ORIGINAL ARTICLE

Fungus isolated from dermatomycoses: a 9-month prospective study at Hospital Melaka

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ABSTRACT

Introduction: Dermatomycoses are common superficial cutaneous fungal infections which affect the skin, nails and human hairs. It affects 20 to 25% of the world population. The causative fungus varies geographically across the globe. Study on dermatomycoses is crucial to identify the aetiological fungus involved locally. The study aimed to determine the causative fungus of superficial fungal infections of the skin, nail and hair in patients presented to Hospital Melaka.

Methods: This was a prospective study conducted from 15th January 2022 till 15th October 2022 at Dermatology Clinic, Hospital Melaka. Subjects with clinical dermatomycoses were included in this study. The samples were collected from skin, nails and hairs clinically affected by tinea corporis/cruris/pedis, onychomycosis and tinea capitis respectively. A potassium hydroxide (KOH) study was performed on the sample in which the fungal hyphae/yeast positive subjects were sent for fungal culture and fungal PCR test.

Result: A total of 222 clinical samples from skin, nails and hairs with a clinical suspicion of dermatomycoses yielded fungal hyphae/yeast in KOH. Majority of the samples were collected from skin (138, 62.2%), followed by nails (65, 29.3%) and hairs (19, 8.6%). Male to female ratio was 1.18: 1. The age ranged from 2 to 87 with the median of 55.5-years-old. Out of 222 samples, 150 (67.6%) were fungal culture positive. From fungal culture positive samples, 87 samples were from tinea corporis, 50 samples were from onychomycoses and 13 samples were from tinea capitis. *Trichophyton rubrum* (39, 44.8%) was the commonest dermatophyte isolated in tinea corporis/cruris/pedis. Non-dermatophyte moulds (NDM, 35, 70%) were the main fungi isolated in onychomycosis. *Microsporum canis* (7/53.8%) was the principal causative fungus among patients with tinea capitis. Among 150 fungal culture positive samples, 76 were fungal PCR positive. Only 38 samples consistently isolated same fungal species in both fungal culture and PCR test.

Conclusion: Majority of tinea corporis and tinea capitis fungal culture isolated dermatophytes, especially *Trichophyton rubrum* and *Microsporum canis*, respectively. Non-dermatophyte moulds were mainly isolated in onychomycosis.

KEYWORDS:
Dermatomycosis, dermatophytes, moulds, Trichophyton rubrum, Microsporum canis

INTRODUCTION

Dermatomycoses are superficial cutaneous fungal infection which affects the skin, nails and hairs of humans. It is caused by dermatophytes, non-dermatophyte moulds (NDM) and yeasts. Dermatophytes consist of *Trichophyton, Microsporum* and *Epidermophyton*. Non-dermatophyte moulds include other filamentous fungi such as *Fusarium* and *Aspergillus*. Yeasts which are round or oval, encompass *Candida, Malasezzia* and *Trichosporon*.

Superficial skin mycosis is a common skin infection that affects 20–25% of the world population.[1] Transmission of dermatomycoses can occur by direct contact with infected people, animals and contaminated soil.[2] The causative fungus varies due to the difference in regions, climate, lifestyles, ages and the affected sites.[3] Dermatophyte was the commonest fungus isolated in United State[4] China[5] and Vietnam.[6] Non-dermatophyte moulds were commonly identified in Malaysia population affected by onychomycosis.[7,8]

To date, the number of studies or audits on the causative agents of dermatomycoses in Malaysia is limited. In addition, they are subjected to bias due to the retrospective nature of the studies. Here we aim to investigate the common pathogenic fungi involved dermatomycoses in our local population.

MATERIALS AND METHODS

This was a prospective study conducted from 15th January 2022 till 15th October 2022 at Dermatology Clinic, Hospital Melaka. We included all patients with clinical dermatomycoses and were treatment naïve. We excluded patients who had already received anti-fungal drugs in the last 6 months. Clinical samples included skin scrapings, hair plucking and nail clippings of the respective diseased sites. After obtaining proper informed consent, skin scrapings were collected from tinea corporis/cruris/pedis; nail clippings from onychomycoses; plucked hairs from tinea capitis.

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The sites of lesions were cleaned with 70% alcohol. The skin scrapings were collected from the active edges of the lesion with a sterile blunt scalpel. The infected nails were clipped with nail clipper. The affected hairs were epilated from the scalp lesion with sterile forceps. All samples were subjected to direct microscopic examination with 10 to 20% potassium hydroxide (KOH) solution. Sample which yielded positive fungal bodies (yeasts and hyphae) will then be seeded on the surface of Sabourouds dextrose agar media from Thermo Fisher Scientific, incubated at 30ºC and culture growth was analysed daily. The isolated fungi were identified based on the examination of the cultural characteristics and microscopic morphology. Cultures without growth during a period of up to 4 weeks were considered negative.

Polymerase chain reaction (PCR) was performed for positive dermatophytes' cultures for comparison study. Using bead bashing method of Fungal/Bacterial DNA MiniPrepTM kit (Zymo Research, USA), the fungal DNA was extracted according to the manufacturer instructions. Thereafter, DNAs were amplified by PCR using a set of universal primers; a forward primer (ITS1: 5′- TCCGTAGGTGAACCTGCGG-3′) and a reverse primer (ITS4: 5′-TCCTCCGCTTATTGATATGC-3′). The use of primers ITS1 and ITS4 have been verified in numerous dermatophyte studies to screen for the presence of fungus in the specimens.9,10 The PCR amplification was done using a Thermal Cycler (Gene Amp, PCR system 9700, Applied Biosystems, USA) in a total volume of 25 µl consisting of 3.0 µl DNA, 12.5 µl MyTaq HS mix (Bioline Meridine Bioscience, London, UK), 0.5 µl of each primer and 8.5 µl of nuclease free distilled water. Quality control DNA were included in the assay. Amplified PCR fragments were analysed to determine DNA bands through which observed by UV transilluminator alongside DNA Ladder (100 bp). The amplified DNA was sent for sequenced by Apical Scientific Sdn Bhd and further analysed to identify the fungal species. Data was presented in table and further analysed using SPSS. Categorical data will be analysed using Chi square(crosstabs) to acquire "*p*" value. Demographic information and fungi isolated were summarised using descriptive statistics.

RESULTS

A total of 222 clinical samples of skin scrapings, nail clippings and hair plucking that showed hyphae or yeasts in KOH staining (KOH+) were included for final analysis (Figure 1). Majority of samples were collected from skin (138/62.2%), followed by nail (65/ 29.3%) and hair (19/ 8.6%). Out of 222 KOH+ samples, 150 (67.6%) had a positive fungal culture. Interestingly, of the 150 fungal culture positive samples, only 76 (50.7%) were fungal PCR positive. Only 38 (50%) samples had same fungal species identified in both fungal culture and PCR test. In another 38 (50%) samples, discordant fungal species was observed between PCR and culture.

As shown in Table I, the male to female ratio was 1.18: 1. The age ranged from 2 to 87 with the median 55.5-years-old. Majority were adults (108/48.6%). The median duration for onset of symptoms before presenting to our clinic was 5.5 months. Patients with weaken immune system such as those with diabetes mellitus, end stage renal failure (ESRF), human immunodeficiency virus (HIV) infection, nephrotic syndrome, malignancy, systemic lupus erythematosus (SLE) and those taking immunosuppressive agents (methotrexate, cyclosporin) yielded a significantly higher rate of *Trichophyton rubrum* compared to patients with normal immune system.

Based on fungal culture positive samples, 87 samples were from tinea corporis/cruris/pedis, 50 samples were from onychomycoses and 13 samples were from tinea capitis. The results were shown in Table II. Dermatophyte, especially Trichophyton rubrum was the most common fungus isolated in tinea corporis. Non-dermatophyte moulds were the main fungi isolated in onychomycosis, in which *Aspergillus species* being the most common species isolated. *Micosporum canis* was the principal causative fungus among patients with tinea capitis. Results were shown in Table III.

Based on the fungal PCR positive result, there was a total of 42 samples from tinea corporis/cruris/pedis, 25 samples from onychomycoses and nine samples were from tinea capitis as shown in Table II. Table III illustrated the fungal species resulted in the superficial fungal infections. *Trichophyton rubrum* was the most common fungus found in PCR result for tinea corporis/cruris/pedis and onychomysosis. While *Microsporum canis* was the most common fungal isolated in tinea capitis. Among 38 concordant fungal culture and PCR result (same fungal species identified in both fungal culture and PCR), dermatophyte *Trichophyton rubrum* was the most common species identified (18, 47.4%) followed by *Trichosporon mentagrophytes* (6,15.8%).

DISCUSSION

Tinea infection (dermatophytosis) are superficial fungal infections caused by dermatophytes that affect skin, nails and hairs. It is a filamentous fungus that invades cutaneous keratinised stratum corneum e. g. skin, hairs and nails. The pathogenesis for dermatophytosis starts with interaction of dermatophyte with host cell, resulting in adhesion to epidermal layer within 1 hour. This process is mediated by adhesins protein present on the fungal cell wall.[11] This is followed by penetration of stratum corneum by secreting keratinolytic proteases, serine subtilisin and fungalysin. Then arthroconidia germinate and the hyphae inoculate through stratum corneum to prevent removal with cell shedding. This happens within 3 to 4 hours. Within 1 to 3 days, the hyphae will spread through over the skin.[11] In the fungal cell wall, there is a glycoprotein-mannan which promotes fungal infection by inhibiting proliferation of keratinocyte. This protein prevents shedding and suppresses inflammatory response. Cell mediated immune response is greatly suppressed by mannans from various species of dermatophytes. For instance, *Trichophyton rubrum* produces more mannan than *Microsporum canis*. Hence, *Trichophyton rubrum* can suppress lymphoproliferation more effectively as compared to *Microsporum canis*. This has contributed to the less inflammation and more chronicity in *Trichophyton rubrum* infection compared to *Microsporum canis*.[11] This probably explained *Trichophyton rubrum* being the most common causative fungus among tinea corporis/cruris/pedis patients in our population.

Table I: Clinical characteristics of patients with clinical suspicious of dermatomycosis

Characteristics			Total (n = 222)	Culture positive n = 150			Culture negative n = 72			*p* value
				skin	hair	nail	skin	hair	nail	
Age in years	Median		55.5							
	Age range	Paediatric (<10):	14	0	12	0	0	2	0	0.016
		Adolescent (1 to 19)	13	4	0	0	6	3	0	
		Adult (20 to 60)	108	59	1	14	27	1	6	
		Elderly (>60):	87	24	0	36	18	0	9	
Gender		Ratio (male:female)			1.18:1					0.981
		Male	120	43	11	27	29	2	8	
		Female	102	44	2	23	22	4	7	
Ethinicty		Malay	163	71	12	29	38	6	7	0.845
		Chinese	45	12	1	15	10	0	7	
		Indian	11	3	0	5	2	0	1	
		others	3	1	0	1	1	0	0	
Occupation		Blue Collar	49	23	0	13	9	1	3	0.181
		White collar	29	12	0	5	7	5	0	
		Retired personnel	18	7	0	5	5	0	1	
		Unemployed	62	18	2	25	12	0	5	
		Student	32	5	11	0	13	0	3	
		housewife	32	17	0	7	5	0	3	
Risk factor For fungal infection		Low immune status*	75	29	1	29	13	0	3	0.015
		No risk of low immune	147	58	12	21	38	6	12	
Animal or plant contact		Animal contact	67	21	8	13	18	3	4	0.463
		Plant contact	30	11	0	10	7	0	2	
		No contact	125	55	5	27	26	3	9	

* Low immune status included those with underlying diabetes mellitus, ESRF, HIV, nephrotic syndrome, malignancy, SLE and those taking immunosuppressive agents.

Table II: Types of fungal isolated from culture or polymerase chain reaction based on sample collected site

Type of test / Type of test	Fungal culture				Fungal PCR			
	Skin	Hair	Nail	Total	Skin	Hair	nail	Total
Dermatophyte	49(56.3)	10(76.9)	1(2)	60	35(83.3)	8(88.9)	7(28)	49
Non-dermatophyte moulds	24(27.6)	3(23.1)	41(82)	67	1(2.3)	1(11.1)	7(28)	14
Yeast	14(16.1)	0	8(16)	23	6(14.3)	0	11(44)	13
Total	87	13	50	150	42	9	25	76

Table III: Fungus isolated from fungal culture or polymerase chain reaction

Fungus isolated from fungal culture/PCR	Tinea corporis/cruris/ pedis n (%)		Onychomycosis n (%)		Tinea capitis n (%)	
	Culture n = 87	PCR n = 42	Culture n = 50	PCR n = 25	Culture n = 13	PCR n = 9
Dermatophyte						
I. *Trichophyton rubrum*	39(44.8)	20(23)	1(2)	4(8)	2(15.4)	2(15.4)
II. *Trichophyton mentagrophytes*	7(8.0)	14(16.1)	0	2(4)	0	0
III. *Trichophyton tonsurans*	2(2.3)	0	0	0	0	0
IV. *Trichophyton species*	0	0	0	1(2)	0	0
V. *Microsporum canis*	0(0)	1(1.1)	0	0	7(53.8)	6(46.2)
VI. *Microsporum gypseum*	0(0)	0	0	0	1(7.7)	0
VII. *Epidermophyton*	1(1.1)	0	0	0	0	0
Yeast						
I. *Candida species*	13(14.9)	0	7(14)	0	0	0
II. *Candida albicans*	1(1.1)	2(2.3)	0	1(2.0)	0	0
III. *Candida orthopsilosis*	0	0	0	1(2.0)	0	0
IV. *Candida parapsilosis*	0	1(1.1)	0	1(2.0)	0	0
V. *Candida parapsilosis complex*	0	0	0	1(2.0)	0	0
VI. *Candida tropicalis*	0	1(1.1)	0	2(4.0)	0	0
VII. *Trichosporon*	0	1(1.1)	1(2)	2(4.0)	0	0
VIII. *Geotrichum*	0	0	1(2)	0	0	0
IX. *Apiotrichum montevideense*	0	0	0	1(2.0)	0	0
X. *Meyerozyma caribbica*	0	0	0	1(2.0)	0	0
XI. *Wickerhamiella shivajii*	0	0	0	1(2.0)	0	0
XII. *Saccharomyces cerevisiae*	0	1(1.1)	0	0	0	0
Non dermatophyte Hyalohyphomycetes						
I. *Aspergillus flavus*	0	0	3(6)	0	0	0
II. *Aspergillus fumigatus*	0	0	2(4)	0	0	0
III. *Aspergillus niger*	1(1.1)	0	7(14)	1(2)	0	0
IV. *Aspergillus terreus*	0	0	1(2)	0	0	0
V. *Aspergillus penicillioides*	0	0	0	0	0	1(7.7)
VI. *Fusarium species*	1(1.1)	0	10(20)	0	0	0
VII. *Fusarium keratoplasticum*	0	0	0	2(4)	0	0
VIII. *Fusarium solani complex*	0	1(1.1)	0	1(2)	0	0
IX. *Scopulariopsis species*	1(1.1)	0	0	0	0	0
X. *Penicillium species*	0	0	2(4)	0	0	0
Phaeohyphomycetes						
I. *Cladosporium*	8 (9.2)	0	1(2)	0	0	0
II. *Curvularia*	1(1.1)	0	3 (6)	0	0	0
III. *Exophiala*	2(2.3)	0	0	0	0	0
IV. *Phialophora species*	2(2.3)	0	0	0	0	0
V. *Scytalidium dimidiatum*	0	0	2(4)	1(2)	0	0
VI. *Hortea werneckii*	0	0	0	2(4)	0	0
Zygomycetes						
I. *Rhizopus*	0	0	4(8)	0	0	0
Non-sporulating hyaline mould	8(9.2)	0	5(10)	0	3(23.1)	0
Total	**87**	**42**	**50**	**25**	**13**	**9**

Interestingly, *Trichopyton tonsurans* was the predominant species described in United States[12] among patients with tinea corporis/cruris/pedis. In our study, we found that dermatophytes were mostly isolated from skin and hair samples. This is consistent with other studies in different countries such as New Zealand[13] and Mexico.[14] *Trichophyton rubrum* was the most frequent dermatophyte identified from skin samples for culture in China[5], French Guiana[15], and Brazil[16] (Table IV). M*icrosporum canis* was the most common fungus isolated from the scalp among the children with tinea capitis in this study. This finding was consistent with the studies among tinea capitis patients in Poland[17] and Northern Greece[18] (Table IV). Only one *Epidermophyton* isolated in the culture of skin sample.

Candida sp. was the most common yeast isolated from skin scrapings and nail clippings. This is consistent with other studies in most regions namely Africa[19], Australia[20], Europe[21] and Asia.[22] Candida is a dimorphic yeast that can exist in unicellular yeast form to filamentous hyphal form which can release hydrolytic enzymes including phospholipase, proteinase, lipase, esterase and haemolysing. These hydrolytic enzymes invade host cell through degradation of tissues cellular component and facilitate their adhesion, invasion, survival and dissemination.[23] Cutaneous candidiasis is characterised by erythematous patches with satellite lesions commonly found at warm, moist, creased areas such as the inframammary and groin skin. The most typical cause of diaper rash in young children is candida as

Table IV: Comparison dermatomycoses pattern in different countries

Author, year, country	n	Dermatophytes	NDM	Yeasts
Current study	150	*Trichophyton rubrum*: 42/60 (70%) *Trichophyton Mentagrophytes*: 7/60	*Fusarium sp*: 11/67 (16.4%)	*Candida sp*: 20/23 (71.5%)
Cai et al,[5] 2016, China	697	*Trichophyton rubrum*:392/588 *Trichophyton mentagrophyte*: 93/588 *Trichophyton violaceum*: 21/588	*Fusarium sp*: 4/5	*Candida albicans*: 54/104
Simonnet et al,[15] 2011, France	449	*Trichophyton rubrum*: 97/268 *Trichophyton mentagrophyte*: 38/268 *Microsporum canis*: 20/268	*Neoscytalidium dimidiatum*: 39/56	*Candida albicans*: 61/125
Silva-Rocha et al,[16] 2012, Brazil	113	*Trichophyton rubrum*: 21/59 *Trichophyton tonsurans*: 15/59 *Microsporum canis*: 8/59	*Fusarium sp*: 8/9	*Candida parapsilosis*: 18/45
Koussidou-Eremondi et al,[18] 2005, Northen Greece	611	*Microsporum canis*: 515/611 (tinea capitis 276/280) *Trichophyton rubrum*: 34/611 *Trichophyton mentagrophyte*: 11/611		*Candida albicans*: 20/611
Lange et al,[17] 2004, Poland	94	*Microsporum canis*: 58/94 (tinea capitis 25/30) *Trichophyton rubrum*: 16/94 *Trichophyton tonsurans*: 15/94		
Lim et al,[40] 1992, Singapore	87	*Trichophyton rubrum*: 17/27 *Trichophyton interdigitale*: 6/27 *Trichophyton mentagrophyte*:3/27 *Trichophyton violaceum*: 1/27	*Fusarium* : 7/13 *Aspergillus*: 3/13 *S. brevicaulis*: 1/13 *Aureobasidium*: 1/13 *Penicillium*: 1/13	*Candida albicans*: 45/47 *Candida parapsilosis*: 2/47

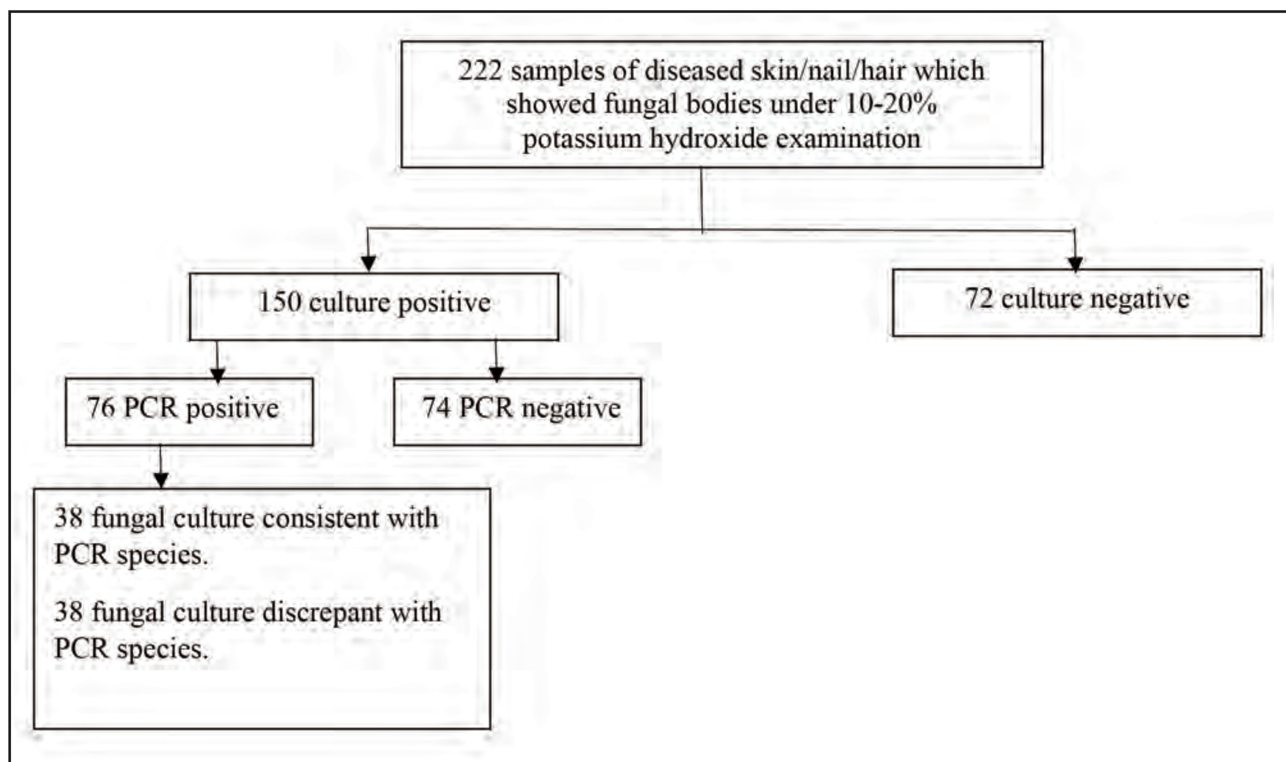

Fig. 1: The study workflow

diaper use provides a warm moist environment suitable for fungal growth. In addition, patients with low immune system e.g., diabetes, cancer, retroviral infection, use of broad-spectrum antibiotic, organ transplant are predisposed to candidiasis.

In onychomycosis, dermatophytes were the predominant fungi identified in Tunisia Afrika[24] and Turkey Middle East.[25] Moulds and dermatophytes were commonly found among onychomycosis patients in tropical country like Thailand.[26] Non dermatophyte moulds (*Hyalohyphomycetes*, *Phaeohyphomycetes* and *Zygomycetes*) were the most frequent fungi isolated among patients with onychomycosis in our study. This was consistent with other studies done in other states in Malaysia.[8,27]

As the spore of NDM can be ubiquitous in environment, therefore it may contaminate clinical specimen. Moulds isolated from nails were thought to be contaminants, however there are many studies observed that moulds could be pathogenic and are causative agents for onycomycosis.[28,29] It becomes pathogenic if it exhibits both positive microscopy and culture results or if a repeated culture yields the same isolated species.[30] Hence, the presence of mould in fungal culture result needs careful assessment and clinical correlation for its pathogenicity[7], especially in immunocompromised population. Non-sporulating hyphae are moulds that are unable to produce spores either due to unsuitable environment of it they have lost ability or need longer period to produce spores.[31] There are (16/10.7%) of non-sporulating hyphae in this study. In nature, fungi that don't sporulate in culture do produce spores. These fungi can result in irritant and allergy as well as systemic infections especially among population with impaired immune systems.[31] In addition, it is not possible to identify the precise non-sporulating hyphae unless sporulation can be induced, in which the process takes weeks to months.[31] Hence clinical judgment plays an important role in determining the clinical significance of non-sporulating hyphae.

In order to minimise false negative result, the technique to obtain good fungal nail samples is crucial. Micro-drilling, proximal sampling, and subungual curettage are the recommended methods to produce high culture yield for fungal nail culture as compared to conventional nail clipping.[32] Common practice of distal nail clipping results in high prevalence of contamination. This can be overcome by proper cleaning of the nail plate with alcohol before sample taking. In our study, majority of onychomycosis grown NDMs consisting of *Aspergillus* (13/26%) species and *Fusarium* (10/20%). Therefore, repeated nail samplings are deemed needed in order to detect the true pathogenic agents.

In this study, nearly half of the fungal culture positive samples were negative in PCR test. Besides, some fungal culture positive and PCR positive samples yielded discordant fungal species. The inconsistencies may be attributed by insufficient fungal cells in the PCR sample, improper storage of the specimen or late arrival of sample to the lab. Sample processing for fungal cultures is ideally within 2 hours following sample collection.[33] Incorrect technique of sample collection may also affect the culture results significantly. Lastly, improper techniques of PCR by lab assistant also contribute to negative PCR results. According to studies done by in Sweden[34] and India[35], the detection rate of dermatophytes for the fungal PCR in nail specimens with suspected dermatophytosis was 44% and 48%, respectively. This was consistent with our detection rate of around 50%. The low detection rate in these studies was attributed to the mutations in the fungal strains recognised by the PCR hybridising[38] and inhomogeneous distribution of fungal DNA in the sample collected.[35] Other possibility included the use of suboptimal methods for DNA extraction.[36] The method of DNA extraction should selectively accumulate the fungal DNA and at the same time reduce human DNA. The larger fungal DNA volume, the more for amplification and hence the more sensitive. On the other hand, the more human DNA in clinical sample used for amplification will resulting in cross-hybridisation and inhibition of the PCR.[36]

Fungal culture remains the gold standard for the microbiological diagnosis of fungal infections and the price to perform a fungal culture is much cheaper compared to PCR test. Nevertheless, the turn-around-time to perform fungal PCR test was shorter (within days) compared to fungal culture (within weeks). Currently, fungal PCR is not widely available for commercial use.

Among the local Melaka population, most patients with dermatomycoses will initially visit general practitioners. Hence, the diagnosis is often clinically based. Laboratory investigations such as fungal culture are rarely performed at primary health care clinics. The turn-around-time (TOT) for fungal cultures is 7 to 21 days. On the other hand, PCR test may be able to detect the superficial fungal infection at a shorter TOT with presumed a more accurate result. However, this test is very costly and technically highly demanding. Our study on the other hand showed that the detection rate of PCR was nearly 50% lower than the gold standard test culture. Hence, the routine use of PCR in the management of superficial fungal infection may not be cost effective at present. Our study nevertheless helped us to monitor the pathogenic fungus encountered locally in superficial fungal infection. This is especially important for onychomycosis where NDM were the most common aetiology observed. The treatment regime for NDM onychomycosis should be extended and prolonged.[37] Accurate diagnosis is important and essential to ensure the treatment is targeted.

The limitations of this study include small in sample size and short study duration. Our current study only involved patients visited to Hospital Melaka. A larger study population with a longer study duration together with involvement of primary healthcare clinics is needed to ensure the study results are more representative of the whole Malaysian population. In addition, due to the budget limitation, we could not perform PCR tests for culture negative sample. Hence the sensitivity, specificity, positive and negative predictive values could not be determined. A study done in Italy has shown a high positive predictive value of 100% and a negative predictive value of 75.9% for the diagnosis of onychomycosis.[38] Therefore, fungal PCR was useful as a confirmation step and for urgent case in diagnosis of onychomycosis.[38] Ndiaye et al in another study reported that

multiplex real-time PCR assay had a 73.3% positive predictive value and a 90.2% negative predictive value in detecting dermatophytes in human hair samples.[39]

CONCLUSION

Trichophyton rubrum was the main causative fungus of tinea corporis/cruris/pedis while tinea capitis was mainly caused by *Microsporum canis* in Hospital Melaka. Non dermatophyte moulds were mainly isolated in onychomycosis in our study.

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