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Dermatomycoses in Kuala Lumpur, Malaysia (Dermatomikosis di Kuala Lumpur, Malaysia)

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ABSTRACT

*Prevalence of dermatomycoses varies from one centre to another due to many factors. Knowledge of local prevalence is useful to aid clinical diagnosis and treatment. Due to lack of data in Malaysia, this study aimed to look at the causes of dermatomycoses in Kuala Lumpur, Malaysia. Dermatological specimens including skin scrapings, hair and nail clippings were collected carefully from clinically suspected cases of dermatomycoses between 2008 and 2010. All cultures of skin, hair and nails that yielded positive fungal growth were included. Any fungal growth outside the streaking area, duplicate and incomplete data were excluded from the study. Three-hundred-fifty-eight patients were included. Male patients were slightly more than females with a ratio of 1.2:1. The median age was 53 years old with interquartile range of 38–64 years. More than half (53.6%) belonged to 20–60 years age group. Rates of culture isolation were 89.0% for nails, 56.2% for hair and 55.6% for skin. Five-hundred-twenty-two fungi were isolated from 358 clinical specimens. Non-dermatophyte moulds (NDMs) represented the largest group (50.5%; mainly *Aspergillus* species 18.7%), followed by yeasts (41.6%; mainly *Candida* species 26.8%) and dermatophytes (7.9%; mainly *Trichophyton* species 7.7%). In conclusion, NDMs and yeasts were more commonly isolated than dermatophytes from dermatological specimens in this centre. Current treatment regime that focuses on dermatophytes may be ineffective to treat dermatomycoses caused by NDMs or yeasts. Antifungal susceptibility study may be needed to guide therapy in recalcitrant cases.*

Keywords: Dermatology; dermatomycoses; dermatophytes; fungi; mould

ABSTRAK

*Prevalen dermatomikosis adalah berbeza dari satu-satu tempat disebabkan pelbagai faktor. Pengetahuan mengenai prevalen setempat adalah berguna bagi membantu diagnosis dan rawatan klinikal. Berikutan ketiadaan data di Malaysia, kajian ini ingin mencari penyebab dermatomikosis di Kuala Lumpur, Malaysia. Spesimen dermatologi seperti kikisan kulit, rambut dan ketipan kuku dikumpul berhati-hati daripada kes-kes dermatomikosis yang disyaki secara klinikal antara tahun 2008 dan 2010. Semua kultur kulit, rambut dan kuku yang positif telah dipilih. Sebarang pertumbuhan kulat di luar kawasan goresan kultur, data yang berulang atau tidak lengkap telah dikeluarkan daripada kajian. Tiga ratus lima puluh lapan pesakit telah dipilih. Lelaki lebih ramai daripada perempuan dengan nisbah 1.2:1. Umur pertengahan adalah 53 tahun dengan julat antara suku 38–64 tahun. Lebih daripada separuh (53.6%) berumur 20–60 tahun. Kadar pemencilan kultur adalah 89.0% untuk kuku, 56.2% untuk rambut dan 55.6% untuk kulit. Lima ratus dua puluh dua kulat telah dipencil daripada 358 spesimen klinikal. Kulapuk bukan dermatofit (NDMs) mewakili kumpulan terbesar (50.5%; terutamanya *Aspergillus* species 18.7%), diikuti oleh yis (41.6%; terutamanya *Candida* species 26.8%) dan dermatofit (7.9%; terutamanya *Trichophyton* species 7.7%). Kesimpulannya, NDMs dan yis adalah lebih kerap dipencil berbanding dermatofit daripada spesimen dermatologi di pusat perubatan ini. Cara rawatan kini yang memfokus pada dermatofit mungkin tidak berkesan untuk merawat dermatofit yang disebabkan oleh NDMs atau yis. Kajian kerentanan antikulat mungkin diperlukan untuk membantu rawatan dalam kes-kes yang sukar.*

Kata kunci: Dermatofit; dermatologi; dermatomikosis; kulapuk; kulat

INTRODUCTION

Dermatomycoses is a term referring to fungal infections of the skin or its appendages. The term covers fungal infections of hair (e.g. ectothrix, endothrix, piedra), skin (e.g. pityriasis, tinea, rashes) and nails (e.g. tinea unguium, onychomycosis). Skin mycoses have afflicted about 20 to 25% of the world's population (Havlickova et al. 2008). While most of skin mycoses originated superficially, some disseminated fungal infections may

also manifest as secondary skin rashes which are usually pustular, erythematous and sometimes umbilicated.

Local population, education, economy, health facilities and culture may influence the prevalence dermatomycoses. Several studies have shown that the types of fungi isolated from dermatological specimens can vary geographically (Clayton & Hay 1994; Havlickova et al. 2008) and temporally (Borman et al. 2007). Other studies also looked at the distribution patterns of fungal isolates based

on specific groups of population such as children and adolescents (Lange et al. 2004), prison inmates (Oyeka & Eze 2007), forestry workers and farmers (Sahin et al. 2005), HIV-positive individuals (Rodwell et al. 2008) and renal transplant recipients (Selvi et al. 1999). Common fungi implicated in these reports include the dermatophytes (*Trichophyton*, *Microsporum* and *Epidermophyton* species), yeasts (*Candida*, *Trichosporon* and *Malassezia* species) and non-dermatophyte moulds (*Aspergillus*, *Fusarium* and *Scopulariopsis* species). Although there are variations among these groups of population, *Trichophyton rubrum* has been consistently reported as the most common dermatophyte in all groups except among children, where *Microsporum canis* was most common (Lange et al. 2004). There is scarcity of data on the causative agents of dermatomycoses in Malaysia and Southeast Asia. Therefore, this study aimed to determine the causes of dermatomycoses in Kuala Lumpur, Malaysia. The collected data may give us better understanding in the epidemiology, aetiology and perhaps pathogenesis, investigation and treatment of dermatomycoses.

MATERIALS AND METHODS

The study was conducted between January 2008 and December 2010 at Universiti Kebangsaan Malaysia Medical Centre (UKMMC), a tertiary level, multi-disciplinary, teaching hospital located in Kuala Lumpur, Malaysia. Dermatological specimens including skin scrapings, hair and nail clippings were collected carefully from clinically suspected cases of dermatomycoses by the clinicians. The specimens were subjected to direct microscopic examination with 10% potassium hydroxide (KOH) solution and cultured onto Sabouraud dextrose chloramphenicol agar and mycobiotic agar (contains cycloheximide) in air at 30°C for a maximum of 30 days. All cultures of skin, hair and nails that yielded positive fungal growth were included. Any fungal growth outside the agar streaking area and duplicate results were excluded from the study. Moulds, including dermatophytes, were identified based on colony features and microscopic examination using scotch-tape technique and lactophenol cotton blue dye as described by Larone (2002); whereas yeasts were identified by colony features, germ tube test and microscopic examination of structures on cornmeal agar. Two mycology-trained laboratory technologists examined the macroscopic and microscopic features and identified the isolates. In the event of discrepancies, the isolates were referred to a specialist trained in mycology to decide on a final identification. Fungi were identified to species level, if possible. Fungi that failed to produce any sporulating structures, even after culturing onto potato dextrose agar, were reported as non-sporulating hyaline or dematiaceous fungi, depending on the features of their hyphae. Fungal isolates were grouped as yeasts, dermatophytes and non-dermatophyte moulds (NDMs). NDMs were further sub-grouped according to features of their hyphae,

which include hyalohyphomycetes, phaeohyphomycetes and zygomycetes. Hyalohyphomycetes are fungi with hyaline hyphae; phaeohyphomycetes are fungi with darkly pigmented hyphae (dematiaceous fungi) and zygomycetes are fungi with sparsely septate hyphae. Patients data were obtained from the laboratory request form, which included gender, age and type of specimen. Incomplete data were excluded from analysis. This study was approved by the UKM Medical Research and Ethics Committee.

RESULTS

Five-hundred-thirty-five dermatological specimens were received by the mycology laboratory from January 2008 to December 2010. The majority of specimens were skin (63.2%), followed by nails (33.8%) and hair (3.0%). Three-hundred-fifty-eight from 535 clinical specimens yielded positive fungal growth (66.9% rate of isolation). Positive culture rates for nails, hair and skin were 89.0%, 56.2% and 55.6%, respectively. Males were slightly more common than females with a ratio of 1.2:1. The median age of males was slightly more compared with females but this was not statistically significant. The most common age groups afflicted with dermatomycoses in both males and females were adults between 20 and 60 years old (Table 1).

Most of the positive cultures (245/358, 68.4%) isolated a single fungus. Seventy-five cultures (20.9%) isolated two fungi, 25 (7.0%) isolated three fungi and 13 (3.6%) isolated four or more fungi. Therefore, the total number of fungal isolates was 522 (Table 2). The mixed cultures tended to be yeast with non-dermatophyte moulds (NDMs) (55%). Other combinations include NDMs with NDMs (21.7%), yeast with yeast (10%), dermatophyte with NDMs (6.7%), yeast with dermatophyte (4.2%) and a mixture of yeast, dermatophyte and NDMs (2.5%). Overall, NDMs were the most common group of fungi isolated (50.5%), followed by yeasts (41.6%) and dermatophytes (7.9%). However, the most common genera isolated were *Candida* (26.8%), followed by *Aspergillus* (18.7%), *Penicillium* (10.2%), *Trichosporon* (8.2%) and *Trichophyton* spp. (7.7%) (Table 2).

DISCUSSION

To diagnose dermatomycosis, most, if not all clinicians rely mainly on the clinical presentation of the patients. The use of Wood's lamp may help in the diagnosis but is not widely available at our centre and it is unable to detect some fungi (Prevost 1983). Gram staining and potassium hydroxide (KOH) preparation are simple and rapid, however lack sensitivity and specificity. Culture is confirmatory, but questions of possible contamination or colonizers are often raised when NDMs are isolated. In this study, we only identified those fungi that grew from the streaking area of the culture plate. Those fungi that grew outside this area were considered contaminants. To minimize the

TABLE 1. Demographic data of patients clinically suspected with dermatomycoses with positive fungal cultures

	Male (n=196)	Female (n=162)	Total (n=358)	P value
Median age (IQR)	54 (40-66)	52 (34-62)	53 (38-64)	0.105
Age group in years*				
< 10 (paediatrics)	17 (8.7)	12 (7.4)	29 (8.1)	0.039
10-19 (adolescents)	4 (2.0)	14 (8.6)	18 (5.0)	
20-60 (adults)	106 (54.1)	86 (53.1)	192 (53.6)	
> 60 (elderly)	69 (35.2)	50 (30.9)	119 (33.2)	
Positive hair culture (%)	7 (58.3)	2 (50.0)	9 (56.2)	1.000
Positive skin culture (%)	105 (57.4)	83 (53.5)	188 (55.6)	0.511
Positive nail culture (%)	84 (89.4)	77 (88.5)	161 (89.0)	1.000

IQR, inter-quartile range

*age categories are according to WHO definition (2013)

possibility of the fungi being colonizers, proper techniques of specimen collection must be adhered to.

'Dermatomycoses' is often confused with 'dermatophytoses' especially among the non-dermatologically-inclined clinicians. Dermatophytoses (synonym: tinea) are fungal infections caused by dermatophytes only (namely *Trichophyton*, *Microsporum* and *Epidermophyton* spp.). Whereas dermatomycoses include all dermatological infections (i.e. of hair, skin and nails) caused by any fungi including yeasts, dermatophytes and NDMs. Epidemiology of dermatophytes causing skin infections in Malaysia was reported in 2001 (Ng et al. 2001) but this study only looked at dermatophytes and not other fungal causes. Another study reported the aetiological agents of fungal nail infection (onychomycosis) only (Ng et al. 1999). There are no reports on the causative agents of other dermatomycoses (i.e. hair and skin infections) in Malaysia. Both studies in Malaysia reported *Trichophyton rubrum* as the most common dermatophyte isolated. In contrast to most other studies on dermatomycoses (Brajac et al. 2003; Mathur et al. 2008; Sellami et al. 2008), our studies have shown that NDMs and yeasts, rather than dermatophytes, were the most common group of fungi isolated.

The NDMs comprise of fungi that belongs to Class Hyalohyphomycetes, Phaeohyphomycetes and Zygomycetes. The clinical significance of Hyalohyphomycetes was the most difficult to ascertain. Their spores are abundant in the environment and can easily contaminate work surfaces or the clinical specimens. Hence, the clinical significance of these isolates needs to be correlated clinically. *Aspergillus niger*, the most commonly identified hyalohyphomycete, was mainly recovered from nails (52/70 isolates, 74.3%); 25.7% from skin and none from hair. *Penicillium* species was another common hyalohyphomycete isolated in this study; most of them (34/53 isolates, 64.2%) were isolated from skin, the rest from nails (30.1%) and hair (5.7%). *Penicillium* species (other than *P. marneffeii*) are usually regarded as contaminants. However, some studies have reported rare cases of true infections caused by *Penicillium* species other than *P. marneffeii* such as onychomycosis (Ramani et al. 1994), cutaneous infection (Lo'pez-Martinez et al. 1999) and even invasive infections (Lyrtatzopoulos et al.

2002). Therefore, isolation of *Penicillium* species from clinical specimens does not always mean contamination. The interpretation of its clinical significance should be made in light of clinical findings. On the other hand, *P. marneffeii* is well documented as an opportunistic fungal pathogen especially among immunocompromised individuals (Sirisanthana & Supparatpinyo 1998). From our study, there was only one isolate of *P. marneffeii* isolated from a skin biopsy of a 32-year-old HIV-positive man. This was most likely due to dissemination from blood as his blood culture was also positive for *P. marneffeii*. Meanwhile, the majority of *Fusarium* species (88.9%) were isolated from nail specimens. Only 11.1% was isolated from skin. This is in contrast to a study on 259 patients (232 immunocompromised, 27 immunocompetent) with fusariosis where 72% and 52% of these patients, respectively, had skin involvement (Nucci & Anaissie 2002).

Dermatomycoses by phaeohyphomycetes showed a more variable pattern where no single organism stood out from the others as the main causative agent. Most of them were isolated from skin (50.0%) and nails (44.8%). However, phaeohyphomycoses have been reported to cause infections at other body sites too (Revankar 2006). Although little is known about the pathogenesis of these infections, the presence of melanin in the cell wall is thought to be the virulence factor. It is believed to act by scavenging free radicals and hypochlorite and bind to hydrolytic enzymes produced by phagocytic cells (Jacobson 2000).

Zygomycetes were rarely isolated in our study, with *Rhizopus* species being the most common species identified. However, there was a report of five cases of primary cutaneous infections with *Aspergillus* species and *Rhizopus* species within a period of 16 months, among patients with haematological malignancy and neutropaenia (Khardori et al. 1989). All of these cases were thought to be associated with moist and humid conditions created by occlusive dressings or excessive perspiration. In our study, three *Rhizopus* species were isolated from nails, two from skin soles and one from hair. All of them were isolated in mixed cultures, raising the question of their true clinical significance.

TABLE 2. Prevalence of fungal isolates according to type of specimens

Isolate	Hair	(%)	Skin	(%)	Nails	(%)	Total	(%)
Yeasts	5	(31.3)	116	(43.1)	96	(40.5)	217	(41.6)
<i>Candida albicans</i>	2	(12.5)	14	(5.2)	16	(6.8)	32	(6.1)
<i>Candida glabrata</i>	0	(0)	3	(1.1)	2	(0.8)	5	(1.0)
<i>Candida parapsilosis</i>	0	(0)	28	(10.4)	29	(12.2)	57	(10.9)
<i>Candida species</i>	1	(6.3)	25	(9.3)	10	(4.2)	36	(6.9)
<i>Candida tropicalis</i>	0	(0)	2	(0.7)	8	(3.4)	10	(1.9)
<i>Geotrichum</i> spp.	1	(6.3)	1	(0.4)	2	(0.8)	4	(0.8)
<i>Malassezia</i> spp.	0	(0)	1	(0.4)	0	(0)	1	(0.2)
<i>Monilia sitophila</i>	0	(0)	0	(0)	1	(0.4)	1	(0.2)
<i>Rhodotorula</i> spp.	0	(0)	17	(6.3)	5	(2.1)	22	(4.2)
<i>Saccharomyces</i> spp.	0	(0)	0	(0)	3	(1.3)	3	(0.6)
<i>Sporobolomyces salmonicolor</i>	0	(0)	0	(0)	1	(0.4)	1	(0.2)
<i>Trichosporon</i> spp.	0	(0)	24	(8.9)	19	(8.0)	43	(8.2)
<i>Ustilago</i> spp.	1	(6.3)	1	(0.4)	0	(0)	2	(0.4)
Dermatophytes	1	(6.3)	36	(13.4)	4	(1.7)	41	(7.9)
<i>Microsporium canis</i>	1	(6.3)	0	(0)	0	(0)	1	(0.2)
<i>Trichophyton rubrum</i>	0	(0)	5	(1.9)	0	(0)	5	(1.0)
<i>Trichophyton</i> spp.	0	(0)	30	(11.2)	4	(1.7)	34	(6.5)
<i>Trichophyton tonsurans</i>	0	(0)	1	(0.4)	0	(0)	1	(0.2)
NDMs	10	(62.5)	117	(43.5)	137	(57.8)	264	(50.5)
Hyalohyphomycetes	5	(31.3)	78	(29.0)	103	(43.5)	186	(35.6)
<i>Acremonium</i> spp.	0	(0)	0	(0)	1	(0.4)	1	(0.2)
<i>Aspergillus flavus</i>	0	(0)	5	(1.9)	8	(3.4)	13	(2.5)
<i>Aspergillus fumigatus</i>	1	(6.3)	3	(1.1)	2	(0.8)	6	(1.1)
<i>Aspergillus niger</i>	0	(0)	18	(6.7)	52	(21.9)	70	(13.4)
<i>Aspergillus</i> spp.	0	(0)	4	(1.5)	2	(0.8)	6	(1.1)
<i>Aspergillus terreus</i>	0	(0)	2	(0.7)	1	(0.4)	3	(0.6)
<i>Chrysosporium</i> spp.	0	(0)	2	(0.7)	1	(0.4)	3	(0.6)
<i>Fusarium solani</i>	0	(0)	1	(0.4)	3	(1.3)	4	(0.8)
<i>Fusarium</i> spp.	0	(0)	1	(0.4)	13	(5.5)	14	(2.7)
<i>Malbranchea</i> spp.	0	(0)	2	(0.7)	1	(0.4)	3	(0.6)
<i>Paecilomyces lilacinus</i>	0	(0)	1	(0.4)	0	(0)	1	(0.2)
<i>Penicillium marneffeii</i>	0	(0)	1	(0.4)	0	(0)	1	(0.2)
<i>Penicillium</i> spp.	3	(18.8)	33	(12.3)	16	(6.8)	52	(10.0)
<i>Sepedonium</i> spp.	0	(0)	1	(0.4)	0	(0)	1	(0.2)
<i>Trichoderma</i> spp.	1	(6.3)	4	(1.5)	3	(1.3)	8	(1.5)
Phaeohyphomycetes	3	(18.8)	29	(10.8)	26	(11.0)	58	(11.1)
<i>Aureobasidium pullulans</i>	0	(0)	1	(0.4)	0	(0)	1	(0.2)
<i>Cladosporium</i> spp.	0	(0)	6	(2.2)	2	(0.8)	8	(1.5)
<i>Curvularia</i> spp.	0	(0)	1	(0.4)	5	(2.1)	6	(1.1)
<i>Exophiala</i> spp.	0	(0)	0	(0)	1	(0.4)	1	(0.2)
<i>Fonsecaea</i> spp.	0	(0)	0	(0)	2	(0.8)	2	(0.4)
<i>Hortaea werneckii</i>	0	(0)	1	(0.4)	2	(0.8)	3	(0.6)
<i>Madurella grisea</i>	0	(0)	3	(1.1)	0	(0)	3	(0.6)
<i>Madurella</i> spp.	0	(0)	6	(2.2)	4	(1.7)	10	(1.9)
<i>Phialemonium</i> spp.	2	(12.5)	3	(1.1)	2	(0.8)	7	(1.3)
<i>Phialophora</i> spp.	0	(0)	1	(0.4)	3	(1.3)	4	(0.8)
<i>Piedra hortae</i>	1	(6.3)	0	(0)	0	(0)	1	(0.2)
<i>Scedosporium prolificans</i>	0	(0)	1	(0.4)	0	(0)	1	(0.2)
<i>Scedosporium</i> spp.	0	(0)	1	(0.4)	0	(0)	1	(0.2)
<i>Scytalidium</i> spp.	0	(0)	4	(1.5)	4	(1.7)	8	(1.5)
<i>Stemphylium</i> spp.	0	(0)	1	(0.4)	1	(0.4)	2	(0.4)
Zygomycetes	1	(6.3)	3	(1.1)	7	(3.0)	11	(2.1)
<i>Conidiobolus coronatus</i>	0	(0)	0	(0)	2	(0.8)	2	(0.4)
<i>Rhizopus</i> spp.	1	(6.3)	2	(0.7)	3	(1.3)	6	(1.1)
<i>Syncephalastrum</i> spp.	0	(0)	1	(0.4)	2	(0.8)	3	(0.6)
Non-sporulating hyaline mould	1	(6.3)	7	(2.6)	1	(0.4)	9	(1.7)
Total	16	(100)	269	(100)	237	(100)	522	(100)

Among the yeasts, it is interesting to note that *C. parapsilosis* has surpassed *C. albicans* as the most common yeast isolated, especially from nail (12.2% vs. 6.8%) and skin (10.4% vs. 5.2%) specimens. However, no *C. parapsilosis* was isolated from hair specimens as compared with *C. albicans* (12.5%). This is in contrast to other reports that *C. albicans* was still the most common yeast isolated in Africa (Ellabib et al. 2002; Lohoue et al. 2004), Australia (McAleer 1980), Europe (Brajac et al. 2003; Ergin et al. 2002; Seneczko et al. 1999) and Asia (Das et al. 2007; Mathur et al. 2008; Ng et al. 1999). The importance of this finding is that in our centre, *C. parapsilosis*, like other non-*albicans* *Candida* spp., were shown to have elevated minimum inhibitory concentrations (MICs) to azoles as compared with *C. albicans* (Tzar & Shamim 2009). This could adversely affect the effectiveness of treatment with azoles. Other yeasts in this study such as *Trichosporon* and *Rhodotorula* species were mainly isolated from skin and nails.

For the dermatophytes, most of them were isolated from skin (87.8%), whereas only 9.8% and 2.4% were isolated from nail and hair, respectively. In our study, *Trichophyton rubrum* was the most common dermatophyte identified. This finding is consistent with most other studies (Rodwell et al. 2008; Singh et al. 2003; Welsh et al. 2006) except for studies among children where *Microsporum canis* was the most common dermatophyte (Lange et al. 2004; Popoola et al. 2006). The only *Microsporum canis* isolated in this study was from a scalp scraping of a 9-year-old boy. There was no *Epidermophyton* species isolated in this study.

Our study was limited in terms of some incomplete documentation, which led to exclusion of some data. The methods of fungal identification used in this study were also not optimum, whereby we mostly relied upon personal expertise of the laboratory technicians in conducting conventional identification methods, which were subjective and may result in identification bias. Carbohydrate assimilation for yeasts and molecular methods (nucleotide amplification and sequencing) for yeasts and moulds, are more objective in confirming the identification but they are not economical and practical to use for identifying fungi from non-sterile specimens in routine diagnostic laboratories. However, we tried to mitigate this issue by having mycologically-trained personnels to identify the fungi by observing characteristic macroscopic and microscopic features of the fungi. The diagnosis of dermatomycosis also ideally needs to be confirmed by histopathological studies. However, this is not always practical for routine practice. Despite these limitations, data from this study may provide a valuable addition to the knowledge gap in epidemiology of dermatomycoses in this region. We would recommend further cohort studies on patients with dermatomycoses to evaluate the effectiveness of current treatment regime of dermatomycoses. A local database on antifungal susceptibility profiles of the causative agents may be useful to guide therapy for recalcitrant cases.

CONCLUSION

Our study showed that non-dermatophyte moulds (NDMs) were more commonly isolated than dermatophytes, in contrast to other studies on dermatomycoses in other parts of the world. Although NDMs are often regarded as contaminants, their true clinical significance should always be interpreted in light of clinical findings. This is in line with many studies reporting NDMs as true pathogens. High prevalence of dermatomycoses caused by NDMs may render current treatment regime for dermatomycoses ineffective. Local database on epidemiology and antifungal susceptibility patterns should be developed to assist clinicians to make the best choice of antifungal agent.

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