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Original Research Article

A SYSTEMATIC CASE STUDY ON FUNGAL INFECTION: TYPES AND DIAGNOSTIC TOOL

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ABSTRACT

Fungi are one of the most well-known microspecies present in wide areas. Specific species as well as variants of fungi are responsible for various types of diseases as the recent one was fungal infections caused after Covid. Types of infections spreads through these variants are one of the most arising problems which have to resolve in order to make easy diagnosis and cure. In given investigation different types of diagnostic techniques define in elaborative forms to generate a better cure and development of advanced medication. Systematic survey regarding effects of cumulative analysis gives us a concise overview to develop a proper vision to understand number of infected individuals in different age groups and from different regions. These surveys help us to clear our vision towards diseases and their diagnosis and a well suitable method which can be design to cure such a vast ill effects of these microorganisms. In our present research, diagnostic tools clear out all possibilities to make a proper analysis. Concise and systematic survey brings positive results which will be helpful in analytical and curative parts of research.

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INTRODUCTION

The prevalence of fungal infection among critically ill patients poses a significant challenge with its exact extent being challenging to ascertain. The EPIC study, which provided a snapshot of infection rates in the intensive care unit (ICU), indicated a high incidence of fungal infection although this may not be consistent across all units due to variations in patient populations. Factors such as the treatment of increasingly unwell patients, widespread use of potent broad spectrum antibiotics, and heightened clinician awareness could potentially contribute to a rising incidence of these infections.[1] A fungal infection, also known as mycosis, is an illness that results from fungal growth and can impact different areas of the body, such as the skin, the tissues beneath it, and more internal organs. The signs of this infection can vary from itchy rashes in the early stages to the formation of lumps and alterations in the skin in more advanced cases, and may also feature symptoms similar to those of pneumonia or meningitis in widespread infections.[2,3] Mycoses involves different types of human diseases include Athlete's foot may progress to severe secondary infections, resulting in significant morbidity and even mortality. Mycoses encompass a range of common human diseases; from normal to severe infections create increased death rates during last decades. The former group includes superficial infections that can become highly prevalent in normal populations, requiring specific public health interventions. The patient with compromised

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immunity is particularly susceptible to fungal infections due to impaired immune function, while this may be a localized issue in a healthy patient. Additionally, the possibility of treatment creates low possibility of a worse condition [4].

Systemic fungal infections can be divided into three main groups: those that occur in compromised patients, those that are persistent to native regions, and opportunistic infections. Opportunistic infections are influenced by different factors and are found in various climates. In tropical regions, systemic fungal infections primarily belong to the endemic respiratory infections group, with the skin being involved in some cases due to bloodstream spread or direct introduction by inoculation. Additionally, infections affecting patients with HIV/AIDS may exhibit cutaneous involvement, and can also occur in travelers visiting endemic areas for a short time [5,6].

Fungal infections are typically categorized based on the affected body part: superficial infections impact the skin, such as tinea and yeast infections; subcutaneous infections affect tissues under the skin, like eumycetoma and chromoblastomycosis; and systemic infections are more severe, including various types of threaten diseases. The characteristic symptoms, ranging from rashes in superficial infections to lumps and skin changes in skin or subcutaneous infections, and pneumonia-like symptoms or meningitis in deeper or systemic infections.[7] In the upcoming section, we will cover the prevalence of Candida infection, which is widespread. Aspergillus is more prevalent in immune-compromised individuals but is generally less common in the intensive care unit (ICU). This article does not cover Cryptococcus, Fusarium, and other fungal issue.

The conventional method of diagnosing and treating fungal infections is based on the strategy used for immuno-compromised patients. However, there are significant distinctions between these patients and the critically ill population, and applying the same approach to both groups may pose challenges [8,9].

Fungi [10]

Fungi are ubiquitous in nature, yet only a limited number are pathogenic to humans. Fungal infections may occur via inhalation of spores, direct contact with the skin, or entry through breaches such as cuts, wounds, or injections. These infections are more prevalent in immunocompromised individuals, including those with HIV/AIDS or patients receiving immunosuppressive therapy. Pathogenic fungi encompass yeasts, molds, and dimorphic species. Among them, *Candida albicans* is notable for causing superficial candidiasis in otherwise healthy individuals, while in immunocompromised hosts, it may lead to life-threatening systemic candidiasis.

Epidemiology of fungal infection [12,13]

- Fungal infections affect over one billion individuals globally each year, resulting in an estimated 3.75 million deaths in 2025 [14,15,16]. This burden has continued to increase, with approximately 1.7 million deaths reported in 2020. Additionally, fungal infections are a significant contributor to morbidity and mortality, particularly among children.
- According to the Global Action Fund for Fungal Infections [17,18], more than 10 million cases of fungal asthma, approximately 3 million cases of chronic pulmonary aspergillosis, 1 million cases of blindness caused by fungal keratitis, over 200,000 cases of cryptococcal meningitis, 700,000 cases of invasive candidiasis, 500,000 cases of pneumocystis pneumonia, 250,000 cases of invasive aspergillosis, and 100,000 cases of histoplasmosis are reported annually. During the COVID-19 pandemic, several fungal infections have been associated with the disease. These infections may mimic COVID-19 symptoms, occur concurrently with it, or complicate its progression [19,20]. Furthermore, fungal infections can develop following the use of antibiotics to treat secondary bacterial infections in COVID-19 patients. Among the most common and serious fungal infections observed in individuals with COVID-19 are aspergillosis and invasive candidiasis [21,22].

MATERIALS AND METHODS

Ethical Clearance for Fungal Studies

The study involving fungal infections has been reviewed and approved by the Institutional Ethics Committee (IEC) in accordance with national and international ethical guidelines for research involving human participants.

Prior to sample collection, written informed consent is obtained from all participants or their legal guardians, explaining the purpose of the study, the procedures involved, potential risks and benefits, confidentiality of data, and the right to withdraw from the study at any time without any consequences to their medical care.

The study ensures that the collection of clinical specimens such as skin scrapings, hair, or nail samples is performed using minimally invasive procedures and with strict adherence to hygiene and safety protocols to prevent infection and discomfort. Special care is taken while working with vulnerable populations, such as immunocompromised patients and children.

All personal and medical information is handled with strict confidentiality, and data is anonymized for analysis. The research does not interfere with standard patient care and treatment and is conducted with the intent to contribute to better understanding, diagnosis, and management of fungal infections.

Ethical clearance has been granted by the Institutional committee with approval number MU/IIP/PO/2025/008, and the study will be conducted with integrity, transparency, and respect for human dignity.

RESULTS

Diagnosis of fungal infection [23,24]

The standard procedure comprises the following steps

Scraping or *rasping* [25]

"During this process, infected lesions are scraped to confirm the presence of fungal elements through microscopic examination. Before sample collection, the lesions should be thoroughly cleaned of debris and topical medications by using alcohol or washing with water. Specimens should be collected

from multiple sites to prepare separate potassium hydroxide (KOH) mounts. The application of a small amount of distilled water at the site can facilitate easier sampling of scales. One hand should be used to gently stretch the skin above the lesion, while the other hand scrapes the edge of the lesion with the scalpel.

For hairy areas such as the scalp and beard, it is important to carefully scrape the edges to effectively remove short hair stubs and crusts. In cases involving the nails, deep scraping of the affected area is recommended to collect keratinous material for better diagnostic yield. Nail clippings or avulsed nail portions can be securely held with forceps, and their undersurfaces or dystrophic edges can be scraped for microscopic evaluation."

Potassium hydroxide mount or fungal wet mount [26]

To prepare a KOH mount, gently scrape the skin using a pre-flamed blunt scalpel from the edge of the lesion, and collect the scrapings either on black paper or directly onto a clean glass slide. Add a few drops of 10% potassium hydroxide (KOH) solution to the specimen, cover it with a cover slip, and gently preheat the slide to enhance the digestion of keratin. After allowing it to stand for a few minutes, examine the specimen under a microscope to identify fungal elements such as hyphae, spores, or yeast cells.

Microscopic Examination of Fungal Elements

This process involves closely observing the specimen under a microscope to detect fungal spores or hyphae. The examination is typically carried out in two stages:

Initial Observation: The specimen is first examined using low-power magnification (×10) with low intensity lighting. The condenser is adjusted appropriately to ensure adequate contrast and visibility.

Detailed Observation: After locating potential fungal elements, the magnification is increased to x 40. The condenser is raised to enhance illumination, allowing for a more detailed study of fungal morphology, including the structure and branching of hyphae. Fungal spores generally range from **2 to 10 µm in diameter**. However, caution must be exercised during interpretation, as certain artifacts may mimic fungal structures. For example:

- The junctions between normal epidermal cells may dissolve, forming patterns resembling a branching network, often referred to as **"mosaic fungus,"** a common artifact.
- Other non-fungal elements such as **cotton fibers** and **synthetic fibers** may appear similar to fungal hyphae and must be differentiated carefully.

Proper sample preparation, magnification, and interpretation are essential to avoid misdiagnosis.

Diagnostic Uses of KOH Examination**[27]**

1. Dermatophytic and Candidial Infections of the Skin[28]

Potassium hydroxide (KOH) examination is commonly used to diagnose dermatophytic and candidial infections affecting the skin. A smear prepared from scales or erosions collected from lesions reveals fungal elements such as **hyphae**, **pseudohyphae**, and **yeasts** under the microscope.

2. Dermatophytic Infection of Hair (Including Black Piedra)

For hair infections, affected hairs—often appearing as broken stubs—are ideal for examination. These hairs can be carefully removed with forceps without causing undue trauma, or collected by gently rubbing with a moist gauze pad or toothbrush.

When examined with KOH mount:

Endothrix infection: Fungal spores are found packed inside the hair cortex and sometimes also outside the cortex. This may be associated with hair shaft dystrophy.

Ectothrix infection: Spores are seen grouped and attached outside the hair cortex, forming a sheath around the hair.

These morphological features help distinguish the type of hair infection and guide appropriate treatment.

Diagnostic Uses of KOH Examination in Nail Infections [29,30]

Dermatophytic and Candidial Infections of the Nails

Potassium hydroxide (KOH) examination is useful for identifying fungal infections of the nails. Scrapings collected from the affected nail plate, when examined under the microscope, reveal **hyphae**, **pseudohyphae**, and **blastoconidiating yeast cells**. These structures are indicative of dermatophyte or candidial infections and assist in confirming the diagnosis.

Diagnostic Use of KOH Examination in Pityriasis Versicolor [31]

Pityriasis Versicolor

In cases of pityriasis versicolor, a potassium hydroxide (KOH) examination of scrapings from the lesions reveals characteristic fungal elements. Under the microscope, the sample shows clusters of **short hyphae** and **round yeast cells**, often described as having a **"spaghetti and meatballs"** or **"banana and grapes"** appearance. This distinctive morphology helps confirm the presence of *Malassezia* species, the causative fungi in pityriasis versicolor.

Diagnostic Use of KOH Examination in Vaginosis [32]

Bacterial Vaginosis

Bacterial vaginosis is a common cause of vaginitis in sexually active women of childbearing age. For diagnosis, a sample of vaginal fluid is tested for increased **pH**, and **10% potassium hydroxide (KOH)** is added to the discharge on a glass slide to check for a characteristic **fishy odor**, known as the **whiff test**.

Additionally, a **wet mount preparation** can be examined immediately under a light microscope to identify **"clue cells"**, which are vaginal epithelial cells coated with *Gardnerella vaginalis* bacteria. The presence of clue cells is a key diagnostic feature of bacterial vaginosis.

Demonstration of Fungi from Cutaneous Lesions in Deep Fungal Infections [33,34]

Deep fungal infections involving cutaneous lesions can be diagnosed by microscopic examination using potassium hydroxide (KOH) preparations:

1. **Cutaneous Cryptococcosis**

In cases of cutaneous cryptococcosis, the presence of *Cryptococcus* encapsulated yeast forms can be demonstrated by mounting a drop of **serum or exudate** from the lesion onto a glass slide with a few drops of **10% potassium hydroxide** and **India ink**. The India ink highlights the capsule as a clear halo surrounding the yeast cells.

2. **Chromoblastomycosis**
   For chromoblastomycosis, **sclerotic (muriform) fungal cells** can be observed by preparing a KOH mount from **skin scrapings** collected from the surface of lesions.
3. **Blastomycosis**
   In blastomycosis, **spherical yeast cells with broad-based buds** can be visualized in a KOH preparation of **pus, skin scrapings, or sputum** samples from the infected area.

These microscopic features are essential for confirming deep fungal infections and differentiating among various fungal pathogens

Advantages:

- **Rapid Results:** KOH preparation provides quick results, often within minutes.
- **Cost-Effective:** The procedure is inexpensive compared to other diagnostic methods.
- **Simplicity:** It is easy to perform and does not require sophisticated equipment.

Limitations:

- **Specificity:** While KOH preparation can confirm the presence of fungal elements, it does not identify the specific species of fungus.
- **Sensitivity:** The sensitivity of the test may vary, and false negatives can occur if the fungal load is low or the sample collection is inadequate.

Sign and Symptoms [35,36]

The severity of fungal infections varies depending on the causative species and the host's immune status. Clinical manifestations are diverse and may include respiratory symptoms resembling asthma, persistent fatigue, headache, myalgia, night sweats, unexplained weight loss, chest discomfort, and dermatological signs such as pruritus or scaly lesions. Fungal pathogens can involve multiple organ systems, with infections affecting the skin, hair, lungs, gastrointestinal tract, bloodstream, and even the central nervous system.

Risk factor [37,38]

Individuals with compromised immune function are particularly susceptible to fungal infections. This includes patients with HIV/AIDS, those receiving corticosteroid therapy, or individuals undergoing chemotherapy for malignancies. Additional risk groups comprise individuals with diabetes mellitus, the elderly, neonates, and patients receiving broad-spectrum antibiotics, as these factors disrupt normal host defenses and microbiota. Furthermore, immunocompromised pediatric populations, such as children undergoing cancer treatment, are at significant risk of developing invasive fungal infections.

Causes of fungal infection [11,39]

Fungal infections, collectively referred to as *mycoses*, are caused by diverse groups of fungi, including yeasts, filamentous molds, and dimorphic species capable of transitioning between yeast and mold forms. These infections are globally prevalent and may arise through inhalation of fungal spores, direct cutaneous contact, or entry via breaches in the skin, such as wounds or invasive medical procedures. Among pathogenic fungi, *Candida albicans* is the most frequently implicated species, commonly producing mucocutaneous infections such as oral and vaginal candidiasis, particularly following disruption of the normal microbiota by antibiotic therapy

Types of fungal strains cause infection

- Candidiasis [40]
- Jock itch [41]
- Cryptococcosis [42]
- Oral Candidiasis [43]
- *Tinea versicolor* [44]
- Valley fever [45] etc.

Rare

- Fungal infection of the eye [46]
- Mycormycosis, also called the zygomycosis [47]

Result for KOH Test on Skin Scraping Study Table 1- In this study involving 120 patients, the KOH test on skin scraping revealed the following results:**Positive and Negative cases obtained in patients, Positive Cases:** 82 patients tested positive for fungal infections. **Negative Cases:** 38 patients tested negative.

Table 1: Potassium Hydroxide Test
The total patients test positive or negative

TEST	DETECTION
1. KOH TEST On sample	**Positive - 82** **Negative - 38**
2. Total sample was 120	

Common Fungal Infection: The most common fungal infection identified was jock itch (tinea cruris), found in 17 patients.

Demographic Distribution: Rural Area: The majority of positive cases were from rural areas. **Urban Area:** The remaining positive cases were from urban areas.

Table 2: The age group distribution of patients under the study

Age Groups	Number	Percentage
18-20	14	17%
21-30	20	24.3%
31-40	24	29.2%
41-50	10	12.1%
51-60	10	11.5%
∠60	4	4.8%
Total	**82**	**100%**

Table 3 describe the Gender distribution of patients under the study. It was observed that 40.24 %of the patients were Females while 59.7%of the patients were males.

Table 3. Gender composition

Gender	patient figure	Percentage
Female	33	40.24%
Male	49	59.7%
Total	82	100%

Table 4 shows the Diagnosis of patients under the study. It was observed that Athletes Foot was 17%, Ringworm Patients was found 12.18% Jack itch was found 23.1%, Scalp ringworm 20.7 % Nail fungus 17.1 % , Yeast infection 4.8 % , *Pityriasis versicolor* 2.4% , and Sporotrichosis 14.6% .

Table 4: Diagnosis of the disease and percentage

S/N	Diagnosis of the disease	No of patient	Percentage (%)
1	Athlete's Foot	15	17
2	Ringworm (*Tinea pedis*)	10	12.19
3	Jack Itch (*Tinea cruris*)	17	23.1
4	Scalp Ringworm (*Tinea capitis*)	8	20.7
5	Nail Fungus (Onychomycosis or *Tinea unguium*)	14	17.1
6	Yeast infection (candidiasis)	4	4.8
7	*Pityriasis versicolor (Tinea versicolor*)	2	2.4
8	Sporotrichosis	12	14.6
	Total	82	100

Table 5 show the Diagnosis of patients under the study. It was observed that Athelte,s Foot (7-female & 8 Male), Tinea Corporis(4-female & 6 Male), Tinea Cruis (2-female & 8 Male), Tinea Capitis (4-female & 6 Male), Tinea Unguium (9-female & 5 Male), Candidiasis (3-female & 1 Male), *Tinea versicolar*(1-female & 1 Male), Sporotrichosis (6-female & 6 Male) while Table No. 6 represents duration.

Table 5. Diagnosis of patients under the study

S/N	Male	Female	No of patent	Clinical diagnosis
1	8	7	15	Athelets Foot
2	6	4	10	Tinea corporis
3	8	2	10	Tinea Cruris
4	6	4	10	Tinea capitis
5	5	9	14	Tinea unguium
6	1	3	4	Yeast infection

7	1	1	2	Tinea Versicolor
8	6	6	12	Sprotrichosis

Table 6. Duration of fungal infection

Duration	Patients' response in Percentage
Under one week	66%
1-2 Week	25%
Upper 2 week	9%
	100%

Advantages regarding studies on fungal infection [48,49]

Fungal studies helps in developing accurate diagnostic methods such as microscopy, culture techniques, and molecular tools. We can create a better treatment approach enables the development of targeted antifungal drugs and therapies, continuous studies reveal how fungi develop resistance to treatments and it leads to better management strategies and design of combination therapies. Data gives us information about how a fungal infection gives us spreading factors, risk factors, and prevalence. Research paves the way for potential vaccines, especially for life-threatening fungal infections.

Disadvantages regarding studies on fungal infection50

Although we have observed considerable positive results and more diagnostic way but there are some challenges arises during research work as fungi have diverse life cycles, sometimes it makes difficult to investigate appropriate results. Handling pathogenic fungi can pose health risks, during experiment and observation; few vaccines are available due to the complexity of fungal pathogens and immune responses as continuous research gives us more diverse way to elaborate pathogenic strains but we can't ignore risk factors during handling.

The study involving fungal infections has been reviewed and approved by the Institutional Ethics Committee (IEC) in accordance with national and international ethical guidelines for research involving human participants.

Prior to sample collection, written informed consent is obtained from all participants or their legal guardians, explaining the purpose of the study, the procedures involved, potential risks and benefits, confidentiality of data, and the right to withdraw from the study at any time without any consequences to their medical care.

The study ensures that the collection of clinical specimens such as skin scrapings, hair, or nail samples is performed using minimally invasive procedures and with strict adherence to hygiene and safety protocols to prevent infection and discomfort. Special care is taken while working with vulnerable populations, such as immunocompromised patients and children.

All personal and medical information is handled with strict confidentiality, and data is anonymized for analysis. The research does not interfere with standard patient care and treatment and is conducted with the intent to contribute to better understanding, diagnosis, and management of fungal infections.

Ethical clearance has been granted by the Institutional committee with approval number MU/IIP/PO/2025/008, and the study will be conducted with integrity, transparency, and respect for human dignity.

DISCUSSION

Potassium hydroxide (KOH) preparation is a widely used and effective diagnostic technique for detecting fungal elements in clinical specimens such as skin scrapings, hair, and nail clippings. The principle of this method relies on the keratolytic action of KOH, which dissolves keratin—the main structural protein of the skin, hair, and nails—while leaving fungal elements, including hyphae, pseudo hyphae, and spores, intact. This case study gives us an effective result with appropriate data helpful in determination of comparative studies and their results.

CONCLUSION

The KOH test on skin scraping proved to be an effective diagnostic tool, with a significant proportion of patients (82 out of 120) testing positive for fungal infections. Jock itch emerged as the most prevalent infection among the patients, affecting 17 individuals. The higher incidence of positive cases in rural areas compared to urban areas highlights a potential disparity in the prevalence or detection of fungal infections based on geographical and possibly socioeconomic factors. This study underscores the importance of accessible and efficient diagnostic methods like the KOH test in both rural and urban settings to promptly identify and treat fungal infections. Further research could investigate the underlying causes for the higher prevalence

in rural areas and explore measures to improve fungal infection management and prevention in these communities.

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AUTHORS' CONTRIBUTION

MG: Conceived the research idea, designed the study framework, and supervised the overall project. **JKP**: Conducted the literature survey, prepared background information, and drafted the introduction section. **AB**: Collected and analyzed case study data, and contributed to the results and discussion. **CM**: Worked on diagnostic tools, methodology validation, and interpretation of findings. **NK**: Assisted in manuscript writing, proofreading, referencing, and final editing of the paper. All authors contributed significantly, reviewed the manuscript critically, and approved the final version for submission.

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CONFLICT OF INTEREST

The authors and coauthors have no conflicts of interest regarding the publication of this research paper. No known financial or personal relationships that could have appeared to influence the work reported in this paper.

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