SUPERFICIAL MYCOSES: A STUDY PERFORMED FOR THE ISOLATION AND IDENTIFICATION OF FUNGAL SPECIES FROM INFECTED PATIENTS

SUMBUL SHAMIM*, S. WASEEMUDDIN AHMED**, SHAMIM ARA SIDDIQUI*** AND IQBAL AZHAR**

*Faculty of Pharmacy, Hamdard University, Karachi
**Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi,
***Department of Microbiology, University of Karachi, Karachi-75270, Pakistan

ABSTRACT:
Present study was performed for the isolation and identification of fungal species from infected patients and to study prevalence of superficial mycoses. The paper also provides comprehensive clinical data of patients suffering from skin disease. Specimens collected from three hundred male, female and children showing symptoms of superficial mycoses were microscopically examined. A sufficient number of fungal species were isolated and identified.

INTRODUCTION
Infectious diseases, particularly those involving the skin and mucosal surfaces, are a serious problem all over the world, mainly children of the “Third World”, due to deficient sanitation and education. An important group of these skin pathogens are fungi (Caceres et al., 1991; Desta, 1993).

Fungal infections may be classified as "Superficial" affecting only the skin, hair, nails and mucous membrane, or "Systemic" affecting the body as a whole. Fungal infections may also be described as "Local" when they are restricted to one body area, as "invasive" when there is spread into the tissue, or as "disseminated" when the infection has spread from primary site to other organs throughout the body (Gupta et al., 1994; Picard, et al., 1996). Dermatophytoses have been reported to be encouraged by hot and humid conditions and poor hygiene and occur throughout tropical and temperate regions of the world (Smith, 1993; Degreen, 1994).

The Dermatophytes, under most conditions have the ability to infect and survive only on dead Keratin, that is, the tope layer of the skin (stratum corneum or Keratin layer), hair and nails. Superficial candidiasis, caused by Candida spp., include infection of the oropharynx, vagina and skin. Oropharyngeal and vulvo vaginal infections are commonly known as thrush (Grabue, 1994).

Dermatophytes are categorized clinically according to the body area affected. For example, tinea barbae refers to infections of the bearded area of the face, whereas tinea pedis is an infection of the foot. Generally, Microsporum spp. attack hair and skin but not nails; Trichophyton spp. attack hair, skin, and nails; and Epidermophyton spp. infect skin and occasionally nails but not hair. It should be noted that each of the body areas may be infected by more than one species of dermatophyte. In some instances, more than one species of dermatophyte may be isolated from the same lesion.
MATERIALS AND METHODS

Collection of samples:
Skin samples were selected for the present study. The samples were collected from center, Institute of Skin Diseases, Karachi, Sindh. The scraping of affected skin of patients was done with the help of a fairly blunt scalpel at the definite edge of lesion and transferred to a sterile petriplate.

The Wood's lamp (ultraviolet [UV] light) was useful for clinical differentiation of the dermatophytoses. Hair that was infected with a dermatophyte gave fluoresce under UV light. Fluorescing hairs was selectively examined and cultured because they contain the fungi. The UV light was also useful for differentiating ringworm of the skin from erythrasma. In addition to culture, a portion of the clinical specimen consisting of skin, nail, or hair was placed into a drop of 20% potassium hydroxide (KOH) preparation on a clean microscope slide. The slide is gently warmed and then examined with both the low and high-power objectives of the microscope.

Microscopic Diagnosis:
For preliminary diagnosis, microscopic examination was carried out. The scraping at the edge of lesion was directly taken on the slide and juggled under microscope to confirm that the causative organisms are dermatophytes. Dermatophytes were identified by the presence of translucent, non-pigmented, septate mycelium, and arthrospores. *Candida* spp. Showed cluster of budding oval yeast with pseudo and true septate mycelium (Rook, 1992).

Isolation medium:
Mycobiotic Agar (Merck) (MBA) was used for the isolation of Dermatophytes; and sabouraud’s dextrose Agar (Difco) (SDA) was used for the isolation of Saprophytes and *Candida* spp. (Rook 1992).

Culture and Isolation:
Clinical Specimens, including skin, hair and nails that were suspected of containing dermatophytes were inoculated onto media. All media were incubated at 30°C. All culture were held for 3 weeks before being discarded as negative. When colonies appeared on primary media, they were immediately transferred to Sabouraud dextrose agar (SDA), incubated at 25° to 30°C, and checked to ensure their purity. Molds, yeasts, and bacteria may contaminate dermatophyte cultures. In some instances a dermatophyte culture may contain two different dermatophytes. The original isolation plates were re-incubated while purification was being formed.

Pure colonies of suspicious dermatophytes were examined grossly and then microscopically in either slide culture or teased preparations.

![Trichophyton rubrum](image1.jpg)

Fig. 1.1: *Trichophyton rubrum*

![Epidermophyton floccosum](image2.jpg)

Fig. 1.2: *Epidermophyton floccosum*
for dermatophytes and 2-6 days for saprophytes and *Candida* (Davis, 1967).

**Purification and maintenance of culture:**
For maintenance of cultures the SDA slants were used for all isolates. The organisms were purified by subculuring on MBA for dermatophytes and on SDA for saprophytes and *Candida* spp. (Jain., 1990).

**Identification of fungal spp.:**
The Dermatophytes were identified by means of macroscopic as well as microscopic characteristics. Macroscopically colonies showed white, cream brown or pink with a distinctive bright pigment on the reverse. Microscopically two different genera were identified:

*Trichophyton*: Macroconidia thin walled smooth and spindle shaped with 4-6 transverse septa (Fig.1.1).

*Epidermophyton*: Macroconidia pear to oval shaped broadened and rounded at its distal pole, fairly thick walled and smooth upto 4 septa (Fig.1.2).

*Candida*: *Candida albicans* is an oval yeast 2-6×3-9 µm in size, which divides by budding, and is not usually found in non-living habitats.

**Further tests for differentiation of *T.rubrum* and *T.mentagrophytes:***
Other tests were also performed for differentiation of *T. rubrum* and *T. mentagrophytes*, since colonial appearance, microscopic morphological features, and nutritional requirements of these two species overlap. Differentiation of these organisms was accomplished with the following three tests:

i) Urea production test:

ii) pigment production test:

iii) In vitro hair perforation test: Fig. (1.3)

Fig. 1.3: Hair perforation test.

**RESULTS**

Out of 315 skin samples, 250 have been observed to be infected with fungi. In total 13 different fungal species were identified from 250 hosts. *Dermatophytes* were noted predominant (in 204 hosts), followed by *Saprophytes* (in 30 hosts) and *Candida* (in 16 hosts). Among *Dermatophytes*, two different genera *Trichophyton* and *Epidermophyton* were isolated. *Trichophyton rubrum* (in 84 hosts) was predominant followed by *Trichophyton verrucosum* (in 48 hosts), *Trichophyton violaceum* (in 24 hosts), *Trichophyton touloucans* and *Trichophyton mentagrophytes* (in 18 hosts) and *Epidermophyton floccosum* (in 12 hosts).

In case of Saprophytes, only *Aspergillus* has been isolated *Aspergillus gloeaus* was observed as predominant (in 12 hosts) followed by *Aspergillus flavius* and *Aspergillus fumigatus* (in 6 hosts). There different species of *Candida*, *Candida albicans* (in 10 hosts) as predominant followed by *Candida glabrata* and *Candida tropicalis* (in 3 hosts) were identified in the present study (Table-3 and Fig.3).

**DISCUSSION**

The present study was very helpful to observed various fungal species, most commonly associated with skin infection in this part of the world as well as to evaluate
three plant extract against the isolated strains to explore the possibility of a good antifungal preparation (cream/ointment) of natural origin.

Most outbreaks of ringworm are caused by species that infect only humans, although there are some species that can infect both humans and the animals. Those dermatophytes that infect only humans are transmitted through either direct or indirect contact.

Fungal spores remain viable for long periods on carrier animals and fomites. Exposure to reservoir hosts harboring different dermatophytes determines the type and incidence of infection in humans. Up to 50% of people exposed to infected cats, both symptomatic and asymptomatic, have acquired infection. Pets may also acquire disease from humans. *T. mentagrophytes* is most commonly transmitted to man from rodents, and *T. verrucosum* from cattle and horses.

Direct or indirect contact with asymptomatic animals or with skin lesions of infected animals. Contaminated bedding Equipment Fungi in air, dust, or on surfaces of room (spores persist on contaminated surfaces) may be the causes of transmittance.

In rodents is often asymptomatic and not recognized until people are affected. In cats is often asymptomatic. Dogs often show classic skin lesions. Varying severity of dermatitis occurs with local loss of hair. Deeper invasion produces a mild inflammatory reaction which increases in severity with the development of hypersensitivity.

In human often mild, self limiting; scaling, redness, and occasionally vesicles or fissures were seen. Thickening & discoloring
of nails may show circular lesions which clear in the center forming a ring. Fungal infections in man are categorized as to the location on the body.

Fig. 2.1: Thursh is characterized by extensive growth of Candida albicans in the mouth (From a child patient # 86).

Fig. 2.2: Distinctive appearance of ringworm on the body, caused by T. mentagrophytes (Obtained from the back of a male patient # 214).

Ringworm infections spread most easily in settings such as schools, summer camps, prisons and families, where there may be overcrowding and concentrated use of facilities such as bath rooms and showers. Sharing of clothing, combs and hair brushes and personal hygiene can contribute to the spread of dermatophytes (Fig.2.2). Outbreaks of ringworm of the scalp (Tinea capitis) have been associated with barbershops, hair salons, and seats in movie theaters and school buses. Athlete’s foot (Tinea pedis) is associated with wearing shoes. The heat and moisture in shoes provide an ideal environment for the growth of dermatophytes. The incidence of athlete’s foot is higher among people who frequently go to swimming pools and participate in sports.

Ringworm of the body (Tinea corporis) can be transmitted by clothing, towels, and bedding that is contaminated with dermatophytes (Fig 2.3).

Fig. 2.3: Tinea Capitis ringworm of scalp obtained from a child patient.

The present study also indicates the possible contributing factors as well as reason of infections of skin. First, as already has been said, the poor hygiene and humid conditions and second the carelessness of the patients and their relatives towards the complete cure of ailment; they do not consider it necessary to take the complete course of antifungal medicines so that reoccurrence of infections is most common in our country so there is need of awareness among people to complete the course of medicine especially for immuno-compro-mised and immuno-suppressed patients. There is strong need of
good sanitary conditions which can be provided by Government. The regular spray of antiseptic and antibacterial and antifungal solutions can be helpful to minimize the probability of infection among people especially among children.

During our study we observed that after *T. rubrum*, *T. verrucosum* was second most prevailing (Table-3) among patients who live in hilly regions and by profession they use to look after their pet cattles and horses, this might be the reason of prevalence of this dermatophyte in such patients.

Candidiasis typically is a superficial infection of mucous membranes. Extensive growth of *C. albicans* develops into a white, cheeselike coating on the mucosal surface. In the mouth, it covers the tongue and palate, and it is referred to as thrush (Fig. 2.1). There usually is no pain or ulceration of the mucosa. Thrush is seen mainly on newborns during the first few weeks of life.

The search for new remedies for the treatment of this type of ailments is necessary. Most antifungal drugs used in Pakistan are imported and very expensive. Therefore, the development of antifungal agents from local raw materials is still required.

**REFERENCES**


