CUTANEOUS FUNGAL PATHOGENS IN PATIENTS AT THE HOSPITAL OF HUE UNIVERSITY OF MEDICINE AND PHARMACY, VIET NAM

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Background: The superficial cutaneous mycoses are usually confined to the outer layers of skin, hair, and nails, and don't invade living tissues. Dermatophytes and yeasts are the main pathogenic agents of these mycoses. In addition, some molds are mentioned as causative agents of these diseases. This study aims to identify the cutaneous fungal species from 181 dermatophytosis patients in Hospital of Hue University of Medicine and Pharmacy, Viet Nam. **Methods:** A cross sectional, descriptive survey on 181 dermatophytosis patients with positive direct examination from samples, including skin, hair and nail scrapings. These specimens were cultured on Sabouraud agar - Chloramphenicol medium or Sabouraud agar - Chloramphenicol - Cycloheximide medium. The culture tubes were incubated at 25 ^{0}C and examined daily for 4 weeks. Dermatophytes and nondermatophyte molds were identified by the microscopic morphology. Identification of *Candida* species and other yeast pathogens based on germ tube tests, cultivation on Corn Meal agar medium and colorimetric sugar utilization tests (Auxacolor test). **Results:** *Dermatophytes* was the most prevalent cutaneous fungal infection (90.64%), followed by yeasts (7.74%) and then *non dermatophytes molds* (1.65%). As the causative dermatophytes species, *Trichophyton rubrum* was the most frequently isolated pathogen (58.01%). *T.rubrum* and *T.mentagrophytes* were isolated from all the dermatophytosis clinical types. *Candida sp* and *Trichosporon cutaneum* were etiological agents of paronychia - onychomycosis. *Fusarium sp.* were pathogenic in onychomycosis and tinea corporis. *Scopulariopsis sp.* caused of onychomycosis. **Conclusion**: In this study, the cutaneous fungal pathogens were the highest rate of anthropophilic *dermatophytes,* and then zoophilic dermatophytes. *Candida albicans* and *Candida non albicans* were also found in paronychia - onychomycosis patients. In addition some nondermatophyte mold infections were found in both of skin and nail.

Key words: *Dermatophytosis, filament, yeast, molds, dermatophytes, Candida sp.*

1. INTRODUCTION

The cutaneous mycoses are superficial fungal infections of the skin, hair or nails. Many studies performed all over the world have demonstrated that most cases of these diseases are caused by dermatophytes [4,18,2,20]. However, other studies have recorded that yeasts and non dermatophyte molds may play a role, particularly in onychomycosis [4,18,3].

Dermatophytes, a group of fungi have the capacity to invade keratinized tissue, so that they are able to invade the hair, skin, and nails of living host. There are 55 dermatophyte species divided into three genera: *Trichophyton, Microsporum, and Epidermophyton*. Relating to human pathogens are 23 *Trichophyton* species, 15 *Microsporum* species and one *Epidermophyton* species (*E.floccosum*) [20]. These species are classified as anthropophilic, geophilic or zoophilic according to their habitat [20]. Some anthropophilic are very popular all over the world such as *T. rubrum, T. tonsurans*, *T. mentagrophytes*, *T. violaceum*, *M. audouinii, M. ferrugineum, E. floccosum*, *M. canis, M. equinum, T. verrucosum* are the main species in zoophilic. *M. gypseum, M. praecox, T. ajelloi* are belong to geophilic group [1]. Dermatophyte infections are divided according to the affected body site into tinea capitis (scalp), tinea pedis (foot), tinea corporis (body), tinea cruris (groin), tinea manuum (hand), and tinea unguium (nail) [20]. Dermatophytes are common in both developed and developing countries. The fungal species distribution depends on geographic region. Not all species of dermatophytes are popular in over the world. Some ones have been isolated from every continent, others have geographically limited in some country or areas. The distribution of

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dermatophytes varies from region to region and over time, tends to influence by some factors, such as age, socioeconomic factors, lifestyle, close to contact to pets, and climate [8,7].

Candida sp, the component of the normal human microbiota flora, is considered opportunistic yeast. It could affect mucous membranes, skin and nails. This fungus has a worldwide distribution and affects people of all ages. In the other hand, *Trichosporon sp., Geotrichum sp*, and other yeasts are mentioned as a yeast causative agent in human [10]. Today, several reasons have been suggested to explain the increased incidence of yeast infections, including the overuse of antibiotics, receiving aggressive regimens of chemotherapy in cancer, or taking immunosuppressive drugs for rheumatologic or autoimmune diseases, as well as the increasing number of AIDS patients.

Molds rarely cause cutaneous infections. Occasionally, secondary skin infections can occur in immunosuppressed patients, such as with leukemia and after stem cell transplantation. Primary skin infections due to molds are even rarer. Nowadays, non dermatophyte molds like *Fusarium sp., Acremodium sp…* are considered emerging pathogens in onychomycosis [3,9].

Viet Nam is a tropical country. Hue city is located in central of Viet Nam, where heat and humidity are good conditions for cutaneous fungal grown. It is suitable for high rate of dermatophytosis. Our study in 2003, the rate of dermatophytosis was 52% in the Hospital of Hue Medicine and Pharmacy University. The etiological agents of dermatophytosis have been well studied in many countries, but there wasn't any data in Vietnam as well as in Hue city.

This study aims to identify the fungal cutaneous pathogens from March 2010 to March 2011 in Parasitology Department of Hue University of Medicine and Pharmacy Hospital, Viet Nam.

2. MATERIALS AND METHODS

Across-sectional descriptive study was carried out in Parasitology Department of Hue Medicine and Pharmacy Hospital, Vietnam. A total of 181 patients included in this study attended the Dermatology Department and had been clinically diagnosed as having dermatophytosis- like lesion from March 2010 to March 2011.

Sampling

The skin scraping and hair specimens were collected from these patients by scraping by the edge of the lesion using the clean slide and Scotch test technique. Nail specimens were clipped with scissors and the debris under the nail plate were collected using a sterile scalpel. Hair the samples were placed in clean sterile Petri dishes for direct examination with KOH 20% solution and cultivation.

We also collected the data regarding name, age, sex, occupation, address, date of sample collection, site of the lesion following the classified clinical form of infection by ICD-10. Clinical forms were divided in eight types, including tinea pedis, onychomycosis, tinea corporis, tinea capitis, tinea manuum and intertrigo, tinea cruris, paronychia, and multiplex clinical type (more one body infected site).

Mycological Examination

All the specimens were firstly subjected to direct microscopic examination using 20% KOH solution for the presence of fungal hyphae, spore or yeast. Then the filamentous fungi positive samples in direct examination were cultured on the Sabouraud's dextrose agar supplemented with chloramphenicol (50mg/l) and cycloheximide (0.5mg/l) for identification dermatophyte by macroscopic and microscopic morphology of the colonies. We also cultivate these samples on the Sabouraud's dextrose agar supplemented with chloramphenicol (50mg/l) without cycloheximide (0.5mg/l) for identification non dermatophyte mold and yeast. Addition cultural Corn Meal agar medium following Dalmau technique and Auxacolor test were using to identify yeast species. The identification of filamentous fungi was based on the observation and description of the macroscopic and microscopic characteristics of the colonies obtained in the culture.

The statistical analysis was performed using the software SPSS version 11.5.

3. RESULT

A total of 181 patients having superficial mycosis features and positive fungal direct examination were included, with 71.27% males (129/181) and 28.73% females (52/181). The mean age of patient was 24 years. The youngest patient was 4 years old and oldest was 74 years old. The distribution of dermatophytosis isolated in relation to different age groups is shown in table 1. Analyzing the number of cases per age group, patients aged 16 - 25 years accounted for most cases (n= 136,75.14%), followed by patients aged 26 - 35 years (n= 14, 7.73%) and patients aged 46 - 65 years (n=13, 7.18%). However,

there were not more patients in each group in our study, exception to group 16 -25 years, so it was difficult to consider the difference in frequency of dermatophytosis according to age groups.

The most frequent agent isolated from culture was dermatophyte (90.64%), followed by yeast (7.71%) and non dermatophyte mold (1.65%). The detail species identification was shown in table 2. In three genera of dermatophytes, the most frequency was *Trichophyton sp* (82.85%), followed *Microsporum sp* (7.18%) and *Epidermophyton floccosum* (0.55%). Regarding in *Trichophyton species*, *T. rubrum* was the most specie isolated (58.01%) and some agent also found such as *T.mentagrophytes*, *T.tonsurans*, *T.violaceum*, *T.erinacei*, *T.schoenleinii*, *T. soudanense*, *T.verrucosum*. In case positive for non dermatophyte mold, three pathogenic were recognized including *Fusarium onysix*, *Fusarium solani* and *Scopurialopsis sp*.

The highest clinical form was tinea cruris and the incidence of the others clinical types was peformed in table 3.

The dermatophyte species most commonly isolated were *T. rubrum*, followed by *T. mentagrophytes,* in all of clinical forms accept for paronychia. In contract, *Candida sp* and *Trichosporon cutaneum* were the causative agent of paronychia. The distribution of fungal causative agent according to clinical types was presented in table 4.

Table 1. Distribution dermatophytosis according to age and gender

Age	Male		Female		Total	
	N	(%)	N	(%)	N	(%)
< 15	5	3.88	2	3.85	7	3.87
16 – 25	105	81.40	31	59.62	136	75.14
26 – 35	10	7.75	4	7.69	14	7.73
36 – 45	3	2.33	7	13.46	10	5.53
46 – 65	6	4.64	7	13.46	13	7.18
> 66	0	0.00	1	1.92	1	0.55
Total	**129**	**100**	**52**	**100**	**181**	**100**

Table 2. Identification the species of fungal cutaneous agents

Agents		Number	Rate (%)
Dermatophytes	*T.rubrum*	105	58.01
	T. mentagrophytes	26	14.36
	T.tonsurans	6	3.31
	T.violaceum	5	2.76
	T. erinacei	3	1.66
	T.schoenleini	2	1.10
	T.soudanense	1	0.55
	T.verrucosum	2	1.10
	M.gypseum	8	4.42
	M.canis	4	2.21
	M.persicolor	1	0.55
	Epidermophyton floccosum	1	0.55
Nondermatophytes molds	*Fusarium solani*	1	0.55
	Fusarium onysix	1	0.55
	Scopulariopsis sp.	1	0.55

Yeast	*Candida albicans*	7	3.86
	Candida parapsilopsis	2	1.10
	Candida tropicalis	1	0.55
	Candida famata	1	0.55
	Candida guilliermondii	1	0.55
	Trichosporon cutaneum	2	1.10
Total		**181**	100

Table 3. Clinical presentations

Clinical types	N	Rate (%)
Tinea capitis	3	1.66
Onychomycosis	7	3.86
Tinea manuum and intetrigo	6	3.31
Tinea pedís	11	6.08
Tinea corporis	52	28.73
Tinea cruris	63	34.81
Multiplex types	28	15.47
Paronychia	11	6.08
Total	181	100.00

Table 4. Causative agent according to clinical presentations

Clinical feature / **Fungal Species**	Tenia capitis	Onycho-mycosis	Tinea manuum and intertrigo	Tinea pedis	Tinea corporis	Tinea cruris	Multiple clinical type	Paronychia	Total
*T.*rubrum	1	4	1	7	30	38	24	0	105
T. mentagrophytes	1	0	1	1	9	11	3	0	26
T.tonsurans	0	0	0	0	1	5	0	0	6
T.violaceum	0	0	0	1	1	2	1	0	5
T. erinacei	0	0	0	0	3	0	0	0	3
T.schoenleini	0	1	0	0	0	1	0	0	2
T.soudanense	0	0	0	1	0	0	0	0	1
T.verrucosum	0	0	0	0	1	1	0	0	2
M.gypseum	0	0	1	0	5	2	0	0	8
M.canis	1	0	0	0	1	2	0	0	4
M.persicolor	0	0	0	1	0	0	0	0	1
E. floccosum	0	0	0	0	0	1	0	0	1
Fusarium solani	0	1	0	0	0	0	0	0	1
Fusarium onysix	0	0	0	0	1	0	0	0	1
Scopulariopsis sp.	0	1	0	0	0	0	0	0	1
Candida albicans	0	0	3	0	0	0	0	4	7
Candida parapsilopsis	0	0	0	0	0	0	0	2	2
Candida tropicalis	0	0	0	0	0	0	0	1	1
Candida famata	0	0	0	0	0	0	0	1	1
Candida guilliermondii	0	0	0	0	0	0	0	1	1
Trichosporon cutaneum	0	0	0	0	0	0	0	2	2
Total	3	7	6	11	52	63	28	11	181

4. DISCUSSION

Cutaneous fungal disease remains a popular clinical condition in humans worldwide. Factors such as age, weather conditions, social practices, and hygiene practices certainly contribute to the epidemiological variations in this disease. In the present study, dermatophytosis affected more 71.27% male (71.27%) than females (28,73%). This result was similar of study of Nishimoto Katsutaro in Japan (2002) [13] and Flores Juan Medina in Peru (2009) [8]. This difference could be explained by the capacity of more body action in men. It related to increase body's sweat, so it is a better factor for fungal grow [19,20]. Althought the mean age of our patients was 24 years, our study showed the range in age of between 4 and 74 years. This result revealed that fungal cutaneous infection was common in all of age. Some study showed that the rate and clinical feature of dermatophyte depended on the age [4,13]. In addition, the study of Pires CA et al in Brazil (2012) showed that the female in age group of 51 to 60 years were the most affected by cutaneous mycoses [14]. However, the distribution of cutaneous mycose was focused on age from 16-25 years old in both genders in this study, which could be understood by more Hue University's students attending in Dermatology department. In order to understand more detail the influence of age and other affecting factors of this disease, it should carry out more study about this subject in Vietnam.

In the table 2 showed that the most of fungal cutaneous agents were the septae hypha fungi 92,29% (*dermatophytes* 90.64% and *nondermatophytes moulds* 1.65%), the yeast was 7.71%. The predominance of dermatophyte in our study was the same result of the data reports in etiologies of superficial fungal diseases from many regions on over the world [1]. Howerver the *nondermatophytes moulds* causative agents should be concentrate on especially in onychomycosis, especially onychomycosis in diabetes mellitus, peripheral vascular disease and activities related to foot trauma patients [3,17,19,20].

In dermatophyte agents, the predominent rate of *Trichophyton sp.* was 82.85%, then *Microsporum sp.* 7.18%, and the lowest rate was *Epidermophyton* 0,55%. In the genus *Trichophyton*, the highest rate was *T.rubrum* (58,01%) and then *T.mentagrophytes* (14,36%), the same data from other study in Asia region such as Thailand, India, Srilanka... [1]. The report of Flores Juan Medina et al in Peru showed that the predominent rate of *T.rubrum* was 59.7% and *T.mentagrophytes* was 9.7% to compare with other species [8]. The study of Nishimoto Katsutaro in Japan showed the same result of commonly rate of *T.rubrum*, then *T.mentagrophytes* [13]. In contract, the study of Bassiri-Jahromi S in Iran showed that the highest rate of *Epidermophyton floccosum* and then *T.rubrum*, *T.mentagrophytes* [2]. In our study, we found that, the most of dermatophyte agents were anthropophilic fungi (84.7%). In contrast, there were only 10 cases (5.5%) of zoophilic dermatophyte including 3 cases of *T. erinacei,* 2 cases of *T. verrucosum*, 4 cases of *M. canis* and 1 case of *M. persicolor*. In cases of *T. erinacei* and *T. verrucosum,* we found them from famers, however we did not know exactly the animal contacting factors, especially contacting with hedgehog [15]. Some *T. erinacei* infectious reported cases come from Asia, but reports from South America and Europe also found [5,11,16]. It showed that the affecting factors such as geographic region, socioeconomic factors, lifestyle, poor hygiene conditions, physical activity should be concentrated on to prevent this disease. In addition it should carry out more study on this subject in our country to understand more about dermatophytose epidemiology.

In addition, there was 1.65% of *nondermatophytic* causeative agents with *Fusarium solani, Fusarium onysix* and *Scopulariopsis* in our research. Some other study showed that the low rate of *nondermatophyte moulds* but there were diversity of agents such as: *Aspergillus sp.(A.flavus, A.fumigatus, A.terreus, A.niger), Fusarium sp., Acremonium sp*., *Scopulariopsis sp., Cladosporium sp., Trichoderma sp*.,...[9,2]. Furthermore, the nondermatophytic agents were often reported in immunocompromised patients such as diabete, especially caused by onychomycosis [12]. However, in our study, *Fusarium onysix* caused by tinea corporis in normal immunocompetent patient, and *Fusarium solani,* and *Scopulariopsis* were found in onychomycosis patients with normal immunocompetent.

The others fungal pathgenic of onychomycosis were *Candida* and *Trichosporon*. The result in table 2 showed that the prevalence of *Candida sp* was higher than *Trichosporon* (6.64% vs 1.10%). This result was the same of Bassiri-Jahromi S, Khaksari AA's study [2]. In Candida species, we reported some species like *Candida albicans* (3.86%), *Candida parapsilopsis* (1.10%), *Candida*

tropicalis (0.55%)*, Candida famata* (0.55%) and *Candida guilliermondii* (0.55%).

We classified the clinical types follow by ICD-10. The table 3 showed that the high rate were tinea cruris (34.81%) and tinea corporis (28.73%), in contract, the lowst rate was tinea capitis (1,66%).

The result on table 4 showed that *T.rubrum* and *T. mentagrophytes* were the most common on every clinical presentation except for paronychia. In contract, *Candida sp* and *Trichosporon cutaneum* often caused of paronychia and onychomycosis. The rare cases tenia capitis caused by *T.rubrum* (1 case), *T. mentagrophytes* (1 case) và *M.canis* (1 case). The study of Bassiri-Jahromi S and Nishimoto Katsutaro also reported *M. canis* and *T. tonsurans* were the commonly agent of tinea capitis [2,13]. We found three agents cause of onychomycosis including *T.rubrum, T. shoenleini* và *Fusarium solani*, but paronychia was caused by *Trichophyton sp.* and *C.albicans*. Eventhough *C. albicans* was the most common caused in paronychia, we also found some *C. non albicans* species such as: *Candida parapsilopsis, Candida tropicalis, Candida famata, Candida guilliermondii* and *Trichosporon cutaneum*. In contract, tenia pedis were caused by *Trichophyton sp.* và *M.persicolor.* Classification fungal species from tenia corporis, we found two genus of *Trichophyton sp, Microsporum sp* and non dermatphyte mould: *Fusarium onysix*. The most common causative agent of tenia crusis were *Trichophyton sp, Microsporum sp* and *Epidermophyton floccosum*. Our study was the first study in Vietnam about etiology of dermatophytose and similar some other studies [2,13].

This study showed that, the most fungal causative agents of skin lession were anthropophilic dermatophyte, and onychomycosis were *Candida sp.* However, some diversity of agents of *nondermatophyte* and *Candida non albicans* should be concetration on.

5. CONCLUSIONS

The most common fungal isolates from cutaneous fungal diseases in Hospital of Hue University of Medicine and Pharmacy was *dermatophytes (*90.64%), in which anthropophilic fungi were 84.7%, and zoophilic dermatophyte were lower rate including: *T. erinacei, T. verrucosum*, *M. canis* and *M. persicolor*. Yeast agents were often found on paronychia - onychomycosis patients with both of *Candida albicans* and *Candida non albicans* such as*: C. parapsilopsis, C. tropicalis, C.famata, C.guilliermondii.* In addition nondermatophyte mold can cause diseases in both of skin and nail including: *Trichosporon cutaneum, Fusarium solani, Fusarium onysix* and *Scopulariopsis*.

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