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Clinical and Pathogenic Analysis in 1393 Cases of Superficial Fungal Diseases in Shanghai

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Abstract: Fungi are a kind of eukaryotic cell organisms widely distributed in nature and are considered as independent branches in existing organisms. There are 1 million to 1.5 million fungi in the world, most of which are beneficial to humans and only a few are harmful to humans. Mycosis is an infectious disease caused by fungi that infect humans and animals. The fungus that infects humans is mainly from the external environment. The route of infection can be contact, inhalation or ingestion. A few fungi can be directly pathogenic, and most fungi are conditional pathogens that require certain conditions to cause disease. Based on the depth of fungal invading tissue, fungal infectious diseases are clinically classified into superficial fungal diseases and deep fungal diseases. Superficial mycosis refers to fungal infections in superficial tissues such as epidermis, hair and nails (toenails). It is a common fungal disease in dermatology clinics and the prevalence rate can reach 20%-50%. According to the different types of pathogenic fungi, superficial fungal diseases can be divided into dermatophytosis, cutaneous candidiasis and Malassezia disease. Among them, dermatophytosis is the most common superficial fungal disease. The pathogenic fungi are dermatophytes, including Trichophyton, Microsporum and Epidermophyton, and Trichophyton rubrum in Trichophyton occupy the first place; Candida is a common human conditional pathogenic fungus, can cause skin candidiasis, the incidence has gradually increased in recent years, still dominated by Candida albicans, but Candida tropicalis, Candida glabrata, Candida krusei with the increase in the detection rate, there are also increasing trends year by year; Malassezia is also a conditional pathogen that easily accumulates in areas rich in sebaceous glands, has lipid dependence, and often causes pityriasis versicolor and Malassezia folliculitis in clinical practice. Malassezia disease is prone to recurrence. With the development of research and improvement of detection techniques, it has been found that Malassezia infection is associated with more and more diseases.

Keywords: Superficial Fungal Infection, Dermatophytosis, Cutaneous Candidiasis, Malassezia, Pathogenic Fungi, Trichophyton Rubrum, Candida Albicans

1. Introduction

Fungi are eukaryotic organisms with membrane bound nucleus, well differentiated apparatus and a cell wall, hence not typical eukaryotic organisms [1]. They are much larger than bacteria. Most fungi are non-motile throughout their lifecycle although spores are carried a great distance by wind. Some can also be parasites on living animals or plants although very few fungi absolutely require a living host [2]. Most fungi are dimorphic, meaning they exist in two forms; they have unicellular and yeast like forms in their host but when growing saprophytic ally in soil or lab medium, they have filamentous forms. Almost all fungi that exhibit dimorphism are pathogenic to man. They replicate sexually by fusion of gametes and asexually by spore formation, and exist in macroscopic or microscopic forms.

Fungal skin infections are categorized into superficial and deep infections. Superficial fungal infections (SFI) of the skin are the most common diseases seen in our daily practice. These infections affect the outer layers of the skin, the nails and hair. In contrary to many of the other infections affecting the other organ systems in humans, the fungi may cause dermatological conditions that do not involve tissue invasion. On the other hand, the skin surface is the habitat of some of these fungi and is liable to environmental condemnation. The main groups of fungi causing superficial fungal infections are dermatophytes, and yeasts. Dermatophytosis (dermatomycosis or ringworm infection) which ranked as one

of the most common cutaneous conditions all over the world is simply a fungal related infection of the stratum corneum of the epidermis and keratinized tissues such as skin, hair and nails of humans and animals [3]**.** The deep fungi are mostly opportunistic pathogens, and infringe people with low immunity. In recent years with the wide use of broad-spectrum antibiotics, corticosteroids, immunosuppressants, the development of organ transplantation, catheters technology, and more people infected with HIV, deep fungus infection is increasing, at the same time also appeared a lot of new pathogenic fungi.

Until now, dermatophytes consisting of about 40 species belonging to three genera: *Trichophyton, Microsporum* and *Epidermophyton* [4]. Based on natural habitat, dermatophytes also classified into three broad categories including the anthropophilic, zoophilic and geophilic species. This differentiation with respect to natural habitats and host preferences is believed to have played a significant role in determining the global distributions of the dermatophytes [5].

Superficial mycoses are common worldwide. They are believed to affect 20 to 25% of the world's population, and the incidence continues to increase [6]. They are predominantly caused by dermatophytes, and the causative species vary with geographic region such as *Trichophyton rubrum*, *T. mentagrophytesvar*, *Microsporum canis* and *Epidermophyton floccosum*. Others have partial geographic restriction, such as *T. schoenleinii* (Africa), T. *Sudanese* (Africa), *T. violaceum* (Africa, Asia and Europe) and *T. concentricum* (Pacific Islands, Far East, and India) [7, 8]. Most cases of tinea unguium, tinea cruris, tinea corporis, and tinea pedis are caused by *T. rubrum* which is the commonest dermatophyte in most developed countries [7].

The superficial fungal infections include those caused by dermatophytes such as tinea faciei for face; tinea manuum for hands; tinea corporis for glabrous skin; tinea cruris for crural folds; tinea pedis for feet; tinea capitis for scalp and tinea unguium for nails [9]. Moreover, non-dermatophytes such as cutaneous candidiasis, pityriasis versicolor, tinea nigra, black piedra, and white piedra, etc [9].

Symptoms and signs of dermatophytes infections of skin and its appendages differ according to the affected site of the body and the source of fungi. Generally, skin lesions are scaly well-defined erythematous plaques with active margin showing more scales with or without vesicles and pustules [10]. The lesions tend to extend peripherally and clear centrally. Zoophilic fungi exhibit more inflammation compared to anthropophilic fungi. Another common SFI is pityriasis versicolor which is mainly caused by *Malassezia species*, which is yeast, and manifested as round or oval patches (macules) in different colours (white, red and brown) with fine scales [11].

Yeasts belonging to the *Candida* genus are also involved in superficial infections and the main species isolated from dermatomycosis are the *Candida parapsilosis*species complex. Candida albicans is the most common candida. The lesion occurs mainly in intertriginous areas of the body such as interdigital, inguinal, intergluteal and inframammary regions as well as nails and periungual regions. The genus *Malassezia* comprises lipophilic yeast-like fungi that are part of the normal skin microbiota in humans and warm-blooded animals [12]. Malassezia is a common parasite in humans, it can cause pityriasis versicolor and Malassezia folliculitis.

The aim of this study is to analyze the clinical and pathogenic superficial fungus disease in Shanghai 10th people hospital affiliated to Tongji University School of medicine, shanghai, China. The superficial pathogenic fungi clinical manifestation, direct microscopic and culture analysis were carried out from January 1-December 31, 2016 using patients who came to our department of dermatology and received mycological examination of superficial fungal disease.

2. Collection of Superficial Fungal Diseases and Classification

2.1. Materials and Methods for Collection of Superficial Fungal Diseases

2.1.1. The Target Patient of the Research

The study was conducted in 1393 participants. All study participants were drawn from patients having superficial fungal diseases examined in the department of dermatology, shanghai tenth People's Hospital, Tongji University. The study duration was limited to January 01- December 31, 2016 from which data was collected based on clinical findings, microscopic and culture examination carried out on samples taken from patients who have visited the hospital for superficial fungal diseases during the study period. A check list was initially prepared to include all the variables required for the analysis. The general categories of the variables are: patient details, fungal species and disease types. A case selection criterion was set as follows to determine the detailed examination process for the identification of clinical infections and causing pathogenic fungal agents.

2.1.2. Classification Criteria

In this study, patients clinically diagnosed and found to be superficial fungi suspected were subjected for further direct microscopic and culture analysis. Based on the results of microscopic and culture results, the superficial fungi diseases were classified as Dermatophytes, Candida, Malassezia and others unclassified pathogenic fungal species.

Dermatophytes belong to a group of organisms that are able to break down the keratin in tissues such as the epidermis, hair, nails, feathers, horns and hooves. Most of these fungi reside in the soil and are involved in decomposition; however, the Dermatophytes can infect living hosts [15]. Dermatophytosis is a common transmissible disease caused by fungi known as dermatophytes. The most common dermatophytes genera which cause dermatophytosis include Trichophyton, Microsporum, and Epidermophyton. Members of Microsporum and Trichophyton cause illness in both humans and animals.

Candida is a type of yeast that normally lives in small amounts in places like mouth, belly or skin without causing

any problems. However, when the environment is right, the yeast can multiply and grow out of control. The infection it caused is called candidiasis, such as mucosal candidiasis, oral candidiasis (thrush, oropharyngeal candidiasis), cutaneous candidiasis and systemic candidiasis. Presently, there are more than 150 known species of Candida. However, only 15 of these species are isolated from patients as infectious agents [19]. These are Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida guilliermondii, Candida lusitaniae, Candida dubliniensis, Candida pelliculosa, Candida kefyr, Candida lipolytica, Candida famata, Candida inconspicua, Candida rugose, and Candida norvegensis. Among these species, C. albicans is still the most common pathogen in spite of its dwindling in humans; it generally colonizes some regions including skin, oropharynx, lower respiratory tract, gastrointestinal tract, and genitourinary system. The C. glabrata is a more common infectious agent among older and neoplastic patients. The C. tropicalis, on the other hand, is more commonly seen among leukemia patients and neutropenic patient. Rhodotorula species, R. mucilaginous, R. glutinis and R. minuta are known to cause disease in humans [20]. Species of Rhodotorula have been recognized as emerging yeast pathogens in humans in the last three decades. While no cases of Rhodotorula infection were reported in the medical literature before 1985, the number of infections increased after that time, most likely because of the wider use of intensive treatments and centralvenous catheters.

Malassezia, as part of the body flora can be isolated from the sebum-rich areas of the skin, particularly the chest, back, and head regions. A study done by Lemming [22], examined clinically normal skin at 20 different sites over theentire body surface. *Malassezia* species were recovered from every subject from the chest, midline back, scalp, ear, and upper inner thigh. The highest mean population densities occurred on the chest, ear, upper back, forehead, and cheeks. Some differences in carriage rates were noted between females and males, with higher population densities from the lower trunk and upper thigh of males. The common endogenous factors linked with pityriasis versicolor include age, poor immune status and genetic predisposition. The age factor contributes significantly to the onset and duration of PV. The age distribution of individuals with pityriasis versicolor in most epidemiological studies falls within the puberty period, 12 to 40 years [24].

2.1.3. Direct Microscopy

Skin, nail or hair samples were collected from the infected area on the patient. For skin samples, a scalpel or edge of a glass slide is used to gently scrape skin scales from the infected area. For hair samples, a forceps is used to remove hair shafts and follicles from the infected site. If the test is being sent to a laboratory, the scrapings are placed in a sterile covered container.

A part of the scrapings were placed directly on to a microscope slide and covered with 10 or 20% KOH. The slide was gently heated to speed up the action of the KOH, and left to stand until clear, normally about two-five minutes, in order to dissolve skin cells, hair and nail. Place the slide under microscope to find the hypha or spore.

2.1.4. Culture Isolation of Fungal Pathogens

The remaining portion of each clinical sample was cultured irrespective of the negative or positive direct microscopic examination results. Each sample was streaked on two plates of SDA oblique medium with chloramphenicol, and the medium were cultured in 25°C or 37°C constant temperature incubator for two weeks. If culture Malassezia, some olive oil should be added into the surface of the SDA medium. Incubated plates were examined twice a week for any fungal growth. If yeast was grown, the yeast can be transferred to the CHROMAGER color plate, and different Candida showed different color.

2.1.5. Data Analysis and Statistical Methods

Data was processed using Statistical package for social sciences (SPSS) version 20. Categorical measurements were analyzed using chi-square test. It was used to test the significance of development of infection in relation to age, gender, direct microscopic and culture examination, and site of infection, the *P*-value of less than 0.05 was taken to be statistically significant. The data was presented in tables, graphs and pie.

2.2. Result for Collection of Superficial Fungal Diseases

2.2.1. General Situation Analysis

A total of 1393 study participants were enrolled in the present study of which 769 (55.2%) were males and 624 (44.8%) were females (Table 1). This value makes the male to female ratio to be 1.23:1. The ages of the study subjects ranged from 1 to 94 years with a mean age of 42±19 years. The most affected age group was found to be 21-30 years which accounted 292 (20.9%). Based on age group, males at the age group of 31-40 years 167 (12.1%) were more affected than the counter female age groups 116 (8.3%). And also the p-value ($P \leq 0.05$) was significant from each gender and age group. However, females at older age (≥61 years) group 139 (10.1%) were more affected groups than the counterpart male age groups (Table 1).

2.2.2. Different Fungal Pathogens Isolated in the Study Subjects

As shown in Figure 1, the culture and direct microscopic positive results have been found 1046 (75.1%) and 1276 (91.6%), respectively. Compared to microscopic results 117 (8.4%), the culture negative results were relatively higher 347 (24.9%). Among the culture positive results, *Dermatophytes* 567 (40.7%) followed by *Candida* 378 (27.1%) have been found the most dominant superficial pathogenic fungi. About 44 (3.2%) and 57 (4.1%) culture positive results were also found to be *Malassezia* and unclassified fungi species (Figure 1b).

2.2.3. The Relationship Between Fungal Pathogens with Gender and Age

Out of the total culture positive subjects (1046), 588 (56.2%) and 458 (43.8%) culture isolates were from male and female,

respectively (Table 2). The predominant isolates in male and females have been identified Dermatophytes fungi which accounts 314 (30.0%) and 253 (24.2%) out of a total of 567 (54.2%) Dermatophytes fungi, respectively. Related to this result the p-value (P≤0.004) was also significant among culture result and gender.

Table 1. *Age and sex distribution of the study patients.*

	Male		Female		Total		P-value
	Number	%	Number	%	Number	%	
Age (year)							
0-10	56	4.0	30	2.2	86	6.2	
11-20	38	2.7	24	1.7	62	4.5	
21-30	155	11.1	137	9.8	292	20.9	
31-40	167	12.1	116	8.3	283	20.3	
41-50	92	6.6	84	6.0	176	12.6	
51-60	112	8.0	94	6.7	206	14.8	
≥61	149	10.7	139	10.1	288	20.7	
Total	769	55.2	624	44.8	1393	100.0	p≤0.05

Table 2. *Culture results of superficial fungi based on gender.*

*Gender	Culture result				Total	P-value
	Dermatophytes	*Candida*	*Malassezia*	Unclassified		
Male	314 (30.0%)	216 (20.7%)	34 (3.3%)	24 (2.2%)	588 (56.2%)	
Female	253 (24.2%)	162 (15.4%)	10 (1.0%)	33 (3.2%)	458 (43.8%)	
Total	567 (54.2%)	378 (36.1%)	44 (4.3)	57 (5.4%)	1046 (100.0%)	P≤0.05

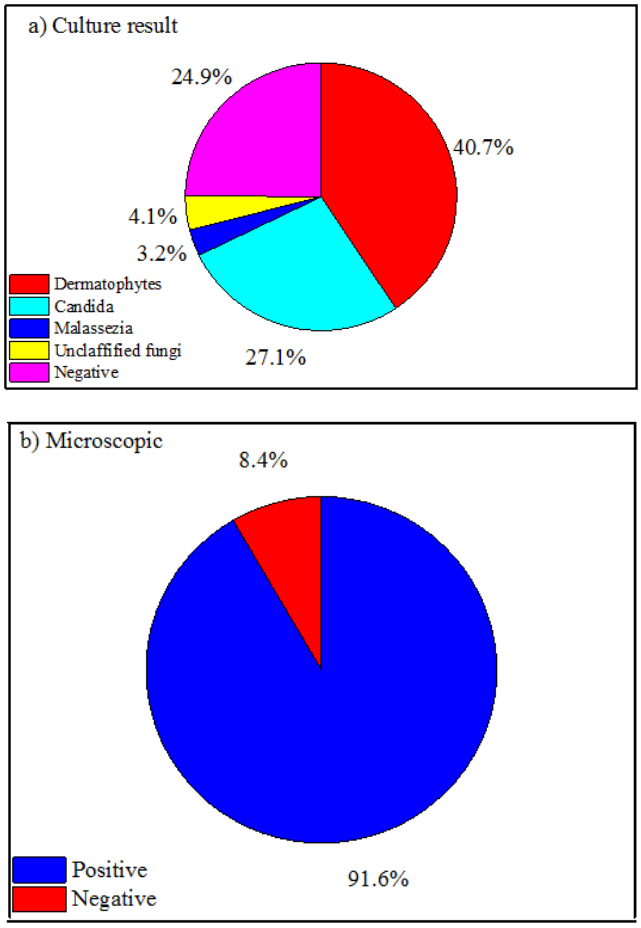


Figure 1. *Superficial pathogenic fungi (a) culture positive and negative (b) direct microscopic positive and negative results.*

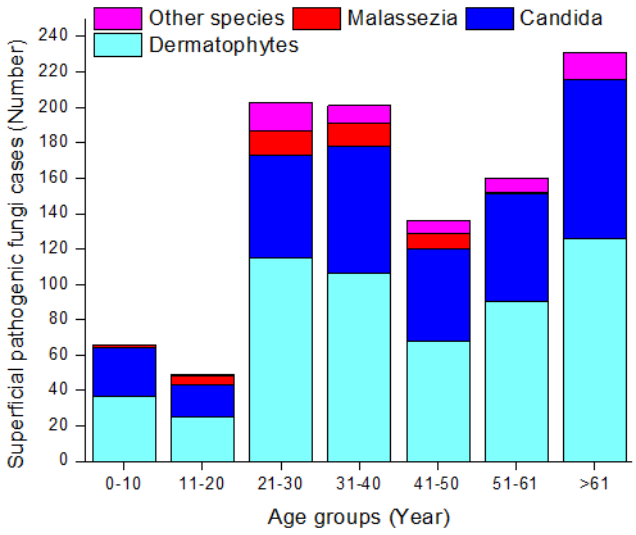


Figure 2. *Distribution of dermatophytes, Candida, Malassezia and other unclassified fungal species in different age groups.*

As shown in Figure 2, the *Dermatophyte* infections at age group over ≥61 years was the highest 126 (38.8%) followed by age group 21-30 years 115 (20.9%) and 31-40 years 106 (14.9%), while the lowest infection rate 25 (4.4%) was at age group of 11-20 years. The second most superficial pathogenic fungi has been also identified *Candida* infections similar at age group ≥61 yeres was the highest 90 (23.8%) followed by age group 31-40 years which was 72 (19.0%).

2.3. Discussion for Collection of Superficial Fungal Diseases

The superficial fungal infection describes a wide spectrum of fungal infections caused by dermatophytes

(Dermatophytosis), *Candida* (candidiasis), and *Malassezia* species. In the present study, the diagnosis of superficial pathogenic fungi has been confirmed by clinical examination and screening of the collected clinical specimen by direct microscopy and fungal culture techniques. In our study we found that culture positive rate in our place was 75.1% which is in agreement to the research done in Singapore (73.4%) [25]. However, it is higher than the result indicated in Brazil (42.7%) [26]. These differences might be due to difference in sample collecting, patient types, laboratory setup and geographical location. Our study showed that male patients are more affected with superficial fungal infections than females with male to female ratio of 1.23:1. A similar study reported in India male to female ratio was 1.24:1 [27]. The higher incidence in males could be due to greater physical outdoor activity with increased sweating. However, our study is inconsistent to the study done in Ethiopia which indicated female (51.96%) were more affected than males (48.04%) [5]. this difference might be difference in geographical location and socio-economic difference of the two countries. Disparity in the prevalence of dermatophytosis in different studies could be resulted from differences in the lifestyle, socioeconomic conditions, risk factors associated with study subjects and environmental factors of study area.

In our study, *Dermatophyte* infections at age group over ≥61 years was the highest 126 (38.8%) followed by age group 21-30 years 115 (20.9%) and 31-40 years 106 (14.92%), and it was more common in both males and females. These results are comparable with other studies for example a study done by Shima [28], Who observed that superficial mycosis was more common in the age group of 21-30 years (28%) followed by 31-40 years (20.8%). In their study they have also revealed that males are predominated (60.8%) by superficial mycosis than female patients (39.2%) with a male to female ratio of 1.55:1. Another study in Northeast Brazil [18] also showed that the prevalence of lesions in glabrous skin was observed in 51% of cases and the age group of 15-50 years (42.1%) was the most prevalent, followed by 50-70 years (26.1%). Glabrous skin lesions relatively higher prevalence (35%) was also observed in the age group >60 in a study conducted by Turner et al [29] and Kaushik et al [30]. They also observed more affected in patients within 21-30 years old patients (23.3%), followed by the age class of 31-40 (20.5%) and 41-50 (14.8%). However, our result is inconsistent with other report that most common age group affected with dermatophytes that ranged from 0 to 10 year [30]. Therefore, our data corroborates with the literature that indicates young and adult patients are more susceptible to develop skin fungal infections,

2.4. Conclusion for Collection of Superficial Fungal Diseases

The prevalence of superficial fungal infection in all study participants were drawn from patients having superficial fungal diseases examined in the dermatology department of the Tenth People's Hospital, which is an affiliate of Tongji University. Culture and microscopic analysis showed that the superficial pathogenic fungi positive results are higher than negative results in both cases. Comparing positive results, microscopic results were found higher than culture positive results. *Dermatophytes*was the commonest superficial pathogenic fungi followed by cutaneous *Candia* and *Malassezia* fungal infections. The results are similar to many reported literatures.

Dermatopytosis was the commonest clinical type which affects all age group particularly the high incidence was recorded in age 61 and above year. The lowest infection rate was seen at age between 11 to 20 years.

We found that male patients were susceptible than female for superficial fungal infection and adults were highly affected. Because of the psychological effects and high morbidity in terms of loss of working days and treatment dermatophytic infection is a public health problem. Therefore, to obtain a true representation of the overall disease pattern of the country more such types of studies should be conducted.

Regular health education needs about fungal infections that highlight their morbidities and modes of spread should be given to the different age group including school children, community and other peoples to reduce the prevalence and burden of superficial fungal infections.

3. Analysis of Dermatophytosis

3.1. Materials and Methods for Analysis of Dermatophytosis

3.1.1. Target Research Object and Clinical Types

From a total of 1393 patients clinically screened for superficial pathogenic fungi and subjected for microscopic and culture analysis, 567 patients were *Dermatophytes* cases. The cases were collected during one year (January 01-December 31, 2016). The *Dermatophytosis* was clinically classified as follow clinical types by position:

1) Tinea pedis: The fungal infection of the feet by dermatophytes.
2) Tinea corporis: The fungal infection of the arms, legs, and trunk by dermatophytes.
3) Tinea unguium: The fungal infection of the fingernails and toenails by dermatophytes.
4) Tinea capitis: The fungal infection of the scalp and hair by dermatophytes.
5) Tinea cruris: The fungal infection of the groin area by dermatophytes.
6) Tinea manuum: The fungal infection of the hands by dermatophytes.

Tinea Pedis

Tinea pedis is a dermatophyte infection that occurs on the soles of the feet and between the toes [32]. This infection is more commonly observed in adults, and incidence of tinea pedis is estimated as 10% in developed countries, 20% in adult men, and 5% among women [33]. Closed shoes facilitate the development of tinea pedis infections. The risk of contraction is high in common areas, and a humid environment, damage in the stratum corneum, and simultaneous growth in the bacterial flora pave the way to

infections. The most common causative agents of tinea pedis infection globally are T rubrum, T mentagrophytesvar interdigital, Tinea pedis has four clinical forms: (a) chronic intertriginous (interdigital), (b) chronic hyperkeratotic, (c) vesiculobullous, and (d) acute ulcerative.

The intertriginous form is the most common. It starts as erosion in the interdigital area with erythema and desquamation and usually settles between the fourth and fifth toes. In the course of time, the infection spreads to the sole and, rarely, also to the dorsum of the foot. The condition may also lead to maceration, itching, and odor in the interdigital area due to occlusion or bacterial coinfection.

Tinea Corporis

Tinea corporis is a skin infection caused by dermatophytes on the hairy skin, face, and other hairless skin except for the anogenital area [34]. Although it is observed worldwide, its prevalence greatly varies between different countries. It is spread either directly by infected humans or animals or through autoinoculation from a body area such as the feet or the inguinal region. In children, the source of infection is usually an infected cat or dog [35]. A dressing style covering the body, heat, and humid climates lead to severe disease and frequent exacerbations. Occlusion at the skin fold, contact of the skin with itself, and traumas facilitate the development of this condition [36]. *T rubrum, T mentagrophytes, M canis*, and *T tonsurans* are the most commonly found agents Classic lesions of tinea corporis are characterized by plaques that are squamous on top and erythematous at the borders. Lesions with vesicular borders usually spread centrifugally. In some cases, the center of the lesions is non squamous and clean [34]. In patients with defective cellular immune responses, the clinical presentation may be different.

The degree of the inflammation in the lesion varies according to the type of the fungus and factors related to the host, as well as the follicular location. Pustules and vesicles are more commonly seen in severely inflammatory lesions. Certain types of dermatophytes may cause more frequent infections in certain body areas, such as M canis, which prefers hairless are as, and M audouinii, which prefers hairy skin.

Tinea Unguium

Tinea unguium is a fungal infection of the nail by dermatophytes. That can involve any component of the nail unit, including the matrix, bed, or plate. It is the most common disease of the nails and constitutes about half of all nail abnormalities [37]. This condition may affect persons of all races and it may affect toenails or fingernails, but toenails are much more likely to be infected than fingernails. Thirty percent of patients with a cutaneous fungal infection (mainly tenia pedis) also have Tinea unguium. The incidence of tinea unguium has been increasing and is related to diabetes, a suppressed immune system, and increasing age. Adults, especially the elderly, are more likely to have onychomycosis than children occurring n about 10 percent of the adult population [33]. The reasons for decrease in the prevalence of onychomycosis in children relative to adults may include reduced exposure to fungus because less time is spent in environments containing pathogens; faster nail growth; smaller nail surface for invasion; and lower prevalence of tinea pedis in children.

The most common symptom of a fungal nail infection is the nail becoming thickened and discolored: white, black, yellow or green. Other than this, Tinea unguium can cause pain, discomfort, and disfigurement and may produce serious physical and occupational limitations. Tinea unguium is not life threatening butpsychosocial and emotional effects resulting from it is widespread and may have a significant impact on quality of life [38].

Tinea Capitis

Tinea capitis is also called ringworm of the scalp. The incidence of this form is not known; however, it occurs most frequently in children exposed through contact with other children or pets [40]. Three types of tinea capitis exist: black dot, gray patch, and favus. Trichophyton tonsurans frequently causes black dot tinea capitis and is the predominant variant observed in the U.S [41]. Gray patch tinea capitis occurs in epidemic andendemic forms; however, the epidemic form is no longer documented in the U.S. The endemic form, which is caused by Microsporum canis, is often spread by cats and dogs. Favus, which rarely occurs in the U.S. is characterized by spores, air spaces, and fragmented hyphae, and occurs more frequently in Eastern Europe and Asia. Black dot tinea capitis is often asymptomatic initially. An erythematous, scaling patch on the scalp enlarges over time, and alopecia occurs. Hairs within the patches break, and a black dot (caused by detritus within the follicular opening) appears [41]. If black dot tinea capitis is left untreated, the alopecia and scarring may be permanent. On occasion, the lesion may change and become elevated, tender, highly inflamed nodules known as kerion. Kerion formation is due to an immune response to the fungus. Lymphadenopathy may occur with kerion. Gray patch tinea capitis presents as circular patches of alopecia with prominent scaling. Kerion formation may occur with gray patch tinea capitisinfection.

Tinea Cruris

The condition is most commonly found in patients with tinea pedis or onychomycosis, featuring auto infection, and is more common in adult males. Itis caused mainly by T. rubrum, E. Floccosum and T. mentagrophytes. Diabetes, obesity and sweating are predisposing factors. It is characterized by macerated, erythematous-squamous lesions, starting in the inguinal fold, which can spread to the thighs, perineum, buttocks, pubic region and lower stomach, typically avoiding the scrotum. It is very itchy and thus, lichenification is common [31].

Tinea Manuum

Tinea manuum is one of the most common dermatophytoses, which is the leading form of infectious disease in dermatology clinics and affects more than 20%–25% of the world's population [42]. Tinea manuum presents as an erythematous scaly patch with an advancing active border, usually located on the dorsum of the hands, or it may occur as diffuse scaly patches with mild hyperkeratosis involving part or the entire surface of the palm and palmar aspect of the fingers. Tinea manuum is often associated with tinea pedis, and it usually appears as the

"the two feet-one-hand syndrome" (bilateral plantar tinea pedis with existent unilateral tinea manuum).

3.1.2. Materials (Reagents, Main Instruments)

(i). Direct Microscopic Examination

The scrapping was done by placing on a microscope slide with one or two drop of 10% KOH solution and a cover slip was placed. The sample was then warmed for 5 minutes over a flame. Each treated slide was then examined under low and high power microscope objective for the presence of fungal hypha or spore.

(ii). Fungal Culture

Each scraping was also cultured in to two SDA mediums. The plates were incubated at 25°C incubator for up to 2 weeks, and examined at 2 to 3 day intervals for fungal growth. The colony which be cultured were also examined visually and microscopically for morphology of fungi using KOH solution. We identified the specific species through the colony morphology and the characteristic conidium.

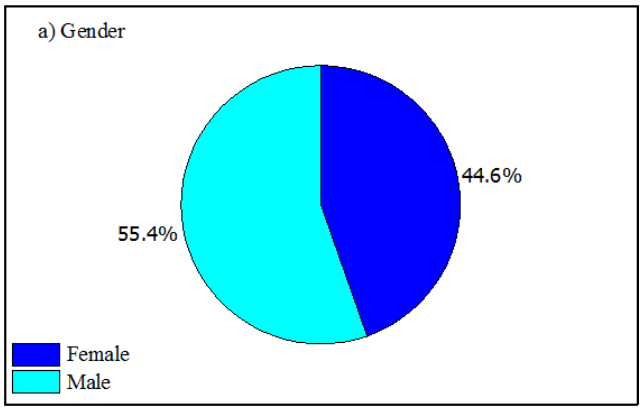
3.1.3. Data Analysis and Statistics Methods

The 567 cases of *Dermatophytes* were analyzed on the following aspects: sex, age, site and pathogenic fungus. Data was processed using SPSS version 20. Categorical measurements were analyzed using chi-square test. It was used to test the significance of development of infection in relation to age and site of infection, the *P*-value of less than 0.05 was taken to be statistically significant. The data was presented in Tables and graphs.

3.2. Result for Analysis of Dermatophytosis

3.2.1. General Situation Analysis

Among 567 cases of *Dermatophytes*, 314 cases (55.4%) were males and 253 cases (44.6%) were females (Figure 3). The ratio of male to female was 1.24:1. The most affected age group was identified to be >61 years 126 (22.2%) followed by age group 21-30 years 115 (20.3%). Relatively least Dermatophytosis cases 25 (4.4%) were observed at the age group of 11-20 years (Figure 3b). The most common clinical type manifestation of dermatophytosis has been identified to tinea pedis 143 (25.2%) followed by tinea cruris 138 (24.3%), tinea corporis 105 (18.5%) and tinea unguium (90=15.9%).



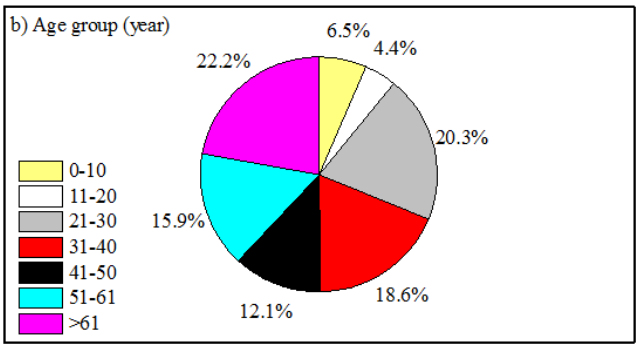


Figure 3. *Dermatophytosis cases based on, (a) gender and (b) age groups.*

3.2.2. The Composition of Various Clinical Types of Dermatophytosis

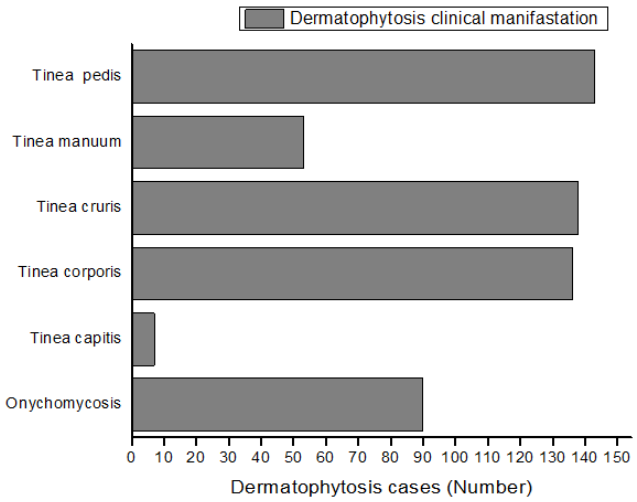


Figure 4. *Clinical manifastation of dermatophytosis.*

A total of 567 dermatomycosis cases were identified by culturing technique. The most predominant superficial fungal disease are tinea pedis which account 143 (25.2%) cases followed by tinea cruris 138 (24.3%) and tinea corporis 136 (24.0%). Tinea unguium, tinea manuum and tinea capitis cases were also identified in 90 (15.9%), 53 (9.4%) and 7 (1.2%) culture samples, respectively (Figure 4).

As evident in Table 3, the superficial fungal isolates identified were found to be T. rubrum 504 (88.9. %), *M. canis* 47 (8.3%), *T. mentagrophytes* (12=2.1%) and *M. gypsum* 4 (0.7%). The most dermatophytosis disease which has been caused by *T. rubrum* were tinea pedis 143 (25.2%) followed by tinea cruris 138 (24.3%) and tinea unguium 90 (15.9%). Relatively low tinea corporis 83 (14.6%) and tinea manuum 50 (8.8%) were also caused mainly by *T. rubrum*. However, there is no tinea capitis caused by *T. rubrum* observed in the current study.

M. canis has been identified in the current study and it was a causative agent of tinea capitis 7 (1.2), tinea corporis 39 (6.9%) and tnea manuum 1 (0.2). *T. mentagrophytes* and *M. gypsum* were also identified the causative agent of tinea corporis 12 (2.1%) and tinea corporis/manuum (2/2=0.4/0.4%), respectively.

Table 3. *Distribution of fungal isolates in relation to clinical manifestations (n=567).*

Pathogenic species	Clinical manifestation of dermatophytosis						Total
	Tinea unguium	*Tinea capitis*	*Tinea corporis*	*Tinea cruris*	*Tinea manuum*	*Tinea Pedis*	
T. rubrum	90 (15.9)	0 (0.0%)	83 (14.6%)	138 (24.3)	50 (8.8%)	143 (25.2%)	504 (88.9%)
M. canis	0 (0.0%)	7 (1.2%)	39 (6.9%)	0 (0.0%)	1 (0.2%)	0 (0.0%)	47 (8.3%)
T. mentagrophytes	0 (0.0%)	0 (0.0%)	12 (2.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	12 (2.1%)
M. gypsum	0 (0.0%)	0 (0.0%)	2 (0.4%)	0 (0.0%)	2 (0.4%)	0 (0.0%)	4 (0.7%)
Total	90 (15.9)	7 (1.2%)	136 (24.0%)	138 (24.3%)	53 (9.4%)	143 (25.2%)	567 (100%)

T. rubrum=Trichophyton rubrum, M. canis= Microsporum canis, T. mentagrophytes= Trichophyton mentagrophytes, M. gypsum= Microsporum gypsum.

3.2.3. Relationship Between Dermatophytosis Based on Gender and Age

The relationship between the clinical types of dermatophytosis and pathogenic fungi based gender and age has been shown in Tables 4 and 5. Among the dermatophytosis cases (n=567), 314 (55.4%) cases were males and 253 (44.6%) were females. The predominant type is tinea pedis 143 (25.2%) followed by tinea cruris 138 (24.3%). Tinea pedis observed 67 (11.8%) in males and 76 (13.4%) females. Tinea cruris is also observed in both males 118 (20.8%) and females 20 (3.5%). The tinea corporis 63 (11.1%) and 73 (12.9%) cases were identified in males and females, respectively. Furthermore, the number of tinea unguium cases observed in males and females were also found to be 34 (6.0%) and 56 (9.9%), respectively. Among the dermatophytosis cases, the last cases were identified tinea capitis 7 (1.2), of which 5 (0.9%) and 2 (0.4%) cases were male and females, respectively.

Table 4. *Distribution of clinical types in relation to gender (n=567).*

Gender	Clinical manifestation of dermatophytosis						Total
	T. unguium	*T. capitis*	*T. corporis*	*T. cruris*	*T. manuum*	*T. pedis*	
Male	34 (6.0%)	5 (0.9%)	63 (11.1%)	118 (20.8%)	27 (4.8%)	67 (11.8%)	314 (55.4%)
Female	56 (9.9%)	2 (0.4%)	73 (12.9%)	20 (3.5%)	26 (4.6%)	76 (13.4%)	253 (44.6%)
Total	90 (15.9%)	7 (1.2%)	136 (24.0%)	138 (24.3%)	53 (9.4%)	143 (25.2%)	567 (100%)

As shown in Table 5, superficial dermatophytosis was more common in the age group of over 61 years (126 cases) followed by 21-30 years (115 cases) and 31-40 (106 cases). Relatively, lower cases (25 cases) of dermatophytosis was identified in age group 11-20 years.

In this study, the decreasing order of dermatophytosis manifestation cases were found to be tinea pedis>tinea cruris> tinea corporis>tinea unguium>tinea manuum>tinea capitis (Table 5). Particularly, tinea corporis has been manifested most commonly at older age group (≥61 years) and at 21-30 years.

Table 5. *Distribution of clinical types of dermatophytosis in relation to Age (n=567).*

Age	Clinical manifestation of dermatophytosis						Total
	Tinea unguium	Tinea capitis	Tinea corporis	Tinea cruris	Tinea manuum	Tinea pedis	
0-10	4	5	6	1	1	20	37
11-20	4	1	9	3	1	7	25
21-30	25	0	27	32	11	20	115
31-40	24	0	16	27	7	32	106
41-50	5	0	19	18	4	22	68
51-60	13	1	23	26	8	19	90
≥61	15	0	36	31	21	23	126
Total	90	7	136	138	53	143	567

Table 6. *The distribution of superficial fungi species based on patient age group (n=567).*

Age	Dermatophyte fungal species				Total
	T. rubrum	M. canis	T. mentagrophytes	M. gypseum	
0-10	26	8	3	0	37
11-20	15	9	1	0	25
21-30	91	14	8	2	115
31-40	97	8	0	1	106
41-50	64	4	0	0	68
51-60	88	2	0	0	90
≥61	123	2	0	1	126
Total	504	47	12	4	567

T. rubrum=Trichophyton rubrum, M. canis= Microsporum canis, T. mentagrophytes= Trichophyton mentagrophytes, M. gypsum= Microsporum gypsum.

The distribution of the patients with SFI based on age is illustrated in Table 6. *Trichophyton rubrum* was the most dominant (123 cases) superficial pathogenic fungal species infecting older age groups (≥61 years) followed by age group of 21-30 years (91 cases) and 31- 40 years (97 cases). *Microsporum canis* species has been also observed higher (14 cases) at the age group of 21-30 years followed by 11-20 years (9 cases). The distribution of *Trichophyton mentagrophytes* species was also 8 cases in age between 21-31 years followed by lower age group (0-10 years). The number of *Microsporum gypseum* species were few, 2 cases in age group between 21-30 and 1 case in each age groups between 31-40 years and ≥61 years.

3.3. Discussion for Analysis of Dermatophytosis

In our study, adults were the most commonly affected age group by *Dermatophytes*. Among the dermatophytosis cases (n=567), 314 (55.4%) cases were males and 253 (44.6%) were females. This finding is well related to the study done in Nigeria [43]. The frequency of the skin infections decreased with either the decrease or increase in age of patients, which agrees to the research findings of India [44]. All clinical manifestations such as tinea pedis, tinea unguium and tinea corporis were higher in females, but tinea cruris and tinea manuum and tinea capitis were higher in males.

The common clinical manifestations of the isolates were tinea pedis (25.2%) and tinea cruris (24.3%). This is similar to the findings of Japan [45], which indicates that tinea pedis and tinea cruris were the most clinical types. And it is also consistent to the findings which revealed that most of the fungal skin infections were found to be from feet (tinea pedis) and groin (tinea cruris) [46] in Saudi Arabia.

Our finding shows *Trichophyton rubrum* was the most common isolate which accounts (88.9%). This finding is agreed with the finding which reported in Southern China (85.2%) [47]. We noted that tinea unguium and tinea pedis predominated in females while tenia cruris predominated in male, this may be partially attributed to the fact that females are more conscious of cosmetic problems and life quality but male make sport sweating problem. This study comparable with study done by northwestern [48]. In Central China, tinea corporis (32.4%) is the most prevalent type of SFI, and T. rubrum (50.7%) is the predominant fungal pathogen [49]. In Northwestern and Northeastern China, tinea pedis (33.1%, 20.0%, respectively) is the most prevalent type of SFI, and T. rubrum is also the predominant fungal pathogen [50]. In South western China, tinea cruris (30.1%) is the most prevalent type of SFI, and T. rubrum is the predominant fungal pathogen [51].

Superficial dermatophytosis was more common in the age group of over 61 years (126 cases) followed by 21-30 years (115 cases) and 31-40 (106 cases). Relatively, lower cases (25 cases) of dermatophytosis was identified in age group 11-20 years. In this study, the decreasing order of dermatophytosis manifestation cases were found to be tinea pedis>tinea cruris> tinea corporis>tinea unguium>tinea manuum>tinea capitis. Particularly, tinea corporis has been manifested most commonly at older age group (≥61 years) and at 21-30 years. Similar results were reported by earlier researches [52]. Tinea pedis was dominant clinical manifestation in age group 45-64. This hand in hand with the findings of a study in Poland [53]. Our result with regards to tinea corporis was not similar to the findings of a study in Libya [54].

3.4. Conclusion for Analysis of Dermatophytosis

Distribution of superficial dermetophytosis varies among the patients. The variation could be due to a number of reasons including immunity, anatomic sites, age, and gender, climatic, socioeconomic and even historical. We found that male patients were susceptible than female for superficial fungal infection and adults were highly affected. Tinea pedis and tinea cruris were the common prevalent SFIs followed by tinea unguium and tinea corporis. In terms of fungal isolates, We noted that Tinea manuum and tinea pedis predominated in females while tenia cruris predominated in male, this could be partially attributed to the fact that females are more conscious of cosmetic problems and life quality but male make sport sweating problems. *Trichophyton rubrum* was the most common dermatophyte species followed by *Microsporum canis*, and it is the most common cause of tinea pedis. *M. canis*, *M. gypseum* and *T. mentagrophytes* are usually transmitted from animals to humans, and young people are susceptible. In clinical practice, *Trichophyton rubrum* has the highest detection rate. It mainly causes tinea pedis and tinea cruris. It is easy to infect and relapse due to its relatives' characteristics.

4. Analysis of Cutaneous Candidiasis

4.1. Materials and Methods for Analysis of Cutaneous Candidiasis

4.1.1. Target Research Object (Patients)

378 cases of cutaneous candidiasis were identified from direct microscopic and culture examinations conducted on 1393 cases of patients visiting Dermatology and Venereology Department of Shanghai 10th people hospital from June 2016 to December 2016. A detailed clinical history including age, sex, site of infection, type of lesions, duration and similar complaints were recorded and used for the classification and distribution of the diseases on the different body sites.

(i). Direct Microscopy

After clinical examination, the suspected patients were requested to give sample for microscopic and culture examination. A portion of each sample was directly placed on a slide and then a drop of 10% KOH solution was added on it. After 5 minutes air drying, the wet mount was examined under low (x10) and high (x40) power magnification lens for the presence of fungal elements such as spores and hypha.

(ii). Culture Isolation of Fungal Pathogens

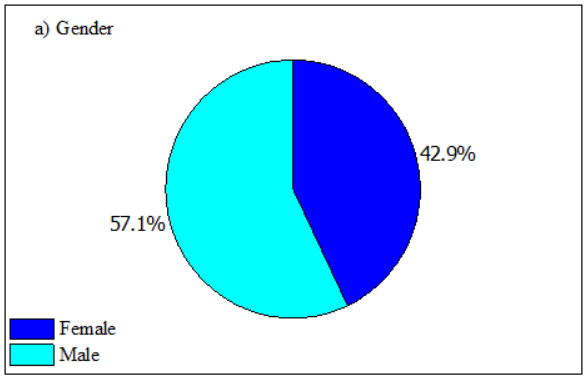
In order to further confirm the superficial pathogenic fungi species, the remaining portion of each clinical sample was cultured irrespective of the negative or positive direct microscopic examination results. Each sample was streaked on two plates of SDA with chloramphenicol. All inoculated plates were then incubated at inverted position for 2 weeks at 25°C thermostatic incubator. Incubated plates were examined twice a week for any fungal growth. Colonies suspected of candida were sub-cultured into CHROMAGER color medium. And through the color of colony, we can know specific species of the candida. Different fungal strains show different colors in CHROMAGER color medium, Candida albicans show emerald green, Candida tropicalis shows blue, Candida krusei show pink, Candida parapsilosis show purple.

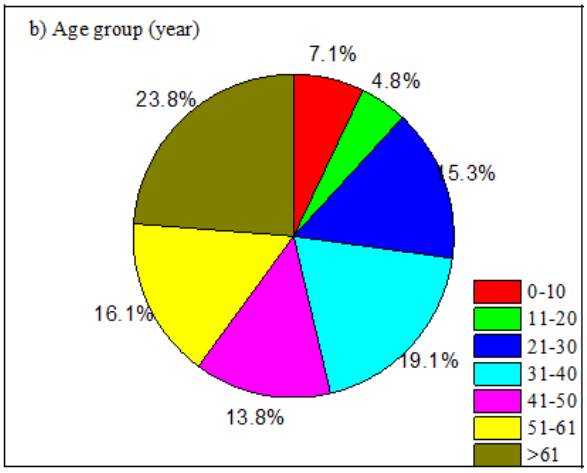
4.1.2. Data Analysis and Statistics Methods

The 378 cases of *Dermatophytes* were analyzed on the following aspects: sex, age, site and pathogenic fungus. Data was processed using SPSS version 20. Categorical measurements were analyzed using chi-square test. It was used to test the significance of development of infection in relation to age and site of infection, the *P*-value of less than 0.05 was taken to be statistically significant. The data was presented in Tables and graphs.

4.2. Results for Analysis of Cutaneous Candidiasis

4.2.1. General Situation Analysis





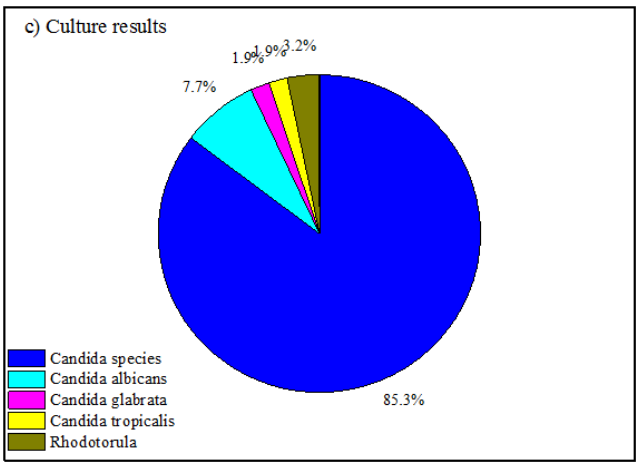


Figure 5. *The analysis of cutaneous candidiasis cases based on (a) gender, (b) age and (c) culture results.*

Among 378 cases of cutaneous candidiasis, 162 cases (42.9%) were females and 216 cases (57.1%) were males (Figure 5a). This gender difference cases make the ratio of male to female to be 1.33:1. The most affected age group was identified to be >61 years 90 (23.8%) followed by age group 31-40 years 72 (19.0%). Relatively least candidiasis cases 27 (7.1%) were observed at the age group of 0-10 years (Figure 5b). As shown in Figure 5c, unclassified *Candida species* has accounted the most cutaneous candidiasis cases 323 (85.3%). Among identified *Candida* species, the most common ones were found to be *Candida albicans* 29 (7.7%) followed by *Rhodotorula* 12 (3.2%), *Candida glabrata* 7 (1.9%) and *Candida tropicalis* 7 (1.9%). Cutaneous candidiasis clinically manifested mostly finger nail 86 (22.8%), hand 76 (20.1%), feet 63 (16.7%) and groin 58 (15.3%).

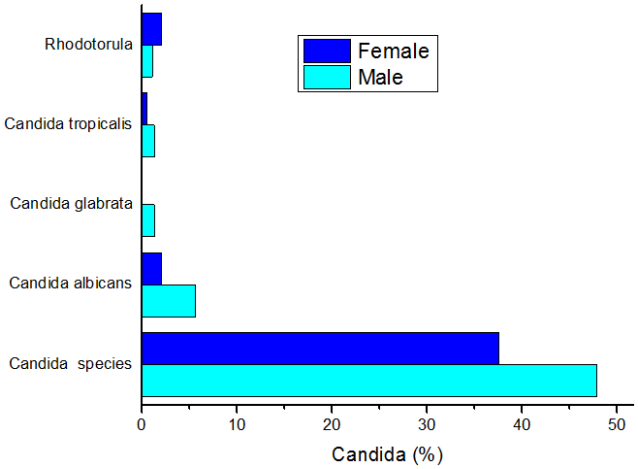


Figure 6. *Distribution of Candida species with gender (n=378).*

4.2.2. Dermatological Candidiasis Pathogens Versus Gender, Age and Site

The relationship between the clinical types of cutaneous candidiasis and pathogenic fungi based on gender has been analyzed and shown in Figure 6. A total of 378 cases were positive for cutaneous candidiasis; therefore, they were recognized as the most frequent type of infection. Among the

unclassified *Candida species*, 47.9% and 37.6% were found to be males and females, respectively. Among the identified *Candida*, the predominant species has been confirmed *Candida albicans* which accounts 5.6% and 2.1% cases in males and females, respectively. *Rhodotorula* (1.1%), *Candida glabrata* (1.3%) and *Candida tropicalis* (1.3%) were also identified in males. In females, except *Candida glabrata* other *Candida species* identified in males such as *Rhodotorula* (2.1%) and *Candida tropicalis* (0.5%) were also identified in males.

According to age, the prevalence of cutaneous candidiasis infections caused by different *Candida species* is shown in Table 7. The most prevalent 82 (21.7%) was observed at older age (>61 years). Moreover, the cutaneous *Candida* infections were also observed at the age group of 31-40 years 55 (14.6%) and 31-40 years 108 (19%). Relatively, Lower prevalence of *Candida* infection was also identified at lower 18 (4.8%) age group (11-20 years).

Candida albicans was the most dominant 11 (2.9%) superficial pathogenic fungal species infecting age group between 31-4011 (2.9%) followed by age group of 21-30 years 7 (1.9%) and 41-50 years 5 (1.3%). *Candida glabrata* has been also observed higher 3 (0.8%) at the age group of 31-40 years followed by ≥61 years 2 (0.5%). The distribution of *Rhodotorula* species was also affected all age groups except 11-20 and 41-50 years (Table 7).

Table 7. *Distribution of Candida infections according to the age (n=378).*

Age	*Candida* types					Total
	Candida sp	*C. albicans*	*C. glabrata*	*C. tropicalis*	*Rhodotorula*	
0-10	25 (6.6%)	1 (0.3%)	0 (0.0%)	0 (0.0%)	1 (0.3%)	27 (7.1%)
11-20	18 (4.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	18 (4.8%)
21-30	49 (13.0%)	7 (1.9%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	58 (15.3%)
31-40	55 (14.6%)	11 (2.9%)	3 (0.8%)	0 (0.0%)	3 (0.8%)	72 (19.0%)
41-50	44 (11.6%)	5 (1.3%)	1 (0.3%)	2 (0.5%)	0 (0.0%)	52 (13.8%)
51-60	50 (13.2%)	3 (0.8%)	1 (0.3%)	4 (1.1%)	3 (0.8%)	61 (16.2%)
≥61	82 (21.7%)	2 (0.5%)	2 (0.5%)	1 (0.3%)	3 (0.8%)	90 (23.8%)
Total	323 (85.3%)	29 (7.7%)	7 (1.9%)	7 (1.9%)	12 (3.2%)	378 (100%)

Candida sp= Unclassified Candida species, C. albicans= Candida albicans, C. glabrata= Candida glabrata, C. tropicalis=Candida tropicalis.

Table 8. *Distribution of Candida infections according to the localization of the involved area (n=378).*

Infection Site	*Candida* types					Total
	Un. *Candida sp*	*C. albicans*	*C. glabrata*	*C. tropicalis*	*Rhodotorula*	
Back	12 (3.2%)	0 (0.0%)	0 (0.0%)	1 (0.3%)	2 (0.5%)	15 (3.9%)
Face	9 (2.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (2.4 %)
Foot	56 (14.8%)	0 (0.0%)	0 (0.0%)	2 (0.5%)	5 (1.3%)	63 (16.7%)
Glans	16 (4.1%)	19 (5.0%)	1 (0.3%)	0 (0.0%)	1 (0.3%)	37 (9.8%)
Groin	53 (14.0%)	5 (1.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	58 (15.3%)
Hand	68 (18.0%)	1 (0.3%)	1 (0.3%)	3 (0.8%)	3 (0.8%)	76 (20.1%)
Leg	5 (1.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (1.3%)
Nail	77 (20.4%)	4 (1.1%)	4 (1.1%)	1 (0.3%)	0 (0.0%)	86 (22.8%
Toe nail	27 (7.1%)	0 (0.0%)	1 (0.3%)	0 (0.0%)	1 (0.3%)	29 (7.7%)
Total	323 (85.3%)	29 (7.7%)	7 (1.9%)	7 (1.9%)	12 (3.2%)	378 (100%)

Candida sp= Unclassified Candida species, C. albicans= Candida albicans, C. glabrata= Candida glabrata, C. tropicalis=Candida tropicalis.

As shown in Table 8, the most commonly affected region in different *Candia species* was found to be the finger nail 86 (22.8%) followed by hand 76 (20.1%), foot 63 (16.7%), groin 58 (15.3%), glans 37 (9.8%) and toe nail 29 (7.7%). Relatively, lower infection results of Candida have been observed for back 15 (4.0%) and face 9 (2.4%) sites.

The most affected sites by *Candida albicans* were glans (5.0%)>groin (1.3%)>nail (1.1) >hand (0.3) (Table 8). *Candida glabrata* has been also observed higher 4 (1.1%) at nail followed by toe nail, hand and glans 1 (0.3% each). The most affected site by *Rhodotorula* species was feet 5 (1.3%) followed by hand 3 (0.8%), back 2 (0.5%) and glans/toe nail 1 (0.3 each). Moreover, sites affected by *Candida tropicalis* include hand 3 (0.8%), feet 2 (0.5%) and nail/back 1 (0.3% each).

4.3. Discussion for Analysis of Cutaneous Candidiasis

Cutaneous candidiasis is usually a secondary infection of the skin (body folds) and nails in predisposed patients. In our study, the most clinical suspected *Candida* characterized by culture techniques was found to be unclassified *Candida species* 323 (85.3%). The identified *Candida species* such as *Candida albicans, Candida glabrata*, *Candida tropicalis*and *Rhodotorulaspecies* all together accounted 14.7%. The reason is that we hadn't color all the candida which cultured in our actual work by CHROMAGER color medium. So I think in actually, the number of Candida albicans should be more than that we get. This result is similarto the works of Abadi and Izadiz [24] and Bongomin, et al [55].

The prevalence of cutaneous candidiasis was higher in male than female with a ratio of 1.33:1. In other study more affected females than male [56]. It has been suggested that differences in different regions may be due to variation in climatic and environmental conditions of the areas being studied and difference in study subjects and methods. In our

study, the most commonly affected region in different *Candia species* was found to be the finger nail 86 (22.8%) followed by hand 76 (20.1%), foot 63 (16.7%), groin 58 (15.3%), glans 37 (9.8%) and toe nail 29 (7.7%). Relatively, lower infection results of Candida have been observed for back 15 (4.0%) and face (9=2.4%) sites similar study in china Although C. albicans remains to be the major pathogen in superficial candidiasis C. metapsilosis and C. orthopsilosis isolates were both found in clinical specimens from skin and nail lesions are affected [57]. Among identified *Candida* species, the most common ones were found to be *Candida albicans* 29 (7.7%). C. albicans is a common member of the human gut flora. It is detected in the gastrointestinal tract and mouth in 40-60% of healthy adults. C. albicans is the predominant cause of invasive fungal infections [58], and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization [59]. Although C. albicans is the most prevalent species involved in invasive fungal infections, the incidence of infections due to non-albicans species is increasing.

4.4. Conclusion for Analysis of Cutaneous Candidiasis

Distribution of cutaneous candidiasis varies among the patients. The variation could be due to a number of reasons including immunity, anatomic sites, age, and gender, climatic, socioeconomic and even historical. We found that male patients were more susceptible than female for *Candida* infection and adults were highly affected. The most affected sites by different *Candida species were* glans, groin, nail, hand, back, leg, feet, face and toe nail. In terms of fungal isolates, Unclassified *Candida species* > *Candida albicans* > *Rhodotorulaspecies* > *Candida glabrata/Candida tropicalis*.

The most prevalent 82 (21.7%) was observed at older age (>61 years). Moreover, the cutaneous *Candida* infections were also observed at the age group of 31-40 years 55 (14.6%) and 31-40 years 108 (19%). The prevalence of cutaneous candidiasis was higher in male than female with a ratio of 1.33:1.

In recent years, the number of superficial fungal diseases caused by Candida is increasing. Candida albicans is the main pathogenic fungus, at the same time, other Candida species have an increasing trend, such as Candida tropicalis and Candida glabrata. In this study, some specimens were not color-coded, resulting in a bias in the classification of Candida species, Candida albicans should account for the vast majority, not other Candida species. There are several cases of Rhodotorula detected in this study. Rhodotorula has been considered as a contaminating fungus, but some species have pathogenicity to humans and animals. The Rhodotorula spp. strains cultured in this study were grown in multiple colonies and had no other contaminating fungi. In this study, the superficial mycosis caused by Candida is second only to Dermatophytes. It is clinically common, especially for infections of the hands and nails, and it is also easy to become a pathogenic fungus in the thigh of infants.

5. The Analysis of Malassezia Disease

5.1. Materials and Methods for Analysis of Malassezia Disease

5.1.1. Target Research Object and Clinical Types

A total of 44 cases of *Malassezia* infections were identified from direct microscopic and culture examinations conducted on patients visiting Dermatology Department of Shanghai Tenth people Hospital from January 01-December 31, 2016. A detailed clinical history including age, sex, site of infection, type of lesions, duration and similar complaints were recorded and used for the classification and distribution of the diseases on the different body sites.

5.1.2. Direct Microscopy

After clinical examination, the suspected patients were requested to give sample for microscopic and culture examination. Skin scraping may be collected directly onto a glass slide with a blade or the edge of a second glass slide. Alternatively, a scotch tape stripping technique can be used. The tape is placed on a glass slide and examined under the microscope. The keratin and debris of the skin scales are first dissolved with the use of 10%-20% KOH and then stained with methylene blue, parker ink or lacto-phenol blue to encourage clear viewing of the fungal element. After 5 minutes air drying, the wet mount was examined under low (x10) and high (x40) power magnification lens for the presence of *Malassezia species*.

5.1.3. Culture Isolation of Fungal Pathogens

In order to further confirm the *Malassezia* species, the remaining portion of each clinical sample was cultured using appropriate media. Each sample was streaked on two plates of SDA with SDA plus olive oil.

This medium was also used for maintenance of culture of the *Malassezia*. Samples were taken from normal looking skin of the upper back and for the patients they were also taken from lesion skin mainly from the upper trunk. All inoculated culture plates were then incubated at inverted position for 14 days at 37°C. The dishes were wrapped in plastic bags to ensure suitable humidity and prevent the medium from drying out. The relative humidity in the incubator was 85% and all culture plates were incubated in plastic bags. This increases the humidity and diminishes the risk of the medium drying out. Incubated plates were examined for any fungal growth.

5.1.4. Data Analysis and Statistics Methods

The 44 cases of *Malassezia* were analyzed on the following aspects: sex, age, site and pathogenic fungus. Data was processed using SPSS version 20. Categorical measurements were analyzed using chi-square test. It was used to test the significance of development of infection in relation to age and site of infection, the *P*-value of less than 0.05 was taken to be statistically significant. The data was presented in Tables and graphs.

5.2. Results for Analysis of Malassezia Disease

5.2.1. General Situation Analysis

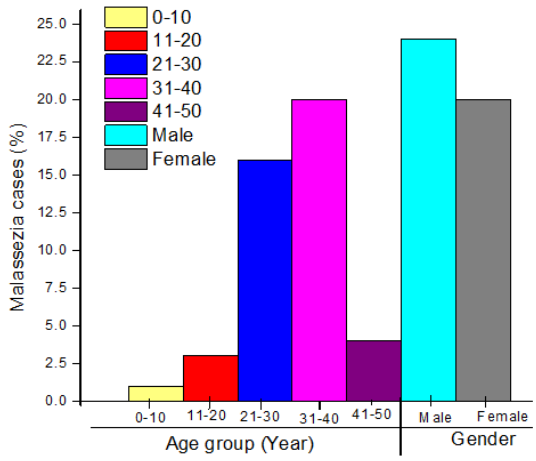


Figure 7. *Malassezia infection based on age group and gender.*

Among 44 cases of infection of *Malassezia*, 24 (54.5%) cases were males and 20 (45.5%) cases were females (Figure 7) which makes the ratio of male to female to be 1.2:1. The most affected age group was identified to be 31-40 years 20 (45.5%) followed by age group 21-30 years 16 (36.4%). Relatively, the least *Malssezia* cases 1 (2.3%) were observed at the age group of 0-10 years.

According to the performance of skin lesions, there has been two kinds of infection namely pityriasis versicolor 33 (75.0%) and *Malassezia* folliculitis 11 (25.0%) The most commonly affected region was the back 20 (45.4%) followed by chest 10 (22.7%), while the least commonly affected region was the abdomen 1 (2.3%). The clinically confirmed *Malssezia* infection has been analyzed through culture techniques and the positive rate of *Malassezia* culture was found to be less 12 (27.7%). The culture negative results were higher 32 (72.5%), suggesting that they are normally found in areas that are rich in sebaceous glands and unable to grow artificial media.

Table 9. *Different characteristics of Malassezia folliculitis and pityriasis versicolor (n=44).*

Characters	Types of *Malassezia*				Total	
	pityriasis versicolor		*Malassezia* folliculitis			
	Number	%	Number	%	Number	%
Age						
0-10	1	2.3	0	0	1	2.3
11-20	1	2.3	2	4.5	3	6.8
21-30	11	25.0	5	11.4	16	36.4
31-40	16	36.3	4	9.1	20	45.4
41-50	4	9.1	0	0	4	9.1
Culture result						
Positive	10	22.7	2	4.5	12	27.2
Negative	23	52.3	9	20.5	32	72.8
Total	33	75.0	11	25.0	44	100

5.2.2. Relationship Between Malassezia Infection with Gender, Age and Site

As shown in Table 9, a total of 44 clinical samples were collected from suspected cases of *Malassezia* infection of which 24 (54.5%) and 20 (45.4%) cases were male and female patients. The ages of study subjects ranged from 0 to 50 years with a mean age of 30.5±8.5 years. *Malassezia* versicolor was the predominant clinical manifestation accounting 33 (75.0%) of the cases, of which 17 (38.6%) and 16 (36.4%) cases were males and females, respectively. The *Malassezia* folliculitis total clinical manifestation also has been found 25% (n=11), of which the number of male and female gender accounted 7 (15.9%) and 4 (9.1%) cases, respectively.

The clinical manifestation of *Malassezia* in relation to age group depicted that patients with age group 31-40 years more affected 16 (36.3%) with *Malassezia* versicolor. Generally, *Malassezia* infection is more prevalent at the age group of 31-40 years 20 (45.5%) followed by 21-30 years 16 (36.4%). The least clinical manifestation of *Malassezia* infection were observed at younger (<10 years) and older (41-50 years) age groups Table 9.

Clinically confirmed *Malassezia* infection has been analyzed through culture techniques and the positive and negative rate of *Malassezia* identified has been found 12 (27.2%) and 32 (72.8%), respectively. The positive culure result of pityriasis versicolor and *Malassezia*folculites were identified to be 10 (22.7%) and 2 (24.5%) cases, respectively. The negative culure result of pityriasis versicolor and *Malassezia*folculites were also identified to be 23 (52.3%) and 9 (20.5%) cases, respectively. The higher culture negative results observed in this study suggesting that *Malassezia* species are normally found in areas that are rich in sebaceous glands and might not be able to grow well in artificial media.

The most commonly affected region in pityriasis versicolor was found to be the back 14 (31.8%) part of the body followed by the armpit 8 (18.2%) and the chest 6 (13.6%). The least affected sites by pityriasis versicolor were also identified to be the neck (9.1%) and the abdomen (2.3%). The most affected region by *Malassezia* folliculitis were also almost the same including the back 6 (13.6%) followed by chest 4 (9.1%) and the armpit 1 (2.3%). The data are shown in table 10.

Table 10. *Distribution of Malassezia infections according to the localization of the involved area in patients (n=44).*

	Armpit	Back	Abdomen	Chest	Neck	Total
Malassezia folliculitis	1 (2.3%)	6 (13.6%)	0 (0%)	4 (9.1%)	0 (0%)	11 (25.0%)
pityriasis versicolor	8 (18.2%)	14 (31.8%)	1 (2.3%)	6 (13.6%)	4 (9.1%)	33 (75.0%)
Total	9 (20.5%)	20 (45, 4%)	1 (2.3%)	10 (22.7%)	4 (9.1%)	44 (100%)

5.3. Discussion for Analysis of Malassezia Disease

Malassezia species is a global resident flora on the skin surfaces of humans and animals, which can cause pityriasis versicolor, *Malassezia*folliculitis and seborrheic dermatitis. The aim of the present study was to identify the various *Malassezia species* from patients suspected from clinical finding.

Superficial fungal infection caused by *Malessieza* has been found predominant in men 24 (54.5%) than women 20 (45.5%). It also showed that the most affected age ranged from 31 to 40 (45.5%) years and more localized in the back (32.6%). However, the *Malssezia species* infection has been found rare on children and older individuals. Differences in the amount and composition of skin lipids at different ages and different body sites may account for part of the variability we found. In addition, hygienic practices change with age and with body site, possibly affecting the habitat of the yeasts. The common endogenous factors linked with *Malassezia* versicolor include age, poor immune status and geneticpredisposition. The age factor contributes significantly to the onset and duration of pityriasis versicolor. The age distribution of individuals with pityriasis versicolor in most epidemiological studies falls within the puberty period (12-40 years) which is usually students and young adults. The high prevalence of pityriasis versicolor in this age group is linked with the increased presence of androgen which stimulates sebum production [60]. The rise in the level of physical activity leading to increased sweating and the probability of harboring the causative yeast as part of skin flora is higher in this age group.

Higher number of cases back, chest and face also confirmed that these sites are rich in sebum (lipid) which is the requirement of *Malassezia species* grow. These findings are comparable with other studies done. A study done by examined clinically normal skin at 20 different sites over the entire body surface. *Malassezia* species were recovered from every subject from the chest, midline back, scalp, ear, and upper inner thigh. The highest mean population densities occurred on the chest, ear, upper back, forehead, and cheeks. Some differences in carriage rates were noted between females and males, with higher population densities from the lower trunk and upper thigh of males. However, the current findings did not agree with previous reports who concluded that the leading cause of skin infections were *Malassezia* (58.1%) [61]. This difference might be attributed to the fact that the prevalent fungal agents causing skin infection varies due to climate of the geographical region and may shift to another due to social demographic factors such as migration and health condition of individuals.

5.4. Conclusion for Analysis of Malassezia Disease

The prevalence of *Malassezia* infection varies among the patients. We found that men patients were more susceptible than women patients for *Malassezia* infection and puberty age range were highly affected. According to the performance of skin lesions, there has been two kinds of infection namely pityriasis versicolor and *Malassezia* folliculitis. Pityriasis versicolor was the predominant clinical manifestation accounting 33 (75.0%) followed by *Malassezia* folliculitis 11 (25.0%). The most affected sites are back, chest and face, which suggests the lipid dependence of the *Malassezia species*. Compared to the culture results, culture positive results were found less than culture negative results. As we know, *Malassezia* is very difficult to culture, and even if add oil, the positive rate of culture is still very low. *Malassezia* is a normal human flora, but middle-aged and young people, especially males, are prone to over-proliferation of *Malassezia* and cause disease due to the exuberant oil secretion. *Malassezia* is easy to relapse, hard to cure, and has a certain heredity. Pityriasis versicolor is seasonal and often occurs in summer. There is no obvious clinical symptoms of pityriasis versicolor, but *Malassezia* folliculitis often causes hair follicle inflammation, itching, etc. no significant seasonal changes, at present, there are few treatments for *Malassezia*, and related diseases caused by *Malassezia* are also under continuous investigation.

6. Conclusions and Prospects

6.1. General Conclusion for Superficial Fungal Disease

The superficial mycosis is mainly caused by dermatophytosis. Cutaneous candidiasis, *Malassezia*, and other unclassified fungus. Thus, species identification is very important to initiate, promote and appropriate antifungal therapy. It is essential that good laboratory methods are available for rapid and precise identification of the pathogenic fungi involved, in order to apply appropriate treatment and preventive measure. The changing profiles of human dermatophytoses among countries have further necessitated the development of improved diagnostic methods for identification of dermatophytes.

The overall objective of this study was clinical and pathogenic fungi to analyze the result of isolated culture of 1393 patients with superficial fungal infections who diagnosed in dermatology clinic in past one year and to assess the prevalence of the infections among patients who has been treated for skin disease in Shanghai 10th people Hospital affiliated to Tongji University School of Medicine, shanghai, China. According to our investigation, the superficial mycosis

was mainly caused by three identified superficial fungal infection agents and unclassified fungal species.

The identified superficial fungal infection agents were: dermatophytosis, cutaneous candidiasis and *Malassezia* diseases: 1) among them, dermatophytosis ranks first in this study. While investigating the distribution of dermatophytes under direct microscopy and culture test, T. rubrum was found to be the most prevalent species followed by M. canis species. The high rate of Trichophyton rubrum was perhaps related to sanitary conditions, traveling, immigration and the use of public facilities. And like many references, Trichophyton rubrum is the most common dermatophyte in clinical practice, and also the most common pathogenic fungus of superficial fungal diseases. Our study also shows that dermatophytosis is the main clinical type of superficial fungal disease in this area. This study revealed that Tinea pedis was the most prevalent superficial fungal infection followed by tinea cruris among dermatophytosis. Microsporum canis is also a common clinical dermatophyte, it is usually transmitted to humans through animals at present, mostly infected by cats and dogs. Trichophyton mentagrophytes are mostly found in soil and can also be carried by animals and infect humans. In recent years, with the improvement of living standards, dermatophytosis caused by Trichophyton mentagrophytes has decreased year by year. In this study, we also found that the proportion of tinea capitis is very low, the pathogenic fungi are mostly transmitted by animals, and infected people are mainly children Tinea capitis are rare in the city, and the incidence is more related to family pets. With the progress of society, such superficial fungal diseases will be less and less; 2) Cutaneous candidiasis is usually a secondary infection of the skin (body folds) and nails in predisposed patients. In our study, the most clinically suspected *Candida* as characterized by culture techniques was found to be unclassified *Candida species.* This is because many Candida species are not colored by the color plate during actual work. This is the limitation of this study, which led to the analysis bias of Candida. In this study, Candidiasis superficial fungal disease is second only to dermatophytosis, and is a common clinical pathogenic fungus. The identified *Candida species* are *Candida albicans, Candida glabrata*, *Candida tropicalis* and *Rhodotorula.* Candida albicans tops the list of cutaneous candidiasis. If it is not due to statistical deviation, Candida albicans should be the highest proportion of Candida species, not other unclassified Candida species. Candida is a conditional pathogen, that can easily cause infections in the hands and nails, especially in the middle-aged and elderly people; 3) the study on *Malassezia* species has showed that the predominantly identified infections were pityriasis versicolor followed by *Malassezia* folliculitis. The positive rate of culture of *Malassezia* is low, and it is usually determined by direct microscopic examination and clinical symptoms. More and more skin diseases have been shown to be related to *Malassezia* infection. Since *Malassezia* is a normal human parasite and has lipophilic properties, pityriasis versicolor or folliculitis caused by *Malassezia* is prone to recurrence, and it is difficult to eradicate, causing life trouble for many people.

We found that male patients were susceptible than female for superficial fungal infection and adults were highly affected. Because of the psychological effects and high morbidity in terms of loss of working days and treatment dermatophytic infection is a public health problem. Therefore, to obtain a true representation of the overall disease pattern of the country more such types of studies should be conducted.

In general, clinical and pathogenic fungi identification is very important to initiate promote and take appropriate antifungal therapy. It is also essential that good laboratory methods should be available for rapid and precise identification of the pathogenic fungi in order to apply appropriate treatment and preventive measures.

6.2. Further Work Direction

This study recommends routine mycological investigations in both adults and children presenting with skin infections suspected to be of fungal nature for better management of infections. Although superficial fungal disease is not life threatening, it should be a public health concern so as to improve the quality of life of those infected. A policy should be formulated on the prevention and treatment of fungal dermatological infections in the country, and I think it is needed for further hospital survey involving a large number of patients to ascertain any association between age, gender and infection.

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