidium dimidiatum* is a dematiaceous mould and can cause opportunistic infections. It has been isolated in our setup in invasive infections in an immune competent soldier. [25] With timely diagnosis and treatment, this case fully recovered.

Cladophialophora carrionii is an agent of chromoblastomycosis a chronic infection involving skin and subcutaneous tissue but can cause opportunistic invasive infections in immunocompromised patients we also encountered this fungus in five cases all were isolated from tissue specimen. [26] Scedosporium species are involved in a wide range of human infections; especially in immune-compromised patients *Scedosporium* spp isolates were isolated from a wide range of geographical origins and from divergent environmental and clinical sources. Cerebral abscesses are relatively frequent reflecting the neurotropic nature of these fungi. [27,28] *Scedosporium*/ *Pseudallescheria* isolates were isolated from a wide range of specimen but predominantly from tissue specimen.

Conclusion

Superficial and deep fungal infections are quite common in our setup. The possibility of fugal etiology must be kept in mind when clinical symptoms are pointing or even vague. The prevailing pattern of fungal isolates thus gains paramount importance for the clinicians to diagnose and modulate treatment of the patients accordingly.

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