RESEARCH ARTICLE

The rising menace of antifungal resistance in dermatophytes among the patients of tinea capitis

Faiza Zeeshan,[1] Muhammad Sabir,[2] Fakhar Uddin[3]

Abstract

Objective: To determine the epidemiological profile of dermatophytes among patients of tinea capitis and their susceptibility pattern to fluconazole and terbinafine.

Method: The cross-sectional study was conducted at the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from August to December 2019, and comprised samples of hair and skin from the scalp of tinea capitis patients regardless of age and gender. Demographic details were collected and the samples were processed for direct microscopy and mycological culture. Antifungal susceptibility testing for fluconazole and terbinafine was performed using broth microdilution method. Data was analysed using SPSS 21.

Results: Of the 207 patients, 115(55.5%) were males, and 114(55.1%) were children. Alopecia was the most common presenting complaint 141(68.1%), while grey patch tinea was the most characteristic clinical form 53(25.6%). Dermatophytes were yielded in 61(29.5%) cases, non-dermatophytes were isolated in 45(21.7%) specimens, and 101(48.8%) were culture-negative. Among the dermatophytes, trichophyton violaceum was the most common pathogen 21(34.4%), followed by trichophyton mentagrophytes 18(29.5%). Resistance to fluconazole and terbinafine among dermatophytes was recorded in 12(19.7%) and 7(11.5%) isolates, respectively.

Conclusion: The frequency of dermatophytes among tinea capitis patients was higher compared to non-dermatophyte species. Antifungal resistance was predominantly seen in trichophyton violaceum and trichophyton mentagrophytes.

Keywords: Antifungal agents, Drug resistance, Fluconazole, Terbinafine, Tinea capitis.

(JPMA 73: 43; 2023) **DOI: 10.47391/JPMA.5307**

Submission completion date: 11-11-2021 **— Acceptance date:** 29-08-2022

Introduction

Tinea capitis (TC) is the fungal infection of the scalp and hair follicles that is caused by a type of molds called dermatophytes. It is estimated that around 10-15% of people around the world get infected by dermatophytes at some point in their lives.[1] TC is one of the most common dermatophytoses of childhood and its incidence decreases with age, being relatively uncommon in adults.[2] According to the World Health Organisation (WHO), TC is the second most common dermatologic infantile infection after pyoderma.[3] This disease is prevalent in fiscally constrained populations where people are compelled to live in closed commodities. Therefore, its transmission from person to person is not surprising. The clinical presentation of TC is not specific. It possesses great diversity from being asymptomatic to large painful inflammatory lesions. TC can be clinically classified into inflammatory and non-inflammatory forms.[4] The inflammatory subtype is characterised by tender plaques covered with broken hairs and painful pustules, while non-inflammatory subtype of TC has various clinical patterns, including grey patch, seborrhoea and alopecia areata. The clinical type of this condition is greatly dependent on the species of dermatophytes involved.

The dermatophytes get access to the body through injured skin, scars and burns. Infection is initiated by exposure to arthrospores or conidia.[5] The first phase of hair shaft invasion in TC is adhesion between fungal cells and keratinocytes. This phase is followed by structural changes in invading dermatophyte, which is presented as swelling of arthrospores, extracellular fibrillar layer formation, and expression of modified intercalary cells.[6] Once the hair is penetrated by a pathogen, variety of keratinolytic enzymes are produced by the invader.[4] The overall detrimental effect on hair and surrounding skin is partly due to keratinolysis, and the remaining is because of delayed type of hypersensitivity and T-cell mediated immune response. Pronounced inflammatory reactions are seen with zoophilic species, and, in contrast,

[1]Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, [2]Karachi Medical and Dental College, Karachi, [3]Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan.

Correspondence: Faiza Zeeshan. Email: dr.faizazeeshan@gmail.com

ORCID ID. 0000-0002-0539-2088

anthropophilic dermatophytes cause non-inflammatory and chronic infections mostly[6]. In European countries, trichophyton (T.) rubrum and T. mentagrophytes constitute >90% of all isolated dermatophytes.[7] In Asia, endemic species of dermatophytes are T. soudanense, T. violaceum and Microsporum (M.) audouinii. Studies have revealed that most common culprit of TC in Pakistan is T. violaceum, followed by microsporum species.[8]

TC can be managed topically as well as systematically with antifungal drugs. The recommended antifungal agents by the United States Food and Drug Administration (FDA) and the British Association of Dermatologist for TC are griseofulvin, azoles, like fluconazole (FLZ), itraconazole, voriconazole and letoconazole, and terbinafine (TER). The emergence of recalcitrant and resistant species of dermatophytes has been observed in the last decade. It is seen due to over-usage of steroids, self-medication due to over the counter (OTC) availability of drugs, increased incidence of immunodeficiency states, and prescription of drugs in under-dosage, incorrect diagnosis and low patient compliance. Various studies have highlighted the increased rate of resistance in dermatophytes towards FLZ. About 3.5% strains of dermatophytes causing TC are found to be resistant to FLZ globally.[9] The molecular mechanism that cause antifungal resistance include reduced uptake of drug, active transportation of the drug out of the cell, modified drug metabolic degradation of the cell and alteration in the interaction of the drug site by point mutation. The development of resistant strains for antifungal agents is clearly becoming a common issue in patients and is inevitable due to the wide use of these drugs. Due to overuse of TER, resistant strains of dermatophytes have also started to evolve. A considerable high rate (32%) of TER resistance has been elucidated in India. Similar resistant cases are also reported globally with TER resistance rate up to 20% in trichophyton species.[10]

Mycological culture and antifungal susceptibility are generally not performed in routine laboratory testing in Pakistan due to limited data regarding epidemiology and antifungal susceptibility testing of dermatophytes. The current study was planned to highlight the current status of antifungal resistance in dermatophytes among the local population.

Subjects and Methods

The observational cross-sectional study was conducted at the Department of Microbiology, Basic Medical Sciences Institute (BMSI), in collaboration with the Department of Dermatology, Jinnah Postgraduate Medical Centre (JPMC), Karachi. After approval from the institutional ethics review board, the sample size was calculated using open-source epidemiological calculator version 3.0 with estimated prevalence 16%, confidence interval 95% and bound of error 5%.[11] Samples of hair and skin from the scalp were collected from clinically suspected TC cases using convenience sampling technique. Patients with history of topical or systemic antifungals use for 30 days prior to presentation and who had applied hair oil and who refused to consent were excluded.

After taking informed consent from all the subjects, demographic and clinical details were recorded using a predesigned questionnaire form. T Hair specimens were collected under aseptic techniques. The surface was first cleaned with 70% alcohol swab and hair follicles from the root were plucked from the affected area by using sterilised forceps. The skin scales were carefully scrapped with the help of glass slides. The hair follicles and skin scales were kept in filter paper, packed and labelled with case number till further processing. The specimens were processed for direct microscopy. On potassium hydroxide (KOH) mount, the hair follicles and epithelial cells were observed for the presence of arthroconidia and fungal hyphae. For Calcofluor white (CFW) staining, the specimens were immersed in KOH-CFW stain solution for 5-10 minutes, and were subsequently observed under fluorescence microscope. Fungal elements in CFW-stained specimens appeared apple green, indicating fungal positivity. The samples were cultured on Sabouraud dextrose agar (Oxoid, UK) with and without antibiotics along with dermatophyte test medium at 25-35°C for four weeks. The samples were periodically checked for colony morphology, pigmentation and pigmentation on reverse. The species of dermatophytes were identified by observing arrangements of fungal hyphae, microconidia and macroconidia with lactophenol cotton blue mount and slide culture techniques.

The antifungal susceptibility testing of fungal isolates for FLZ and TER was performed by micro broth dilution method proposed by the Clinical Laboratory Standard Institute (CLSI, 2008).[12] The inoculum was prepared by adding 1ml 0.85% normal saline on the surface of fungal colony and the suspensions were made by gently probing the surface with the tip of sterile swab. The heavy particles of mixture were allowed to settle for around 10 minutes at room temperature, while upper homogenous suspension was taken into use for further testing.

The test was performed in sterile microdilution trays with 96 U-shaped wells. A growth control well was made by adding inoculum without antifungal agent while the sterility control contained only antifungal drug solution.

Aspergillus flavus American Type Culture Collection (ATCC) MYA-3631 was used as quality control (QC) strain (CLSI, 2008).[12] Each well was inoculated with 100µl of the 2x conidial inoculum suspension of the tested organism along with two-fold drug concentration diluent. The plates were incubated at 35°C and were observed after four days for the presence or absence of growth. The minimum inhibitory concentration (MIC) values were visually determined by comparing growth in the wells with drug-free control. The minimum drug concentration that inhibited 80% growth was taken as MIC value.

Data was analysed using SPSS 21. Descriptive statistics and chi-square test were employed as appropriate. P<0.05 was considered significant.

Results

Of the 207 patients, 115(55.5%) were males, and 114(55.1%) were children. Besides, 196(94.7%) participants were from low socio-economic strata, 10(4.8%) belonged to middle-income group, while 1(0.5%) patient was from high-income group. Further, 22(10.6%) subjects were related to animal handling and fungal positivity on culture was significantly higher (p=0.0001). Correlation of family history and common apparel usage among study subjects was also observed (Table-1).

Non-inflammatory type of lesions were found in 107(51.7%) cases (p=0.004). Cervical and occipital lymphadenopathy was noted in 31(15%) and 11(5.3%) cases, respectively. The most common presenting complaint was alopecia 141(68.1%), followed by dandruff or scaling 132(63.6%), pruritus 108(52.2%) and painful pustules 87(42%). The most common observed sign was grey patch in 53(25.6%) cases, black dot in 32(15.5%) and kerion in 11(5.3%) individuals (For clinico epidemiological relation: Table-2).

Overall, 106(51.2%) samples showed fungal growth and 101(48.8%) specimens were growth-negative. Among the growth-positive cases, 61(57.6%) constituted dermatophytes, while non-dermatophytes were isolated in 45(42.4%) cases. T. violaceum accounted for 21(34.4%) cases, followed by T. mentagrophytes 18(29.5%), T. tonsurans 9(14.8%), T. soudanense 3(4.9%), T. verrucosum 3(4.9%), M. canis 3(4.9%), T. rubrum 2(3.3%) and M. gypseum 2(3.3%) (Figure-1).

For T. violaceum, mean MIC for FLZ was 36.95(±34.24) ?g/ml, whereas MIC for TER was 0.16(±0.26)?g/ml. Similar values for other isolates were FLZ and TER were worked out (Table-3).

The rate of resistance in dermatophytes to FLZ was 12(19.7%). Among dermatophytes, highest resistance to FLZ was noted in T. mentagrophytes 7(38.9%), followed by T. violaceum 4(19%) and T. rubrum 1(50%). Besides, 7(11.5%) dermatophytes showed resistance to TER. Highest rate of resistance was seen in T. mentagrophytes 4(22.2%), followed by T. violaceum 3(14.3%).

Table-1: Clinical characteristics (n=207).

S. No	Clinical attribute	Children (n=114)	Adolescents (n=53)	Adults (n=40)	Total (n=207)	p-value*
1	Duration of illness (mean in months ±SD)	3.3±0.2	4.6±0.6	5.4±1.0	4.0±4.1	0.03
2	Age (Mean in years ± SD)	7.01±2.3	14.04±1.2	34.5±1.7	14.1±11.6	0.001
3	History of recurrence n (%)	21(18.4%)	13(24.5%)	5(12.5%)	39(18.8%)	0.33
4	Cases with comorbidity n (%)	2(1.7%)	3(5.6)	8(20%)	13(6.3%)	0.10
5	Family history of similar lesions on scalp n (%)	18(15.8%)	11(20.8%)	6(15%)	35(16.9%)	0.68
6	Sharing of household items n (%)	16(14%)	9(17%)	5(12.5%)	30(14.5%)	0.81

SD: Standard deviation. *P-value <0.05 is significant.

Table-2: Clinico-aetiological correlation of tinea capitis (TC).

S. No.	Dermatophyte species	Alopecia n(%)	Grey patch n(%)	Black dot n(%)	Dandruff or scaling n(%)	Kerion/Pustules n(%)	Pruritus n(%)
1	T. mentagrophytes	19(94.4)	5(27.8)	9(44.4)	18(88.9)	10(50)	12(66.7)
2	T. violaceum	16(76.2)	2(9.5)	16(76.2)	14(66.7)	12(57.1)	12(42.9)
3	T. tonsurans	6(88.9)	2(22.2)	4(55.6)	6(88.9)	6(77.8)	4(44.4)
4	T. soudanense	2(66.7)	-	2(66.7)	1(33.3)	-	2(66.7)
5	T. verrucosum	1(33.3)	1(33.3)	1(33.3)	2(66.7)	1(33.3)	2(66.7)
6	T. rubrum	2(100)	-	1(50)	1(50)	2(100)	2(100)
7	M. canis	2(66.7)	1(33.3	-	2(66.7)	2(66.7)	2(66.7)
8	M. gypseum	1(50)	1(50)	-	1(50)	-	-

T: Trichophyton, M: Microsporum.

Table-3: Antifungal susceptibility profile of dermatophytes against fluconazole (FLZ) and terbinafine (TER).

Tested strains	Concentration range for FLZ (µg/ml)	Mean MIC for FLZ (±SD) (µg/ml)	Concentration range for TER (µg/ml)	Mean MIC for TER(±SD)(µg/ml)
T. violaceum(21)	4-128	36.95(34.24)	0.030-1.00	0.16(0.24)
T. mentagrophytes (18)	2-128	44.45(36.91)	0.025-1.00	0.19(0.26)
T. tonsurans(9)	1-32	15.44(13.51)	0.030-0.250	0.08(0.09)
T. soudanense(3)	2-16	11.33(8.08)	0.030-0.060	0.23(0.32)
T. rubrum(2)	4-16	34.00(4.24)	0.030-0.30	0.03(0.13)
T. verrucosum(3)	2-32	12.66(16.77)	0.06-0.60	0.32(0.27)
M. canis(3)	4-32	19.00(16.16)	0.030-0.060	0.05(0.28)
M. gypseum(2)	8-16	12.00(5.65)	0.03-0.25	0.14(0.15)

T: Trichophyton, M: Microsporum, MIC: Minimum inhibitory concentration.

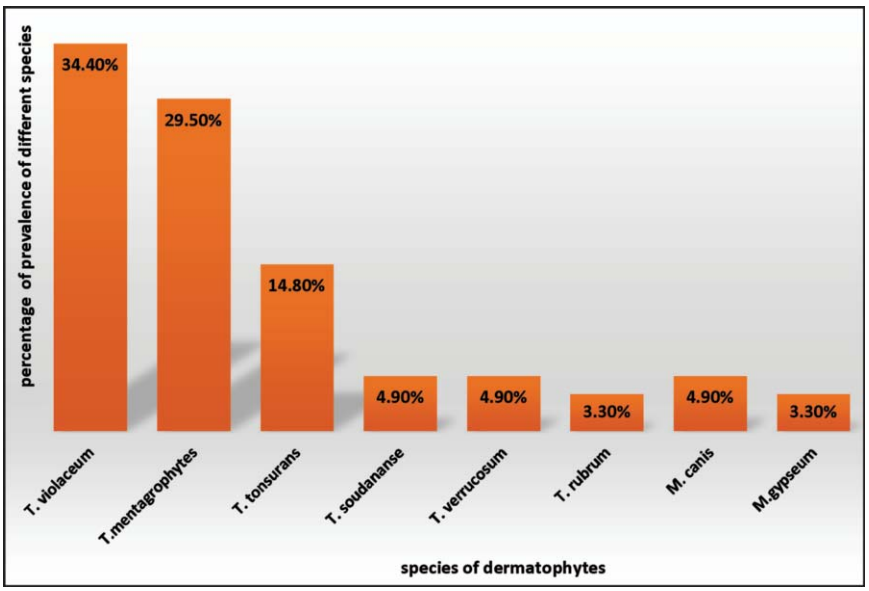

Figure-1: Distribution of various isolated species of dermatophytes.

Correlation analysis indicated that there was no statistically significant difference between the MICs of FLZ and TER for T. mentagrophytes and T. violaceum (p=0.52). However, for T. verrucosum, T. soudanense, T. rubrum, M. canis and M. gypseum, TER showed low MICs (p=0.000).

Discussion

The current study highlighted the epidemiology of TC and dermatophytes along with rising antifungal resistance to FLZ and TER. The frequency of TC in children, adolescents and adults was 55.1%, 25.6% and 19.3% respectively. The highest frequency of TC was observed among children, which is in agreement with Kechia et al.[13] The children of this age are usually school-enrolled and play with their peers, and on account of this, they are vulnerable to catching fungal infections. Another noticeable reason behind this finding is the decreased production of sebum among children. As the child hits puberty, the sebaceous glands start producing copious amount of sebum under the action of androgens. The sebum possesses fungistatic properties which makes adolescents and adults less susceptible to TC in comparison with children. In the present study, a slight male preponderance was observed. The male-to-female ratio was 1.2:1. In children, 56.1%, among adolescents 54.7% and in adult age group 57.5% males were involved. However, the gender predilection was not found to be statistically significant (p=0.06). The findings are in agreement with Farooqi et al[14] and Gopi et al.[15] However, Park et al.[16] reported female predominance with male-to-female ratio of 1:3. The reason for male predominance in the current study is multifactorial. The male population in local society is more vulnerable to catching fungal infections due to frequent exposure to environment on account of physical activities and for employment purposes. Secondly, males keep their hair short and trimmed, making them more susceptible to environmental pathogens compared to females who tend to braid hair and cover their heads. Another possible reason is the patriarchal social system in the country where males tend to avail medical advice more frequently in comparison with females, thus their turnout is higher in outpatient departments (OPDs) than female patients.

The mycological culture yielded 61(29.5%) dermatophytes and 45(21.7%) non-dermatophytes, while 101(48.8%) specimens showed no growth. Among the dermatophytes, the most common isolated species from genera trichophyton was T. violaceum (34.4%), followed by T. mentagrophytes (29.5%), T. tonsurans (14.8%), T. soudanense (4.9%), T. verrucosum (4.9%) and T. rubrum (3.3%). From genera microsporum, only two species were

identified; M. canis (4.9%) and M. gypseum (3.3%). Similar findings were reported by Hussain et al.[8] from Pakistan. Also, the results are in consensus with a study from southern India.[17] The dermatophytes show great diversity of regional distribution, and local epidemiology changes with changes in climate and geography of a particular region. Therefore, a difference in epidemiology is observed even within the same country. The microsporum species are mostly zoophilic, and are associated with domestic animals. As keeping pets is not a common activity in society, isolation of microsporum species was low in comparison with trichophyton species. The anthropophilic species of genera trichophyton are related with poor hygienic conditions and substandard lifestyle. The increased rate of inflation and poor economic growth in Pakistan has generated a flux in population dynamics. This has escalated urbanisation and compelled people to live in compact spaces. Consequently, it promotes human-to-human transmission of fungal infections. On that account, the current study observed considerable domination of trichophyton species.

The current study observed antifungal susceptibility patterns of isolated dermatophytes to FLZ and TER. Overall, 19.7% species of dermatophytes were resistant to FLZ. The highest rate of resistance was recorded in T. mentagrophytes (38.9%), followed by T. violaceum (19%) and T. rubrum (50%). A study in Egypt demonstrated the resistance rate as high as 20% in dermatophytes to FLZ.[18] Burmester et al.[18] presented similar results.

FLZ resistance is a complex mechanism and involves various factors, including drug efflux, drug target modification and stress response.[19] Decreased drug accumulation within fungal cells is one of the reasons behind FLZ resistance. It is mainly due to mutations in genes for adenosine triphosphate (ATP)-binding cassette (ABC) transport system which affects the performance of drug efflux system and hence causes frequent expulsion of drug from fungal cells. Another reason for dermatophyte resistance is the point mutation in the ERG11 gene that codes for lanosterol 14α-demethylase which causes the blockage of the binding capacity of the azole drug to its target.

The resistance to TER in dermatophytes was rarely observed until recently. According to current findings, 11.5% dermatophyte isolates showed resistance to TER. The resistance to TER was noted in T. mentagrophytes (22.2%) and T. violaceum (14.3%). Higher MICs were observed in T. mentagrophytes and T. violaceum. A majority of isolates showed lower MICs for TER. Ebert et al.[20] mentioned an alarming increase in resistance to TER among dermatophytes. A study in India[21] highlighted high MICs (>1μg/ml) among 85% T. mentagrophytes isolates. The resistance to TER has been associated with mutations in squalene epoxidase (SQLE) genes.[10] The reported mutations affect the drug-binding site of SQLE and may lead to failure of drug enzyme interaction. Other reasons for drug resistance to fungal isolates need to be further investigated.

In terms of limitations, the current study did not use techniques like Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) and Multilocus Sequencing Typing (MLST) due to budget constraints.

Conclusion

T. violaceum and T. mentagrophytes were the major TC triggers. One of the most paramount findings was the noticeable escalation in resistance among dermatophytes against FLZ and TER.

Disclaimer: The text is based on an academic thesis.

Conflict of Interest: None.

Source of Funding: None.

References

1. Pires CA, Cruz NF, Lobato AM, Sousa PO, Carneiro FR, Mendes AM. Clinical, epidemiological, and therapeutic profile of dermatophytosis.An Bras Dermatol. 2014; 89:259-64. doi: 10.1590/abd1806-4841.20142569.
2. Jehangir F, Vohra EA. Frequency of Tinea Capitis in Children 5-15 Years of Age Presenting to Primary Health Care Centre in Karachi, Pakistan.Infect Dis J. 2013; 592-5.
3. John AM, Schwartz RA, Janniger CK. The kerion: an angry tinea capitis.JAAD Int.2018; 57:3-9. doi: 10.1111/ijd.13423.
4. Kaplan E, Gonca S, Kandemir H, Döğen A, Hilmioğlu-Polat S, Ilkit M, et al. Genes encoding proteolytic enzymes fungalysin and subtilisin in dermatophytes of human and animal origin: a comparative study. Mycopathologia. 2020; 185:137-44. doi: 10.1007/s11046-019-00367-2.
5. Faway É, Lambert de Rouvroit C, Poumay Y. In vitro models of dermatophyte infection to investigate epidermal barrier alterations.Exp Dermatol. 2018; 27:915-22. doi: 10.1111/exd.13726.
6. Hay RJ. Tinea capitis: current status. Mycopathologia. 2017; 182:87-93. doi: 10.1007/s11046-016-0058-8.
7. Colosi IA, Cognet O, Colosi HA, Sabou M, Costache C. Dermatophytes and Dermatophytosis in Cluj-Napoca, Romania—A 4-Year Cross-Sectional Study.J Fungi. 2020; 6:154. doi: 10.3390/jof6030154.
8. Hussain A, Zakki SA, Qureshi R. Epidemiological Study Of Dermatophytosis In Okara, Pakistan.J Pharmacol. 2016; 4:184-7.
9. Ghannoum, M. Azole resistance in dermatophytes: prevalence and mechanism of action. J Am Podiatr Med Assoc. 2016; 106:79-86. doi: 10.7547/14-109.
10. Singh A, Masih A, Khurana A, Singh PK, Gupta M, Hagen F. et al. High terbinafine resistance in Trichophyton interdigitale isolates in Delhi, India harbouring mutations in the squalene epoxidase gene. Mycoses. 2018; 61: 477-84. doi: 10.1111/myc.12772
11. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source

Epidemiologic Statistics for Public Health, Version 3.01. [Online] 2013 [Cited 2022 July 16]. Available from: URL: https://www.openepi.com/Menu/OE_Menu.htm

12. Kechia FA, Kouoto EA, Nkoa T, Nweze EI, Fokoua DCM, Fosso S, et al. Epidemiology of tinea capitis among school-age children in Meiganga, Cameroon. J Mycol Med. 2018; 24:129-34. doi: 10.1016/j.mycmed.2013.12.002
13. Farooqi M, Tabassum S, Rizvi DA, Rahman A, Awan S, Mahar SA. Clinical types of tinea capitis and species identification in children: an experience from tertiary care centres of Karachi, Pakistan.J Pak Med Assoc. 2014; 64:304-8.
14. Gopi A, Harindranath D, Arun KR. Mycological profile of dermatophytes isolated from clinical samples in KIMS hospital, Bangalore. J Evol Med Dent Sci. 2015;4; 835-43.
15. Park SK, Park SW, Yun SK, Kim HU, Park J. Tinea capitis in adults: an 18-year retrospective, single-centre study in Korea. Mycoses. 2019; 62:609- 16. doi: 10.1111/myc.12916.
16. Poluri LV, Indugula JP, Kondapaneni SL. Clinicomycological Study of Dermatophytosis in South India.J Lab Physicians. 2015; 7:84-9. doi: 10.4103/0974-2727.163135.
17. Abd El Alim M, Halim RMA, Habib SA. Comparison of broth micro dilution and disk diffusion methods for susceptibility testing of dermatophytes. Egypt J Hosp Med. 2017; 69:1923-30. doi: 10.1080/13693780410001711972.
18. Burmester A, Hipler UC, Uhrlaß S, Nenoff P, Singal A, Verma SB, et al. Indian Trichophyton mentagrophytes squalene epoxidase erg1 double mutants show high proportion of combine fluconazole and terbinafine resistance. Mycoses. 2020; 63:1175-80. doi: 10.1111/myc.13150.
19. Khurana A, Sardana K, Chowdhary A. Antifungal resistance in dermatophytes: Recent trends and therapeutic implications.Fungal Genet Biol. 2019; 132:103255. doi: 10.1016/j.fgb.2019.103255.
20. Ebert A, Monod M, Salamin K, Burmester A, Uhrlaß S, Wiegand C, et al. Alarming India-wide phenomenon of antifungal resistance in dermatophytes: A multicentre study. Mycoses. 2020; 63:717-28. doi: 10.1111/myc.13091.
21. Veasey JV, Muzy GD. Tinea capitis: correlation of clinical presentations to agents identified in mycological culture.An Bras Dermatol. 2018; 93:465-6. doi: 10.1590/abd1806-4841.20187435.