

Research Article

Superficial mycoses among patients presenting at the dermatology outpatient department, Teaching Hospital, Kurunegala, Sri Lanka from December 2014 to March 2015

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Abstract

Introduction: Superficial mycoses (SM) are common in tropical countries.

Objective: This study aimed to investigate SM in a group of patients attending the dermatology clinic at the Teaching Hospital, Kurunegala, Sri Lanka.

Methods: The study included 125 clinically diagnosed SM patients who visited the dermatology clinic for the first time from December 2014 to March 2015. Demographic data of the participants were recorded using an interviewer administered questionnaire. Suitable samples were examined using the light microscope after 10-20% KOH digestion and were cultured on Sabouraud's dextrose agar. Fungi were identified on microscopic and macroscopic features.

Results: The study population consisted of 47(37.6%) males and 78(62.4%) females with the highest number of patients (27; 21.6%) in the 50-59-year age group. Onychomycosis (47; 37.6%) was the commonest clinical presentation followed by pityriasis versicolor (37; 29.6%), tinea corporis (21;16.8%) and cutaneous candidiasis (8; 6.4%).

Direct microscopy was positive in 82 (65.6%) samples. Out of 90 cultured samples, 46 (51.1%) produced significant growth. *Malassezia* (35) was the commonest identified aetiology followed

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by dermatophytes (25), *Candida* (16) and non-dermatophyte molds (10). Among dermatophytes, *Trycophyton mentagrophytes* 7 (35%) and *Trycophyton rubrum* 7 (35%) were isolated in higher numbers.

Conclusions: SM were common among females in the 50-59-year age group. Onychomycosis and pityriasis versicolor were the commonest clinical presentations. *Malassezia* species (identified by direct microscopy only) was the commonest aetiology for SM followed by dermatophytes. The leading dermatophytes isolated in this study were *T. mentagrophytes* and *T. rubrum*.

Keywords: *Superficial mycoses, dermatophytes, nondermatophyte molds, Candida, pityriasis versicolor, onychomycosis*

Introduction

Superficial mycoses (SM) represent a substantial amount of global skin diseases. Approximately 20-25% of the world population suffers from SM and the incidence is rising continuously.^{1,2} Listed as the fourth commonest disease globally in man³, SM seems to be highly prevalent among people living in tropical countries including Sri Lanka as highlighted by several investigators.⁴⁻⁸

Treatment of SM usually commences upon clinical diagnosis rather than mycological evidence, due to the shortage of facilities in many hospitals in Sri Lanka.^{4,5,8} Comprehensive studies on SM in Sri Lanka are limited despite the increased disease prevalence and data on SM in the country is insufficient.^{4,9} Understanding the aetiology and clinical presentation of SM helps early diagnoses and proper management of patients in community settings.^{8,10} This study aimed to investigate the aetiology and clinical presentations of SM in a group of patients attending the dermatology clinic, Teaching Hospital, Kurunegala, Sri Lanka.

Methods

Study population

A descriptive, prospective study was carried out on all the patients who were clinically diagnosed with SM at their first visit to the dermatology clinic from December 2014 to March 2015 at the Teaching Hospital, Kurunegala. Non-consenting patients or those who had been treated with antifungals (oral or topical) within the last three months were excluded. Informed written consent was obtained from the participants or parents/ guardians prior to data and sample collection.

Data and sample collection

Demographic data of the participants were recorded using an interviewer administered questionnaire and the samples were collected and transported as described.^{4,5,11,12} In summary, skin samples were obtained by scraping the most active outer layer of the lesion after cleaning with 70% alcohol with a blunt sterile scalpel. Full thickness of the affected nails was cut with sterile scissors and subungual debris and discolored, brittle or dystrophic nails were scraped using a sterile scalpel after cleaning the affected area with 70% alcohol. Infected hair was removed with intact root using sterile forceps and the scalp was scraped with a sterile blunt

scalpel. Two swabs were collected from painful infected lesions in toe web interdigital spaces and palms where scraping was not feasible.

Direct microscopy

All specimens were subjected to direct microscopy for fungal elements following 10% KOH (20% for nail samples) digestion. A portion of the scrapings was transferred to a clean slide and 1-2 drops of KOH were added and covered with a sterile glass cover slip. The slide was kept at room temperature for 30 min or more to allow digestion of keratin, following which a cover slip was pressed down to make a thin layer of the specimen and examined under $\times 10$ and $\times 40$ magnifications. With swabs, one was used for Gram staining and KOH smear preparation and the other was used for culture.

Culture

All samples except those that were direct microscopy positive for *Malassezia* species were cultured on Sabouraud's dextrose agar (SDA) containing chloramphenicol alone and SDA containing both chloramphenicol and cyclohexamide. Cultures were incubated at 26 °C and were examined every other day for any growth up to 3 weeks before they were discarded.

In case of doubtful identification, a slide culture was performed. Microscopic examination of culture positive isolates was done by preparation of lactophenol cotton blue mounts.

Fungal identification

Fungal isolates were identified by macroscopic features such as colony characteristics, including morphology, colour, texture, rate of growth, and diffusible pigments and microscopic features, including macroconidia, microconidia, and spiral hyphae.¹²⁻¹⁴ Culture positive non-dermatophyte molds (NDM) which were not observed under direct microscopy were considered as non-significant growths due to the high possibility of contamination.^{4,8,14,15} Presence of *Malassezia* species was confirmed by direct microscopy of the KOH smear that yielded short, broad, unbranched hyphae with yeast cells.¹⁶

Data analysis

Demographic data, clinical and mycological findings were analysed using descriptive statistics and presented with frequencies and proportions.

Results

Demographic data

Of the 125 patients, 47(37.6%) were males and 78(62.4%) females with a male to female ratio of 1:1.7. The age of the patients ranged from 2-73 years with a median age of 44 years. The majority of patients were within the age group of 50-59 years (N=27). SM was less common at the extremes of age (<10 and >70 years) (Figure.1).

Clinical diagnosis

Clinical diagnosis of 125 SM patients included 8 clinical entities as shown in Table.1.

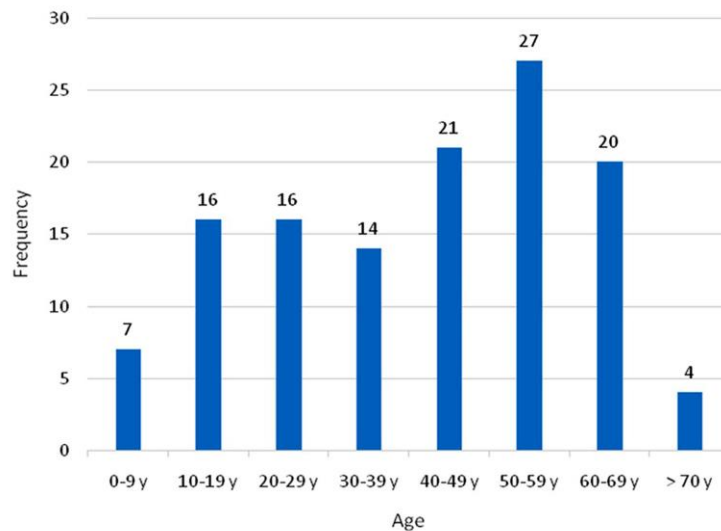


Figure 1. Age distribution of the study participants

Table 1. Results of direct microscopy and culture of SM samples in relation to clinical diagnosis

| Clinical diagnosis | No of cases | % | No of DM positive | % | No of culture positive | % |
|-----------------------|-------------|------|-------------------|------|------------------------|------|
| Onychomycosis | 47 | 37.6 | 25 | 53.2 | 25 | 53.2 |
| Pytriasis versicolor | 37 | 29.6 | 35 | 94.6 | 00 | 00 |
| Tinea corporis | 21 | 16.8 | 10 | 47.6 | 11 | 52.4 |
| Cutaneous candidiasis | 08 | 6.4 | 08 | 100 | 08 | 100 |
| Tinea capitis | 05 | 4.0 | 01 | 20 | 00 | 00 |
| Tinea cruris | 04 | 3.2 | 03 | 75 | 01 | 25 |
| Tinea pedis | 02 | 1.6 | 00 | 00 | 01 | 50 |
| Tinea manuum | 01 | 0.8 | 00 | 00 | 00 | 00 |
| Total | 125 | 100 | 82 | 65.6 | 46 | 51.1 |

Mycological diagnosis

From the total 125 samples, 82 (65.6%) became DM positive. Of them, 35 were identified as pityriasis versicolor. The remaining 90 samples were cultured, of which 46 (51.1%) yielded significant growth.

Specific aetiological agents identified from the culture were categorized as dermatophytes, non-dermatophyte molds (NDM) and *Candida* species. Six species were identified from the 25 dermatophyte isolates (Table 2).

Table 2. Dermatophytes isolated from the SM patients

| Dermatophyte species | Number of isolates |
|-----------------------------------|--------------------|
| <i>Trycophyton mentagrophytes</i> | 07 |
| <i>Trycophyton rubrum</i> | 07 |
| <i>Trycophyton violaceum</i> | 01 |
| <i>Epidermophyton floccosum</i> | 02 |
| <i>Microsporium gypseum</i> | 02 |
| <i>Microsporium canis</i> | 01 |
| Identified only with DM* | 05 |
| Total | 25 |

*These samples showed typical dermatophyte appearance on direct microscopy. However, culture yielded no growth.



Figure 2. Typical DM features of dermatophytes. Note smooth, undulating, branching and septate hyphal filaments with arthroconidiospores (arrowed).

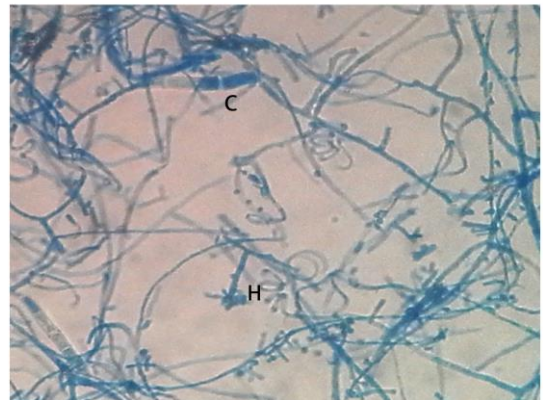


Figure 3. Microscopic appearance of *T. mentagrophytes* in lactophenol cotton blue smear preparation. Note the presence of spiral hyphae (H) and multicellular, septate, club-shaped macroconidia (C)



Figure 4. Microscopic appearance of dermatophytes in lactophenol cotton blue smear preparation of *T. rubrum*. Note the presence of tear drop shaped microconidia (C)

There were five samples that showed typical direct microscopic features of dermatophytes as shown in Figure 2 with a negative culture. *Trycophyton mentagrophytes* (Figure 3) and *Trycophyton rubrum* (Figure 4) were identified as predominant isolates.

There were 10 isolates of NDM which were both DM and culture positive. They included *Fusarium* species (6) *Aspergillus flavus* (2) and *Aspergillus niger* (2). There were 16 *Candida* isolates.

Clinical vs. mycological diagnosis

Although there were 47 cases of clinically diagnosed onychomycosis, only 30 (63.8%) were confirmed mycologically. Onychomycosis was caused by three main aetiological agents including dermatophytes (12; 40%) NDM (10; 33%) and *Candida* (08; 27%).

From the 37 patients with clinically suspected pityriasis versicolor (PV), 35 (94.6%) were laboratory confirmed by DM.



Figure 5. Clinical appearance of a "kerion" lesion found in the study with characteristic boggy erythematous mass with localized alopecia

Of 21 patients clinically diagnosed with tinea corporis, 13 (61.9%) were confirmed mycologically. According to culture findings, *T. rubrum* was the commonest 5 (45.5%) followed by *T. mentagrophytes* 4 (36.4%) while *M. gypseum* and *T. violaceum* were cultured one isolate each.

From five patients clinically diagnosed as tinea capitis, only one had mycological evidence of dermatophyte infection. This patient had a kerion (Figure 5) which showed characteristic endothrix infection confirmed by DM though its culture was negative. There were 8 patients who were diagnosed clinically as superficial candidiasis and all were both DM and culture positive. Notably,

five (62.5%) of the patients with superficial candidiasis were females.

Discussion

There are only a few studies published on the aetiology and clinical presentation of SM in Sri Lanka. When the demographic data of this study sample was considered, nearly 62% of SM patients were females which indicate a female preponderance for SM (male to female ratio of 1:1.7). Our observation of female preponderance of SM may be because the majority of the rural female population are involved in farming, gardening and domestic wet work that promote fungal infections. Furthermore, traditional clothing of Sri Lankan females (sarees and wrap-arounds) may contribute to colonisation by fungi like dermatophytes and *Candida* due to accumulation of sweat and moisture in the body.⁴ On the other hand, females are more concerned about the cosmetic effects of superficial skin lesions and may seek treatment more often than males.⁸

Nearly 70% of the SM patients in this sample were in the 40-69-year age group with the highest number of patients in the 50-59-year range. The proportions in extremes of age were relatively

low (Figure 1). This could be due to the increased physical activities and high chance of environmental exposure in adults which predispose them to SM.^{12,17}

This study showed that 65.6% (82/125) samples were DM positive which is comparable with Bhavsar, *et al.*¹⁷ and Jha & Murthy¹⁸ who reported 68% and 72% DM positivity. However, a lower proportions of DM positivity (53% and 38%) were reported by Ellabib & Khalifa¹⁹ and Lyngdoh, *et al.*²⁰ This type of variation in DM positivity could be a result of varying skills involved in sample collection and microscopic examination that may differ from place to place.

The culture positivity rate in our study was 51.1% (46/90), corroborating some other studies.^{5,14,19} However, Jha & Murthy¹⁸ reported higher culture positivity rate (65%) while some investigators^{12,17,20} reported lower culture positivity rates (20%-37%). The method of culture, use of selective culture media and the skills involved in sample collection may contribute to these variations.¹⁸

Interestingly, there were 5 samples which showed typical DM appearance of dermatophyte infections but yielded no growth. This could be due to non-viability of fungus before inoculation or prior antifungal treatment which inhibits growth. Also, there were 9 samples that yielded dermatophyte growth but their DM were negative. This could be due to the presence of the inactive, sporulating phase of fungi during examination which is difficult to visualise microscopically but are able to grow in favorable culture media. These findings agree with other studies conducted locally^{5,7} and abroad^{17,20} that have emphasised the importance of performing both culture and DM for diagnosis of SM.

Considering the pathogens isolated on culture in the present study, dermatophytes (n=20) were predominant followed by *Candida* (n=16) and NDM (n=10). These findings support previous investigations which reported dermatophytes as the leading cause for SM.^{1,4,5,14} Nevertheless, a recent ten-year retrospective study performed by Jayasekara, *et al.*²¹ to evaluate fungal pathogens isolated from skin, hair and nail samples received at the Department of Mycology, Medical Research Institute, Sri Lanka, reported higher numbers of *Fusarium* spp. and *Candida* spp. than dermatophytes. It is important to note that the Medical Research Institute generally receives fewer samples annually than the major hospitals⁹ which are predominantly from patients who were nonresponsive to empirical treatment at peripheral dermatology clinics. Hence, the above finding may not be comparable with the data from hospital settings like ours.

The most common dermatophyte infection in our study was tinea corporis which is consistent with many other Sri Lankan^{4,5} as well as overseas studies.^{14,17,18} The main etiological agent for tinea corporis in this study was *T. rubrum* which is comparable with some prior studies.^{4,17} However, *M. gypseum* has been identified as the predominant pathogen for tinea corporis in children in an earlier Sri Lankan study.⁵ This discrepancy may have been caused by the differences in life styles, socioeconomic conditions and the geographical locations of the study populations.

Patients with tinea capitis were remarkably low in the current study resembling findings of some other local studies.^{4,5,7,22} The low incidence of tinea capitis may be because dermatophytes responsible for tinea capitis, like *T. schonleinii*, *T. soundense* and *M. audouinii* are uncommon in

Sri Lanka.⁴ In addition, the Sri Lankan custom of frequent bathing using soap, as well as application of hair oil may reduce the incidence of tinea capitis in Sri Lankans.^{9,22}

This study identified only a few patients with tinea pedis confirming previous studies.^{4,5} The low number of patients with tinea pedis may be because many rural Sri Lankans do not use foot wear or that they use open foot wear which reduces the chances of fungal growth and infection.⁴ Further studies are necessary to confirm the above hypothesis.

When the overall results of dermatophytes in specimens taken from patients with SM are taken into account, both *T. mentagrophytes* and *T. rubrum* were equally prevalent in our study. In contrast, Attapattu⁴ reported *T. rubrum* as the primary dermatophyte responsible for SM. However, Perera & Perera⁵ isolated *M. gypseum* as the leading pathogen followed by *T. rubrum* in a Sri Lankan paediatric population. Another study done by Bhatia & Sharma¹² showed *T. mentagrophytes* as the predominant dermatophyte followed by *T. rubrum*. It is likely that both *T. mentagrophytes* and *T. rubrum* are important dermatophytes with regard to SM in our study population.

There were 47 (38%) patients who had clinical features of onychomycosis in our study. However, only 30 patients (30/47; 63.8%) were mycologically confirmed. These findings are in accordance with Ranawaka, *et al*⁶ who showed a 66.4% mycological confirmation rate in onychomycosis. Differences in clinical and mycological diagnosis of onychomycosis may occur due to various factors. Onychomycosis may mimic other nail conditions like chronic paronychia, viral warts, chronic dermatitis, lichen planus, psoriasis, or repeated nail trauma.²³

Considering aetiological agents for onychomycosis, dermatophytes were the leading cause followed by NDM and *Candida* in this study. Some local studies however have previously identified NDM as the major cause of onychomycosis.⁸ Dermatophytes causing onychomycosis in our study were *T. mentagrophytes* (5/12) followed by *T. rubrum* (3/12) highlighting their importance in this study population. The commonest NDM identified in our patients with onychomycosis were *Fusarium* species in contrast to some other studies which suggested *Aspergillus* species as the predominant NDM.^{7,8} *Fusarium* species have also been recognized as the commonest NDM associated with onychomycosis in the USA.²⁴ It is possible that both *Fusarium* species and *Aspergillus* species are important NDM causing onychomycosis.

PV has been previously reported as the leading SM in the Sri Lankan population.⁴ In the present study too, *Malassezia* species was the main aetiological agent for SM as identified by direct microscopy (35/37; 94.6%). The high DM positivity among clinically diagnosed PV reflects the importance of specific clinical characteristics such as hypo or hyperpigmented scaly lesions of the skin affected by PV.^{16,25} DM alone may therefore be sufficient for routine diagnosis of PV rather than fungal culture.

There were 8 (6.4%) patients clinically diagnosed with cutaneous candidiasis and the laboratory confirmation was 100% in this study; possibly due to the typical appearance of the lesions and affected body sites that helped precise clinical diagnosis. There were more females (5; 62.5%) than males (3; 37.5%) among the patients affected by cutaneous candidiasis in our study, supporting findings reported previously.¹⁹

Conclusions

In conclusion, SM was common among females and those who were in the 50-59 year age group in the current study population. Onychomycosis and PV were the most common clinical presentations. *Malassezia* species was the predominant etiological agent followed by dermatophytes, *Candida* and NDM. *T. mentagrophytes* and *T. rubrum* were the leading dermatophytes identified. Direct microscopy alone seems to be sufficient for routine diagnosis of PV in local settings.

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Ethics statement: Ethics approval was obtained from the Ethics Review Committee of the Medical Research Institute (MRI) of Sri Lanka (53/2014 on 13/11/2014).

Authors' contributions: Jayatilake JAMA- prepared the proposal, conducted data and sample collection, performed all the laboratory experiments, analyzed data and prepared the manuscript.

Ranasinghe G – supervised laboratory investigations and provided intellectual input in the experimental protocol and manuscript writing.

Nagahawatta A – provided intellectual input in experimental protocol and manuscript writing.

Munasinghe DM – performed clinical diagnosis and provided intellectual input in manuscript writing.

Jayatilake JAMS – supported manuscript writing and final editing.

All authors have seen and approved the final version of the manuscript.

References

1. Ameen M. Epidemiology of superficial fungal infections. *Clin Dermatol.* 2010;28:197-201. PMID: 20347663. doi: 10.1016/j.clindermatol.2009.12.005.
2. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. *Mycoses.* 2008; 51:2-15. doi: 10.1111/j.1439-0507.2008.01606.x.
3. Urban K, Chu S, Scheufele C, et al. The global, regional, and national burden of fungal skin diseases in 195 countries and territories: a cross-sectional analysis from the Global Burden of Disease Study 2017. *JAAD Int.* 2021; 2:22-27, ISSN 2666-3287. doi:10.1016/j.jdin.2020.10.003.
4. Attapattu MC. The pattern of superficial mycotic infections in Sri Lanka. *Ceylon Med J.* 1980; 25: 86-95. PMID: 7185497. No doi
5. Perera J, Perera C. Fungal skin infections in a paediatric dermatology clinic. *Ceylon Med J.* 1993; 38: 75-77. PMID: 8370091. No doi.
6. Perera A, Atukorale DN, Sivayogan S, et al. Prevalence of skin diseases in suburban Sri Lanka. *Ceylon Med J.* 2000; 45:123-128. PMID: 11192992. doi: 10.4038/cmj.v45i3.8112
7. Perera WPSSS, Rajakulasooriya RSR, Weerasekera MM, et al. Proportion of superficial fungal infections among members of the cleaning staff at the University of Sri Jayewardenepura, *Sri Lankan Journal of Infectious Diseases* 2014; 4(1):38-47. doi:10.4038/sljid.v4i1.6061
8. Ranawaka RR, Silva N, Rangunathan RW. Non-dermatophyte mold onychomycosis in Sri Lanka. *Dermatol Online J* 2012;18:7. PMID:22301044 No doi.
9. Jayasekera PI, Denning DW, Perera PD, et al. Is fungal disease in Sri Lanka underestimated? A comparison of reported fungal infections with estimated disease burden using global data. *Sri Lankan Journal of Infectious Diseases* 2015; 5(2):73-85. doi:10.4038/sljid.v5i2.8055
10. Drago L, Micali G, Papini M, et al. Management of mycoses in daily practice. *G Ital Dermatol Venereol* 2017; 152:642-50. doi:10.23736/S0392-0488.17.05683-8
11. Chaya AK, Pande S. Methods of specimen collection for diagnosis of superficial and subcutaneous fungal infections. *Indian J Dermatol Venereol Leprol* 2007; 73:202-5. doi: 10.4103/0378-6323.32753

12. Bhatia VK, Sharma PC. Epidemiological studies on dermatophytosis in human patients in Himachal Pradesh, India. *SpringerPlus* 2014; 3:134. doi: 10.1186/2193-1801-3-134
13. Larone DH. *Medically important fungi a guide to identification*, 4th ed. Washington, DC, USA. ASM press, 2002. No doi.
14. Khadka S, Sherchand JB, Pokharel DB, et al. Clinicomycological characterization of superficial mycoses from a tertiary care hospital in Nepal. *Dermatol Res Pract.* 2016; 2016:9509705. PMID: 28003819. doi:10.1155/2016/9509705.
15. Borman AM, Johnson EM. Interpretation of fungal culture results. *Curr Fungal Infect Rep* 2014; 8:312-321. doi:10.1007/s12281-014-0204-z
16. Erchiga CV, Martos OA, Casaño VA, et al. *Malassezia globosa* as the causative agent of pityriasis versicolor. *Br J Dermatol.* 2000; 143(4):799-803. PMID: 11069459. doi: 10.1046/j.1365-2133.2000.03779.x.
17. Bhavsar HK, Modi DJ, Sood NK, Shah HS. A Study of superficial mycoses with clinical mycological profile in tertiary care hospital in Ahmedabad, Gujarat. *National Journal of Medical Research.* 2012; 2(2);160-164. No doi.
18. Jha BK, Murthy SM. Increasing incidence of Dermatophytic infections among patients. *International Journal of Science and Research (IJSR)* 2013; 2(1):437-441. No doi.
19. Ellabib MS, Khalifa ZM. Dermatophytes and other fungi associated with skin mycoses in Tripoli, Libya. *Ann Saudi Med.* 2001; 21(3-4):193-5. PMID: 17264550. doi: 10.5144/0256-4947.2001.193.
20. Lyngdoh CJ, Lyngdoh WV, Choudhury B, Sangma KA, Bora I, Khyriem, AB. Clinico-mycological profile of dermatophytosis in Meghalaya. *Int J Med Public Health* 2013; 3(4):254-256 doi:10.4103/2230-8598.123442
21. Jayasekera PI, Kudavidanage S, Perera PD. A ten year retrospective study to evaluate the fungal pathogens isolated from skin, hair & nail samples received at Department of Mycology, Medical Research Institute. *Book of Abstracts of the 2nd Annual Conference and Scientific Sessions of Sri Lankan Society for Microbiology (SSM)* 2013; 1:28 No doi.
22. Attapattu MC. A study of tinea capitis in Sri Lanka. *J Med Vet Mycol.* 1989; 27:27-32. PMID: 2754578. doi:10.1080/02681218980000041
23. Westerberg DP, Voyack MJ. Onychomycosis: Current Trends in Diagnosis and Treatment. *Am Fam Physician* 2013; 88:762-770. No doi.
24. Ghannoum MA, Hajjeh RA, Scher R, et al. A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. *J Am Acad Dermatol.* 2000; 43:641-8. PMID: 11004620. doi:10.1067/mjd.2000.107754.
25. Terazooie B, Kordbacheh P, Zaini F, et al. Study of the distribution of *Malassezia* species in patients with pityriasis versicolor and healthy individuals in Tehran, Iran, *BMC Dermatol* 2004; 4:5. doi:10.1186/1471-5945-4-5