ASSESSING THE MYCOLOGICAL SPECTRUM OF INFECTIONS OVER A COURSE OF THREE YEARS

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ABSTRACT

Objective: To determine the spectrum of fungal isolates in superficial as well as deep seated infections in samples received from Federal Capital and Rawalpindi.

Material and Methods: It is a cross sectional study conducted over duration of three years at Department of Microbiology, Islamabad Diagnostic Center. The samples included specimens, collected from superficial and deep seated tissues/fluids.

Results: Skin (n=71) and nail (n=22) samples were the most common specimens from superficial body sites. In deep seated specimen's sputum/oral cavity samples and body fluids comprised of predominant samples, contributing 46.6% and 18.3% respectively. Overall out of total 60 positive cultures for deep seated infections 53.3% were positive for Candida and 23.3% for Aspergillus. Whereas the isolates found in superficial infections comprised mostly of Aspergillus (42.7%) and Epidermophyton (28.1%).

Conclusion: In different geographical locations, fungal infections and their spectrum vary considerably and in cases with high probability and clinical suspicion of fungal infection, mycological workup for the exact diagnosis is direly needed.

Key words: Fungal isolates, superficial infections, Candida.

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INTRODUCTION

Human beings live in amicable harmony with the microorganisms that surround them. Infections only emerge when the concentration of pathogens reaches an exceptionally high density or the human defense system is damaged. Etiology of infectious diseases includes bacteria, viruses, parasites, fungi, prions, worms and helminthes.

Due to the emergence of effective strategies to control bacterial infections in patients, fungi have become the most hazardous pathogens now. In patients admitted in Intensive Care Units, yeasts and molds are now among the 10 most frequently isolated pathogens. In approximately 7% of all patients with neutropenia febrile episodes are due to invasive fungal infections[1]. Whereas, in general population, fungal infections are reportedly affecting 3%-5% of people[2].

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The geographic location and climate clearly affects the prevalence and pattern of fungal isolates. The hot and humid climate in our region erroneously favors fungal growth in the environment[3]. Fungal infections comprise a significant health problem in our country. A major percentage of these infections are undiagnosed due to inadequate mycological diagnostic facilities[4]. The exact burden of fungal infection in Pakistan is not known, however fungal keratitis has an estimated rate of 44/100,000. After reviewing published data from Pakistan reporting fungal infections, it was found out that Pakistan has probably a high rate of unreported life-threatening fungal infections[5].

Cutaneous fungal infections are superficial infections typically affect skin, hair, and nails. Most commonly, these fungal infections are caused by dermatophytes (fungal organisms that causetinea) and non-dermatophyte fungi and yeast (Candida species). Three dermatophyte genera that are identified in U.S are Microsporum, Epidermophyton, and Trichophyton. Among them approximately 80% of dermatophytic infections are caused by Trichophyton6. As the airways are constantly exposed to environmental fungi, the most common ones isolated in chronic respiratory diseases are Candida albicans and Aspergillus spp. Out of these, various species of Aspergillus have the greatest pathogenic potential[7,8,9]. During reproductive

ages among women, Vulvovaginal candidiasis is a common fungal infection and studies have shown that different species of Candida are among the most common etiological agent of vaginitis[9,10,11].

In critically ill patients, invasive fungal infections are a leading cause of mortality and morbidity. Despite the advent of recent, more effective and less toxic antifungal agents, death rates from invasive fungal infections remain high[7,8]. In hospitals in the USA, Candida has emerged as the fourth leading bloodstream isolate, surpassing many bacterial pathogens[9].

Proper identification and treatment of fungal skin infections is a growing health concern. That is why, continuous surveillance of fungal isolates may lead to a better understanding about fungi and their pathogenic potentials ultimately leading to improvement in infection control practices[4,10,11]. Our study was conducted in order to look for the latest patterns of fungal isolates in our set-up as an effort of updating the clinicians.

MATERIAL AND METHODS

The study was conducted over a course of three years (Aug'2016- Aug'2019) at Islamabad Diagnostic Center (IDC). Permission was taken from Institutional Ethical Committee. All clinical samples for fungal culture received at IDC were included in the study. A total of 375 clinical specimens were part of the study. Skin, hair, nails were collected from patients who were having a clinical suspicion of superficial fungal infections. Whereas, samples like respiratory fluids, body fluids and tissues were collected from patients, who were suspected of having deep seated fungal infections.

The proper collection of relevant clinical material was done as per following recommendations.

Skin scrapings: The skin was cleaned thoroughly with 70% ethanol. A disposable sterile scalpel was used to scrape across the inflamed margin of the lesion into the apparently healthy tissue.

Skin stripping: A water-proof transparent vinyl tape was firmly applied to the affected area and then peeled off. This tape was then applied to a sterile glass slide for transport to the laboratory

Nail: Friable material was removed from under the nails. Clippings were also taken from the distal border of the nail with scissors or nail clippers.

Hair: Infected hair was removed by plucking with epilating forceps.

Sputum: Purulent sample were obtained if pulmonary fungal infection was suspected.

CSF: blood and other body fluids: They were collected with precautions so as to avoid skin yeast contamination.

Sample Storage & Transportation: The skin, hair and nail were allowed to dry to prevent the over growth of saprophytic bacteria for which a black paper packet was used for storing and transporting[12]. Clinical material was inoculated on Sabouraud's dextrose agar (SDA) (Oxoid, UK), containing chloramphenicol and cycloheximide and placed in incubator at 22◦C and evaluated daily for any fungal growth. Species identification was done through colony morphology and microscopic examination of fungal isolates by cellophane tape mount method[13]. The slides were then examined by the consultant microbiologist.

Data was collected with standardized forms and transferred daily to a server. The descriptive data was analyzed using SPSS version 25.0.

RESULTS

Total of 375 patients were included in our study. Out of them 156 were positive for fungal isolates (41%). These patients comprise of 63 male (40%) and 93 (60%) females (figure.1). 144 patients (92%) were adults (>17 age) and rest were children (figure.2).

Out of the total 156 positive cultures, 96 samples were from superficial sites while 60 samples were from deep seated locations. Overall the isolates found in superficial infections comprised mostly of Aspergillus (42.7%) and Epidermophyton (28.1%). Candida and Trichophyton were positive in 8.3% each where as Penicillium was detected in 6.6% samples.

In superficial infections most of the samples received were those of nails (n=71) and skin (n=22), making percentages of 74% and 23% respectively. Out of 71 positive isolates of nail 33(46.4%) were positive for tAspergillus, 20(28%) for Epidermophyton, 5(07%) for Penicillium and Trycophyton 4(5.6%) for Candida. The 22 positive skin samples were also showing significant yield for Aspergillus 8(36.6%) and 6(27.2%) for Epidermophyton. Three patients were found positive for Trichophyton (13.6%) (Table.1). Identification of the strains was done on the basis of distinctive morphological and growth patterns.

In deep seated samples, sputum/ oral cavity samples and fluids made significant percentages of specimen (i.e. 46.6% and 18.3% of patients respectively). Out of 28 samples of sputum, majority of them i.e. 19/28 (67.8%) were showing positivity for Candida. 8 out of 11 samples of body fluid were positive for aspergillus (72.7%) and 2 (18.1%) for Candida. Out of the 8 samples of bronchioalveolar lavage (BAL), most of them were showing growth of Candida (62.5%) see Table.2 for details.

Table 1: Site wise distribution of superficial infections.

Superficial Infections	Aspergillus	Aspergillus and candida	Aspergillus and epidermidis	Bipolarisspp	Candida	Epidermophyton	Epidermophyton and penicillium	Penicillium	Rhizopus	Trichophyton	Aspergillus and trichophyton	Total
Ear	-	-	-	-	1	-	-	-	-	-	-	1
Hair	-	-	-	-	1	1	-	-	-	-	-	2
Nail	33	-	1	1	4	20	1	5	1	5	-	71
Skin	8	1	-	-	2	6	-	1	-	3	1	22
Total	41	1	1	1	8	27	1	6	1	8	1	96

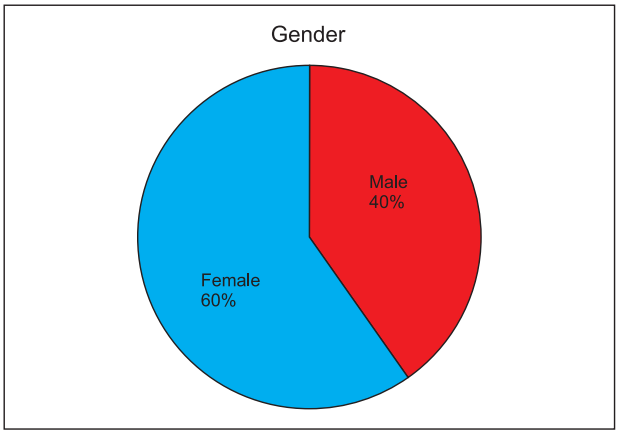

Fig 1: Gender distribution among positive isolates.

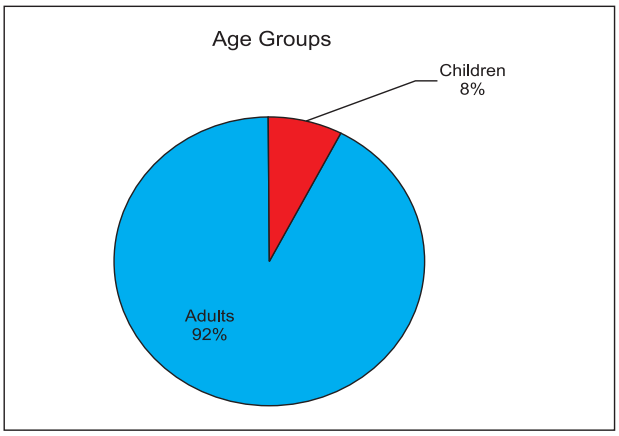

Fig 2: Age wise distribution in positive isolates.

DISCUSSION

Nowadays, fungal infections are among the most difficult diseases to be managed in human beings[14]. Early accurate diagnosis allows prompt initiation of antifungal therapy, however if it is delayed or unavailable, it may lead to serious outcomes and is always challenging especially in under resourced settings[15,16] like ours.

Superficial fungal infections are important because of their frequency, global distribution, person to person transmission, and morbidity[17,18]. Invasive fungal infection involve blood or invade into organ tissue and are also referred to as deep, deep-seated, disseminated, and systemic fungal infection[19]. Although superficial infections account for much of the global prevalence of fungal infections[20], invasive fungal infections are associated with high morbidity and mortality[21]. Many factors have contributed to the emergence of invasive fungal infections, e.g. HIV infection, patients receiving immunosuppressive treatment, indwelling prostheses, diabetes mellitus, burns, patients taking broad-spectrum antibiotics and individuals with frequent nosocomial exposure.

In the past few years, fungal diseases caused almost over 1.6 million deaths annually and over one billion people suffer from severe fungal diseases worldwide.

Nearly a billion people are estimated to have superficial fungal infections, and more than 150 million people have serious fungal diseases, which have significant morbidity or mortality[16,22,23]. Recent global estimates suggest 3,000,000 cases of chronic pulmonary aspergillosis, 223,100 cases of cryptococcal meningitis, 700,000 cases of invasive candidiasis, over 500,000 cases of Pneumocystis jirovecii pneumonia, 250,000 cases of invasive aspergillosis, 100,000 cases of disseminated histoplasmosis, and 1,000,000 cases of fungal keratitis occurring annually[24].

Candida, Aspergillus, and Cryptococcus are among the most common fungal pathogens that cause life-threatening infections across the world each year 25 and goes in parallel with our study which also shows a significant prevalence of fungal infections, both deep seated and superficial especially due to Candida and Aspergillus.

Previous data published in Pakistan show that most of superficial infections are due to dermatophytes[11,26]. However, recently non-dermatophytes have emerged as an important cause of onychomycosis here as well as in India, Sri Lanka, Brazil and Argentina[27]. Our study is also revealing non dermatophytes (predominantly Aspergillus) as the predominant pathogen in skin, nail and hair specimens.

A study conducted in Iran had reported that fungal isolates in deep seated infections are mostly Candida followed by Aspergillus[28]. The same spectrum of fungi was isolated from our samples of deep seated infections as well.

There is limited knowledge about the global incidence of fungal diseases because of lack of regular national surveillance systems, poor clinician suspicion, diagnostic tests, and well-designed research studies[29,30].

CONCLUSION

Superficial and deep fungal infections are quite prevalent in our set up, most likely due to the climatic conditions, unhygienic circumstances, use of contaminated tools and compromised immunity. In case of the slightest clinical suspicion, mycological etiology must be worked up on. Thus, the assessment of pattern of fungal isolates is important for the clinicians to diagnose and treat the patients accordingly.

RECOMMENDATIONS

However, more studies, with larger sample sizes are required for a better approach to study the epidemiology of fungal infections nationwide. Mycological assessment should be done without delay on the basis of clinical doubt.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Khan AA:	Concept, study design, manuscript writing, facilitation of the reagent and materials.
Uppal R:	Study design, Facilitation of the reagent and materials, critical review.
Rehan GE:	Analysis, interpretation, manuscript writing, study conduction.
Zaib H:	Study conduction, discussion, manuscript writing.
Khurshid F:	Critical review, study design , study conduction.
Ahmad K:	Planning, study conduction, critical review.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.