STUDY OF DERMATOPHYTOSIS AND THEIR ANTIFUNGAL SUSCEPTIBILITY IN NATIONAL MEDICAL COLLEGE AND TEACHING HOSPITAL, BIRGUNJ, NEPAL.

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ABSTRACT

Dermatophytosis refers to superficial fungal infections of keratinized tissues caused by keratinophilic dermatophytes. It is the most common of the superficial fungal infections. It is common in tropics and may present in epidemic proportions in areas with high rates of humidity.The aim of the study was to identify and assess the antifungal susceptibility of the dermatophytes in Department of Microbiology, NMCTH, Birgunj. The study included 349 cases of clinically diagnosed dermatophytosis from outpatient department of Dermatology of National Medical College, Birgunj during September 2019 to August 2020. Clinical and epidemiological data were collected as per proforma and skin scraping, hair plucking and nail clipping were done and materials were examined microscopically by KOH mount then cultured on Sabouraud dextrose agar and antifungal susceptibility were done by disk diffusion test in Department of Microbiology, National Medical College,Birgunj. Among 349 cases, overall male preponderance was observed with male to female ratio of 1.6:1. The most common age group of tinea infection was observed between 20 to 29 years of age. Tinea corporis was the dominant clinical type observed (38.1%). Potassium hydroxide positivity was seen in 288 samples (65.3%) and culture positivity was found in 57.9% of cases. The most common species identified was *Trichophyton rubrum* (55%). Itraconazole was found to be the most effective drug with 75.2% sensitive. Fluconazole was the least effective drug among the tested drug with 91.6% resistant.

Key words: Antifungal susceptibility, Dermatophytosis, Fluconazole, Itraconazole, *Microsporum* spp, Tinea, *Trichophyton* spp,

Introduction

Dermatophytes are fungi that cause superficial infections of the skin, hair and nails that require keratin for growth. Dermatophytosis commonly referred to as ringworm. Dermatophytes spread by direct contact from other people (anthropophilic), animals (zoophilic) and soil (geophilic), as well as indirectly from fomites.(1)

Microsporum, *Trichophyton*, and *Epidermophyton* species are the most common pathogens in skin infections.(1) *Trichophyton rubrum*, *T. mentagrophytes var. interdigitale*, *Microsporum canis*, and *Epidermophyton floccosum* are distributed worldwide. *T schoenleinii* (Eurasia, Africa), *T soudanense* (Africa), *T violaceum* (Africa, Asia, and Europe), and *T concentricum* (Pacific Islands, Far East, and India) have partial geographic restriction. *T rubrum*, which is the commonest dermatophyte in developed as well as urban areas of some developing countries.(2, 3) In Asia, *T rubrum* and *T mentagrophytes* are the most commonly isolated dermatophytes.(4,5)

Clinically, tinea can be classified according to the site of involvement including tinea capitis, tinea corporis, tinea cruris, tinea pedis, tinea barbae, tinea manuum, tinea faciei and tinea unguium.(6)

They can be readily diagnosed based on the history, physical examination, and potassium hydroxide (KOH) microscopy. Diagnosis occasionally requires Wood's lamp examination and fungal culture or histological examination.

There are several antifungal drugs used to treat dermatophytosis. Current therapeutics options for treatment of superficial dermatophytosis rely on topical or oral drugs including griseofulvin (targets microtubules), terbinafine (allylamine), miconazole (imidazole) and triazoles such as itraconazole and fluconazole (all of which target ergosterol biosynthesis of fungal membranes). Some infections respond well to topical antifungal therapy only, whereas others like tinea capitis, tinea unguium and more extensive or severe types may require systemic therapy.(7)

The present study was undertaken to assess the clinico-epidemiological profile of dermatophytic infection, to identify the species of fungi and to compare the clinical diagnosis with potassium hydroxide (KOH) smear positivity and culture positivity and also to look for the sensitivity of most commonly used antifungal drugs.

Objectives of the study

To identify various dermatophyes through KOH and culturing specimen in Sabouraud's Dextrose Agar and to assess their antifungal susceptibility.

Materials and Methods

Study Design

This study is a prospective hospital based study conducted at NMCTH, Birgunj for a period of twelve months from September 2019 to August 2020. Samples were tested in Microbiology department of National Medical College and Teaching Hospital, Birgunj,Nepal

Ethical Approval

Informed verbal consent was obtained from the participants prior to the study before preceding the questionnaire and specimen collection. Ethical approval was obtained from the IRC Board of National Medical College and Teaching Hospital.

Study population:

Sample size – sample size is 349.

Justification of sample size

Calculation of sample size done using

$n = z^2 \times p\ (1-p)/e^2$

Where,

n = required sample size.

z = confidence level at 95% (Standard value of 1.96).

p = estimated prevalence of clinically diagnosed dermatophytosis patients coming to OPD.

e = margin of error at 5% (Standard value of 0.05).

Thus, required sample size calculated as

Sample size (n) $= z^2 \times p\ (1-p)/e^2 = (1.96)^2 \times 0.35(1-0.35)/ (0.05)^2 = 349$.

Method

The study included 349 cases of clinically diagnosed dermatophytosis from Department of Dermatology National Medical College, Birgunj from September 2019 to August 2020. The data were collected in the pre structured proforma regarding detailed clinical history including age, sex, occupation, nationality, duration of disease, family history, history of recurrence and type of lesion, site of lesion, adnexal structure involvement and co-morbid status. Samples were send to Microbiology department of National Medical

College and Teaching Hospital, Birgunj,Nepal for culture in Sabouraud's Dextrose Agar , direct microscopy in KOH mount and Anti-fungal sensitivity in agar based disk diffusion method.

METHODOLOGY

Cases were selected on the basis of clinical diagnosis in outpatient department of dermatology. Selected patients were thoroughly informed about the research and were assured for the confidentiality. Consents were taken for the questionnaire and photograph and those who were willing to give consent were selected. Clinical data of patients were recorded on proforma with particular reference to onset of disease, duration, site of involvement, type of initial lesion and progression, associated dermatosis and concurrent systemic illness and familial occurrence of similar diseases. Required investigations were performed for all the cases as per proforma.

Skin scrapings from active margin, nail clipping and hair plucking of suspected lesions were taken after cleaning with 70% alcohol to remove dirt and contaminant bacteria. Samples for culture was collected in sterile paper, folded, labeled and transported to the Department of Microbiology and was inoculated on Sabouraud's Dextrose Agar in test tubes. The culture was maintained in the room temperature and checked daily on a routine basis for 4 weeks. If no growth observed at the end of 4 weeks, culture was labeled negative. Subsequently fungus was identified from each growth under light microscope after mounting over Lacto phenol Cotton Blue (HIMEDIA S016-100ml, laboratories Pvt, ltd, Mumbai).

The identified dermatophyte colony was then grown in separate petridishes containing Mueller Hinton Agar +2% Glucose + 0.5mcg/ml Methylene Blue Dye Medium. Standard protocol for the Disk-Diffusion Method[8] was used to determine the sensitivity for the commonly used antifungal drugs (Clotrimazole, Fluconazole and Itraconazole). The disks with antifungal drugs (HiMedia, laboratories Pvt, ltd, Mumbai, India) used had following potencies: itraconazole (10mcg/disc), fluconazole (10mcg/disc) and clotrimazole (10mcg/disc).

The inoculum was evenly spread on the surface of 10cm Petri dishes containing Mueller Hinton Agar +2% Glucose + 0.5 mcg/ml Methylene Blue Dye. Then the antifungal discs were applied to the plates, after which the plates were incubated in the incubator at 37° C under aerobic condition for 24 hours. After the colonies grew, the zones of inhibition around the discs were measured and recorded. Criteria of susceptibility and resistance of antifungal discs were measured according to the standard protocol.[88] In case of Clotrimazole and Itraconazole, they were considered sensitive if (Inhibition Zone Diameter) IZD ≥12mm. Similarly IZD ≥15mm was considered sensitive in case of Fluconazole. In case of Clortimazole and Itraconazole, they were considered intermediate sensitive if IZD was between 8-12 mm, and for fluconazole intermediate sensitive was considered if IZD was between 6-10mm. Fluconazole was considered resistance if IZD was ≤6 and Clotrimazole and Itraconazole if IZD was ≤ 7. Clinical photographs of patients along with culture characteristics and zone of inhibition were taken.

SELECTION CRITERIA

Inclusion Criteria

- Patients with clinical diagnosis of dermatophytosis.
- Patients willing to give consent.

Exclusion Criteria

- Recent use of antifungal therapy and any topical preparation within one week of study.
- Patient not willing to participate in the study.

Data Collection

Data was collected by questionnaire method, focusing on the object of the study and was put on the structured proforma. On receiving a case, fulfilling the inclusion criteria, patient was explained about the study in detail. Patient and patient' party was assured of confidentiality and an informed written consent was taken. For all the cases, history was taken and complete examination was done .All baseline investigations were done where necessary.

Data analysis and statistical Analysis

Data was entered in excel master-sheet with coding of the variables. SPSS was used for the evaluation. Findings were presented as tables, bar diagrams and pie charts. Frequencies and percentages were calculated. Then results were reviewed.

RESULTS

CLINICAL DATA

Most common clinical pattern in our study was tinea corporis 133 cases (38.1%) followed by multiple type of infection 83 cases (23.7%).

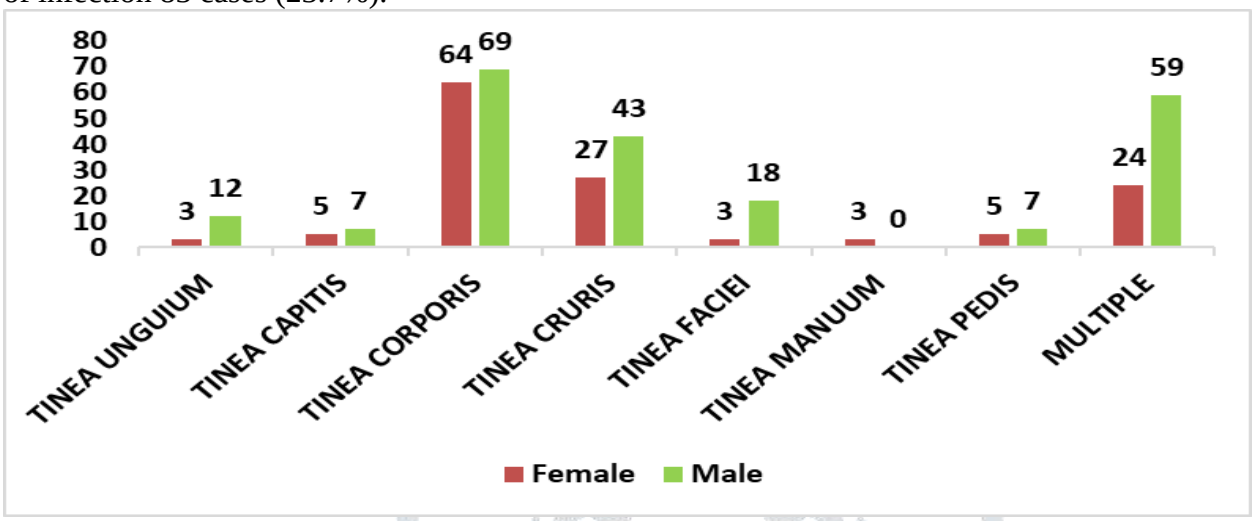

Figure 1: Diagram showing distribution of cases according to clinical types and gender

AGE AND SEX

In this study, age of youngest patient was 1 year whereas age of the oldest patient was 70 years. Cases between the age group 20-29 years were observed to be predominant.

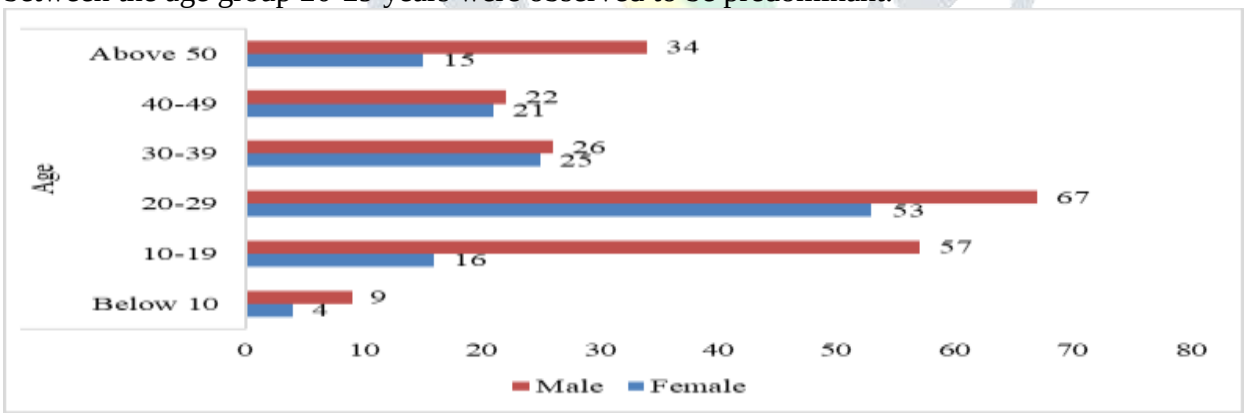

Figure 2: Bar diagram showing Gender distribution of cases according to Age

INVESTIGATION

In the study, overall positivity of direct microscopy was 228(65.3%) out of 349 and culture positivity was 202(57.9%) out of 349 cases.

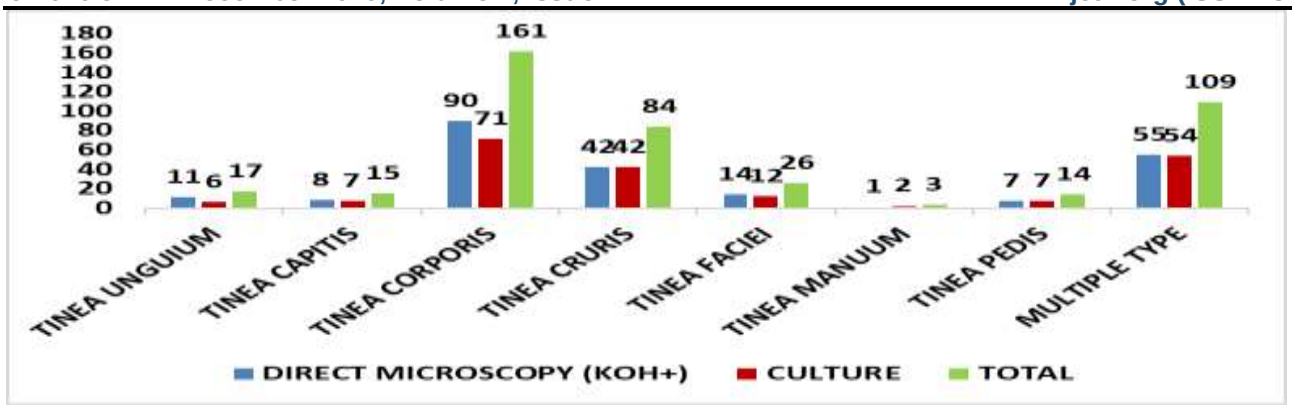

Figure 3: Bar diagram showing distribution of cases according to direct microscopy (KOH) and culture positivity

In our study, most sensitive drugs were Itraconazole (75.2%) and most resistant were Fluconazole (91.6%).

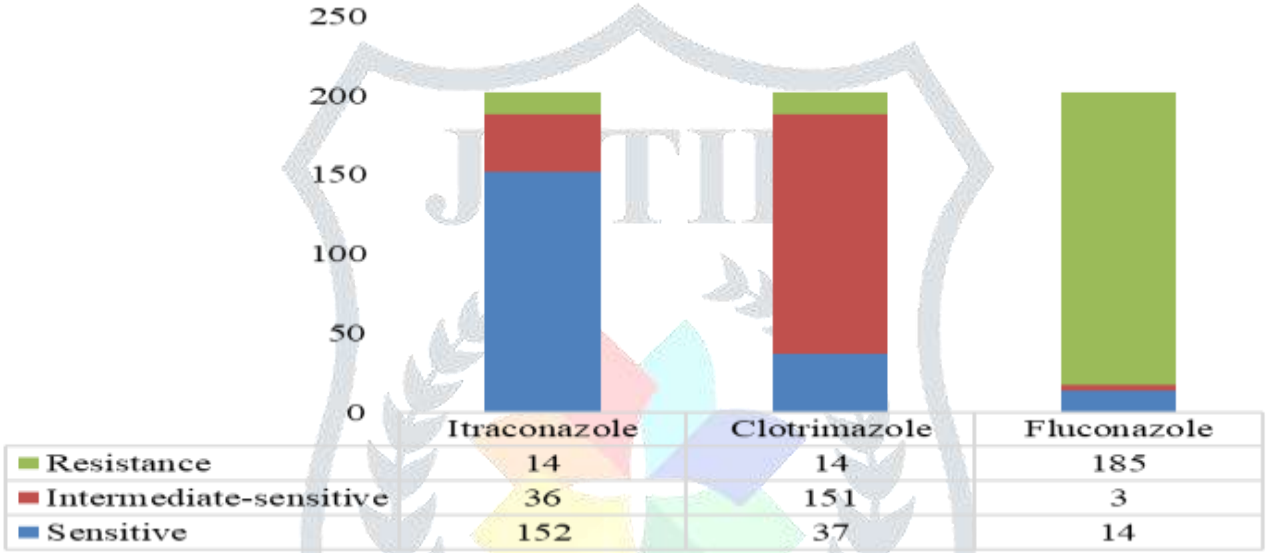

Figure 4: Bar diagram showing pattern of sensitivity and resistance to antifungal

Table 1: Showing distribution of cases according to clinical type of disease in relation to species identified.

Diagnosis	Species			Total
	M. gypseum	*T. mentagrophyte*	*T. rubrum*	
TINEA CAPITIS	7	0	0	7
MIXED TYPE	2	21	31	54
TINEA CORPORIS	5	26	41	72
TINEA CRURIS	3	12	27	42
TINEA FACIEI	1	7	4	12
TINEA MANUUM	1	0	1	2
TINEA PEDIS	1	3	3	7
TINEA UNGUIUM	0	2	4	6
TOTAL	20	71	111	202
PERCENTAGE	9.9%	35.1%	55.0%	100.0%

In this study, Trichophyton *rubrum* (55%) was predominantly isolated followed by Trichophyton *mentagrophyte* (35.1%).

DISCUSSION

In our study, over all male (61.6%) preponderance was observed with male to female ratio of 1.6:1 (Fig.2) which is consistent to other studies, Bindu *et al.*[9] and Bhatia *et al.*[10] also reported male predominance, the higher prevalence of dermatophytosis in males may be due to the differences in occupational exposure as males are more involved in exhaustive physical work and prolonged exposure to sun leading to excessive sweating. In addition, the tight fittings and synthetic clothing particularly in males provide damp, sweaty and warm skin conditions leading to infection. However, Shahindokht *et al.*[11] from Tehran reported female preponderance in their study.

Tinea corporis (38.1%) was the dominant clinical type of dermatophytosis observed in our study followed by mixed type (23.7%) and tinea cruris (20.6%) (Fig.1). It is consistent with other studies reported by Putta S *et al.*, Balakumar S *et al.*, Kaur R, Shrestha S , Sundar Khadka *et al.*, Lakshmi Vasantha Poluri *et al*[12, 13, 14, 15, 16,17] which also show tinea corporis as the most common infection among various clinical types.

Gupta *et al.*[18] have reported mixed type of infection as one of the common clinical variants along with tinea corporis and tinea cruris. According to our study, (17.4%) of cases presented with mixed type of infections and are one of common variants. Similar findings were noted in our study as well, the commonest being tinea corporis followed by mixed clinical type of infection and tinea cruris.

In present study, overall 65.3% (228 out of 349) cases were positive for fungal element on direct microscopic examination (KOH) (Fig 3). This study showed high positive direct microscopy in tinea corporis 39.5 %(90 out of 228 cases) followed by mixed type, tinea cruris, tinea faciei and tine unguium 24.1%, 18.4%, 6.1%, and 4.8% respectively. Among tinea corporis 67.6% (90 out of 133 cases) showed positive direct microscopy. Similarly Bhatia *et al.*[18] , their study showed high positive direct microscopy in tinea corporis (39.1%) which is consistent to our study. The possible reason for high KOH positivity in our study for tinea corporis among the patients could be because most of the patients attended the clinic within 5 weeks of onset of disease before using any antifungal drugs and the possible reason for comparatively low positivity in tinea unguium was probably because nail specimen takes longer time to dissolve and the fungal element might not get released.

In this study, overall positive culture was observed in 57.9% of cases (Fig 3). Similar findings were noted in study of Kaur R, Shrestha S, Sundar K *et al.*, Lakshmi Vasantha Poluri *et al.*[13, 14, 15, 16] . The culture positive were nearly 46%, 57.2%, 55.5%, 56.3% respectively. High positivity in cases of present study might be due to good laboratory support.

Present study showed *T. rubrum* 55% (111 out of 202 cases) as a predominant species identified followed by *T. mentagrophytes* (35.1%) and *M. gypseum* (9.9%) (Table.1). But did not observed any involvement of *Epidermophyton spp*. in the study. Similarly Kak Surendran *et al.*, Arun Agarwalla *et al.*, V Bindu *et al.*, Shukla DAS *et al.*, Lakshmi Vasantha Poluri *et al.* [19, 20, 9, 21, 13] found T. *rubrum* as predominant species. *T. rubrum* was isolated in 67.5%, 45.74%, 66.2%, 55.2%, and 58.6% respectively. The possible explanation to this may be that *T. rubrum* is generally linked to chronic dermatophytosis. Further, this organism is a slow growing organism, there is a possibility that this dermatophyte species might overgrow or mask the growth of *T. rubrum* while attempting isolation.[16] Some investigators believe that this is due to the different enzymatic pattern of this anthropophilic fungus in its negative anomorphic state; the positive strain being considered extinct.[22] Recent studies show that the mannan moiety of this dermatophyte is capable of suppressing lymphoproliferation and keratinocyte proliferation.[23]

In vitro activity of six antifungal drugs tested against clinically important dermatophytes by Pakshir *et al.* [8] reported susceptibility of antifungal drugs as follows: ketoconazole 31 (77.5%) susceptible, Miconazole 36 (90%) sensitive, Clotrimazole 39 (97.5%) susceptible and Fluconazole 39 (97.5%) resistance. S. Mahajan *et al.*[20] found Itraconazole was the most effective drug, followed by ketoconazole, terbinafine and fluconazole. Similarly in our study, test results of the susceptibility to antifungal drugs were as follows: Itraconazole (75.2%) sensitive, clotrimazole (74.8%) intermediate sensitive and fluconazole (91.6%) resistant (Fig 4). In accordance to our study, various studies reported fluconazole as the least active antifungal agent.[24]. Antifungal susceptibility of terbinafine (one of the commonly used drug) could not be done to shortage of its disc. Our study is a single center study; similarly nationwide multi-centre trial should be carried out to address the severity of antifungal drug resistance.

Finally, in view of high level of resistance of commonly used antifungal drugs, treatment plans should be revised. Recent studies reported that plants have limitless ability to synthesize secondary metabolites to treat various bacterial and fungal infections. Antimicrobials of plant origin are efficient in the treatment of infectious disease mitigating simultaneously many of the side effects that are often associated with synthetic ones. The use of medicinal plants in the treatment of dermatophytosis will help to reduce the dependence on the use of microbial or chemically synthesized antimicrobials and thus overcome the problem of the emergences of fungi being resistant to antifungal chemicals on various etiological agents of dermatophyte infections and further work is under progress to understand this issue.[12]

REFERENCES

1. Hainer BL. Dermatophyte infections. American family physician. 2003; 67(1):101-10.
2. Borman AM, Campbell CK, Fraser M, Johnson EM. Analysis of the dermatophyte species isolated in the British Isles between 1980 and 2005 and review of worldwide dermatophyte trends over the last three decades. Medical mycology. 2007;45(2):131-41.
3. Foster KW, Ghannoum MA, Elewski BE. Epidemiologic surveillance of cutaneous fungal infection in the United States from 1999 to 2002. Journal of the American Academy of Dermatology. 2004;50(5):748-52.
4. Tao-Xiang N, Zhi-Cheng L, Sao-Mao W, Wen-Zhu L. Analysis of dermatodermatophytosis in Lanzhou district of northwestern China. Mycopathologia. 2005;160(4):281-4.
5. Tan H-H. Superficial fungal infections seen at the National Skin Centre, Singapore. Nippon Ishinkin Gakkai Zasshi. 2005;46(2):77-80.
6. Ameen M. Epidemiology of superficial fungal infections. Clinics in dermatology. 2010;28(2):197-201.
7. Esteban A, Abarca M, Cabanes F. Comparison of disk diffusion method and broth microdilution method for antifungal susceptibility testing of dermatophytes. Medical mycology. 2005;43(1):61-6.
8. Pakshir K, Bahaedinie L, Rezaei Z, Sodaifi M, Zomorodian K. In vitro activity of six antifungal drugs against clinically important dermatophytes. Jundishapur Journal of Microbiology. 2009;2(4):158-63.
9. Bindu V, Pavithran K. Clinico-mycological study of dermatophytosis in Calicut. Indian Journal of Dermatology, Venereology, and Leprology. 2002;68(5):259.
10. Bhatia VK, Sharma PC. Epidemiological studies on dermatophytosis in human patients in Himachal Pradesh, India. Springerplus. 2014;3(1):134.
11. Bassiri-Jahromi S, Khaksari AA. Epidemiological survey of dermatophytosis in Tehran, Iran, from 2000 to 2005. Indian Journal of Dermatology, Venereology, and Leprology. 2009;75(2):142.
12. Balakumar S, Rajan S, Thirunalasundari T, Jeeva S. Epidemiology of dermatophytosis in and around Tiruchirapalli, Tamilnadu, India. Asian Pacific Journal of Tropical Disease. 2012;2(4):286-9.
13. Poluri LV, Indugula JP, Kondapaneni SL. Clinicomycological study of dermatophytosis in South India. Journal of laboratory physicians. 2015;7(2):84.
14. Khadka S, Sherchand JB, Pokharel DB, Pokhrel BM, Mishra SK, Dhital S, et al. Clinicomycological Characterization of Superficial Dermatophytosis from a Tertiary Care Hospital in Nepal. Dermatology research and practice. 2016;2016.
15. Mathur M, Shrestha S. Clinicomycological Profile and Antifungal Sensitivity Pattern of Commonly Used Azoles in Dermatophytosis. Journal of the Nepal Medical Association. 2015;53(198).
16. Kaur R, Panda PS, Sardana K, Khan S. Mycological pattern of dermatodermatophytosis in a tertiary care Hospital. Journal of tropical medicine. 2015;2015.
17. Putta SD, Kulkarni VA, Bhadade AA, Kulkarni VN, Walawalkar AS. Prevalence of dermatophytosis and its spectrum in a tertiary care hospital, Kolhapur. Indian J of Basic and Applied Medical Research. 2016;5(3):595-600.
18. Gupta AK, Mohan A, Singh SK, Pandey AK. Studying the clinic mycological pattern of the dermatophytic infection attending OPD in tertiary care hospital in eastern Uttar Pradesh and Bihar. International Journal of Research in Dermatology. 2018;4(2):118-25.

19. Surendran K, Bhat RM, Boloor R, Nandakishore B, Sukumar D. A clinical and mycological study of dermatophytic infections. Indian journal of dermatology. 2014;59(3):262.

20. Agarwalla A, Jacob M, Sethi M, Parija SC, Singh NP. A Clinico-Mycological Study of Dermatophytoses in Nepal. The Journal of dermatology. 2001;28(1):16-21

21. Das S, Goyal R, Bhattacharya S. Laboratory-based epidemiological study of superficial fungal infections. The Journal of dermatology. 2007;34(4):248-53.

22. Roberts SO, Hay RJ, Mackenzie DW. A clinician's guide to fungal disease: Marcel Dekker, Inc.; 1984.

23. Dahl M. Immunological resistance to dermatophyte infections. Advances in dermatology. 1987;2:305-20.

24. Fernández-Torres B, Carrillo A, Martın E, Del Palacio A, Moore M, Valverde A, et al. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrobial agents and chemotherapy. 2001;45(9):2524-8.