Original Article

Clinico-mycological study of dermatophytic infections and their sensitivity to antifungal drugs in a tertiary care center

Soniya Mahajan, Ragini Tilak[1], Satyendra K. Kaushal[1], Rabindra N. Mishra[2], Shyam S. Pandey

Departments of Dermatology and Venereology, [1]Microbiology and [2]Community Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Abstract

Background: Worldwide, dermatophytic infections are running a chronic course either due to ineffective treatment or emerging drug resistance. In the past three decades, there has been an increase in incidence and non-responsiveness to conventional antifungals, which suggests that there is a need of antifungal sensitivity testing.

Aims: This study was aimed at identifying clinico-mycological pattern of dermatophytic infections in patients attending thedermatology outpatient department of a tertiary care hospital, and to obtain the sensitivity pattern of isolates against six commonly used oral antifungals (fluconazole, terbinafine, itraconazole, ketoconazole, griseofulvin and voriconazole).

Methods: Patients with suspected dermatophytoses attending the outpatient department of Sir Sunderlal Hospital, Varanasi, were enrolled in the study. A detailed history, clinical examination and sample collection for mycological examinations was done. *In vitro* antifungal sensitivity testing was done on species isolated from culture as per the Clinical and Laboratory Standard Institute M38-A standards, with broth microdilution method.

Results: There were 256 patients recruited in the study, with a male: female ratio of 3:1. The most commonly affected age group was 20–40 years (52.4%). Tinea corporis et cruris was the most common type observed (27.2%). Potassium hydroxide positivity was seen in 211 samples (79.6%) and culture positivity was found in 139 samples (52.4%). The most common species identified was *Trichophyton mentagrophytes* (75.9%). Sensitivity testing was done on fifty isolates of *T. mentagrophytes*. Minimum inhibitory concentrations of itraconazole, ketoconazole, terbinafine and voriconazole were comparable, while griseofulvin showed the highest minimum inhibitory concentration. Itraconazole was found to be the most effective drug, followed by ketoconazole, terbinafine and fluconazole. Griseofulvin was the least effective drug among the tested antifungals.

Limitations: This is a hospital-based study, and may not reflect the true pattern in the community. Sensitivity pattern of only one species *T. mentagrophytes* was carried out.

Conclusion: Inadequate and irregular use of antifungal drugs has led to the emergence of resistant strains, which cause poor treatment outcomes. Thus, it is very important to test for antifungal sensitivity to check for resistance to antifungals.

Key words: Antifungal, dermatophytes, sensitivity

Correspondence:
Dr. Soniya Mahajan,
Room No. 43. Lady Doctors' Hostel, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221 005, Uttar Pradesh, India.
E-mail: soniya.mahajan@yahoo.com

Introduction

The dermatophytes are hyaline septate molds, 42 species in three genera, *Trichophyton*, *Microsporum* and *Epidermophyton,* known to infect keratinized tissues. Ajello, in 1960, said "species not only differs from region to region but may change with the passage of time.[1]" By the end of 20th century, fungi were reported to be developing drug resistance. Resistance to griseofulvin

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was detected for the first time in 1969 by Lenhart.[2] Mukherjee *et al*. in 2003 from Cleveland, USA, first reported resistance to terbinafine.[3] In this background of increasing resistance of dermatophytic infections to therapy, this study was conducted mainly to obtain the sensitivity pattern of dermatophytes for commonly used systemic antifungals.

Methods

The study was conducted on patients with dermatophytic infection attending the dermatology outpatient department of Sir Sunderlal Hospital, Banaras Hindu University, from January 2014 to October 2014. Detailed history and clinical examination was carried out. Scales obtained by skin/nail/hair scrapings were taken to the laboratory for potassium hydroxide examination and culture on Sabouraud's dextrose agar. All potassium hydroxide-positive and/or culture-positive samples were included for further data analysis.

Specimens were inoculated on culture media (Sabouraud's dextrose agar) with cycloheximide (0.05 g/L) and chloramphenicol (0.005 g/L). Test tubes were incubated at 28°C for 4 weeks before labeling it negative. Isolation was done by further subculture on Potato dextrose agar for the preparation of conidial suspension for antifungal sensitivity testing. Most fungi grew within 1 week of incubation. Species identification was done by colony morphology and microscopy on lactophenol cotton blue mount. Antifungal sensitivity testing was done with broth microdilution test according to the Clinical and Laboratory Standard Institute M38-A standards.[4]

Stock solutions of concentration 1 mg/ml were made by dissolving powdered drugs in normal saline for fluconazole and in 100% dimethyl sulfoxide for terbinafine, itraconazole, griseofulvin, ketoconazole and voriconazole. Drug double dilutions were prepared from 0.125 to 64 μg/ml for fluconazole and 0.03 to 16 μg/ml for rest of the drugs with the help of RPMI-1640 ([HiMedia] with L-glutamine but without sodium bicarbonate and buffered at pH 7.0 with 3-[N-morpholino] propanesulfonic acid, monosodium salt). The fungal colony grown on Potato dextrose agar after 7 days was used for *in-vitro* sensitivity testing. The slant was flooded with 1 ml of sterile normal saline and few drops of 1% Tween 80. Colony was scraped gently with the help of sterile loop. The heavy particles were allowed to settle for 3–5 min. The upper homogeneous suspension containing mixture of nongerminated conidial and hyphal fragments was mixed for 15 s with vortex. The turbidity was measured using a spectrophotometer at 530 nm and adjusted to final optical density range of 0.09–0.11 or visually containing standard 1,000,000 cells/ml of fungi counted on Neubauer's chamber. Stock inoculum suspension was diluted at 1:50 in RPMI-1640 medium. This test was performed in round-bottomed 96-well microdilution trays. Columns 1–9 were filled with double dilutions of 100 μL of respective antifungal drugs in rows in each well. Column 10 was a sterility control, containing 200 μL of RPMI-1640 and column 11 acted as a growth control (drug free), having 200 μL of pure conidial suspension. Now, 100 μL of conidial suspension was filled in 1–9 well of serially diluted drugs. This tray was incubated at 30°C for 48–96 h of incubation and minimum inhibitory concentrations were determined, read visually. The growth in each well was compared with that of drug-free growth control and negative control. For most of the drugs, the minimum inhibitory concentration end point criterion for fungi was the lowest drug concentration, showing 50% inhibition and 90% inhibition of growth.

Results

Out of 265 patients, 199 (75.1%) were males and 66 (24.9%) were females. Male:female ratio was approximately 3:1. Age of the patients ranged from 2 to 70 years with mean of 29.08 ± 13.46 years in males and 37.03 ± 13.70 years in females. The common age group involved was 20–30 years in 76 males (38.2%), while it was 30–40 years in 24 females (36.4%). Multiple site involvement (mixed type of infection) was the common presentation in 124 (46.8%), followed by tinea corporis in 55 (20.8%) and tinea cruris in 50 (18.9%). Most of the cases presented with prolonged duration of illness, 107 (40.4%) patients had a history of intermittent or continuous infection which varied from 1 to 6 months, even longer duration of up to 2 years in 95 (35.8%) patients. Associated conditions such as diabetes were seen in 2.6% patients, hyperhidrosis in 2.2%, immunosuppression in 1.5% and atopy in 0.3%. Family history was positive in 82 (30.9%), out of which 25 (9.4%) patients had conjugal transmission. Application of topical steroid alone or in combination with antifungal or antibacterial was reported by 187 (70.6%) patients. Only 15 (5.7%) patients applied antifungal creams as azoles (clotrimazole/miconazole/sertaconazole) or terbinafine. Half of the patients (54.7%) did not take any systemic treatment. One-fourth (26.8%) of the patients gave a history of taking fluconazole 150 mg weekly (37, 20, 6 and 8 patients took this drug for ≤4, 5–8, 9–12 and >12 weeks, respectively). History of taking terbinafine 250 mg daily was given by 8.7% (n = 23) patients (2, 14 and 7 patients took for ≤1, 2–3 and ≥4 weeks, respectively). Griseofulvin was taken by 2.3% (n = 6) patients for 10–25 days. There was no history of taking multiple antifungals orally simultaneously. Treatment regimens in succession were followed by 20 (7.5%) patients, in which fluconazole, terbinafine or itraconazole were commonly administered.

Potassium hydroxide examination was positive for fungal elements in 211 (79.6%) patients. Culture results showed fungal growth in 139 (52.4%) samples inoculated. Thirty-eight (14.34%) samples were contaminated. Sensitivity and specificity of potassium hydroxide, considering culture as a gold standard were 94.2% and 31.8%, respectively. The most common species identified were *Trichophyton mentagrophytes* in 104 samples (75.9%), followed by *Trichophyton rubrum* in 30 samples (21.9%) and *Trichophyton tonsurans* in 1 (0.7%) sample. *Aspergillus fumigatus* and *Fusarium solani,* which are nondermatophytes, were isolated from one nail sample each.

Antifungal sensitivity testing was done on fifty strains of *T. mentagrophytes*. Minimum inhibitory concentration of fluconazole ranged from 0.25 to >64 μg/ml. It was 0.25 μg/ml in 13 (26%) and 16 μg/ml in 12 (24%) strains. Minimum inhibitory concentration ≥64 μg/ml was considered resistant.[5,6] Hence, in the present series, 11 (22%) strains were found resistant to fluconazole.

Minimum inhibitory concentration of terbinafine ranged from 0.03 to >16 μg/ml. More than two-third (70%) of the strains had minimum inhibitory concentration of 0.03 μg/ml. Sensitive strains of terbinafine had minimum inhibitory concentration ranged from 0.01 to 1 μg/ml. Minimum inhibitory concentration >1 μg/ml was found in 9 out the 50 (18%) strains, considered resistant to terbinafine.[4,7]

Minimum inhibitory concentration of itraconazole ranged from 0.03 to >16 μg/ml and 31 (62%) strains showed minimum inhibitory concentration of 0.03 μg/ml. According to the Clinical

and Laboratory Standard Institute standards, sensitive strain has minimum inhibitory concentration between 0.01 and 8 μg/ml. Only three strains (6%) had minimum inhibitory concentration ≥8 μg/ml which were resistant to itraconazole.[4]

Minimum inhibitory concentration of griseofulvin ranged from 0.03 to 8 μg/ml. In 19 (38%) strains, it was 4 μg/ml and 11 (22%) had lowest minimum inhibitory concentration, i.e., 0.03 μg/ml. Minimum inhibitory concentration of 0.06–3 μg/ml was considered a limit of effectiveness.[8] Accordingly, 25 strains (50%) were found resistant to griseofulvin.

Minimum inhibitory concentration of ketoconazole ranged from 0.03 to >16 μg/ml. In 14 strains (28%), it was 0.03 μg/ml and 9 (18%) strains had 0.5 μg/ml. The Clinical and Laboratory Standard Institute guideline for filamentous fungi is that minimum inhibitory concentration ≥8 μg/ml is resistant to ketoconazole.[4] According to this guideline, only four strains (8%) had shown resistance to ketoconazole.

Minimum inhibitory concentration of voriconazole ranged from 0.03 to >16 μg/ml. Ninety percent of the isolates had minimum inhibitory concentration ≤8 μg/ml, only 2 (4%) isolates had minimum inhibitory concentration >16 μg/ml [Table 1].

There was a statistically significant difference in the sensitivity of itraconazole as compared to terbinafine, fluconazole and griseofulvin (P = 0.12, 0.04 and <0.001, respectively). Although fungi were more sensitive to itraconazole than ketoconazole, the difference was not statistically significant (P = 1.0). There was higher sensitivity to terbinafine than to griseofulvin and the difference was statistically significant (P = 0.002). Terbinafine was less effective than ketoconazole, but the difference was statistically insignificant (P = 0.23). Sensitivity of dermatophytes to fluconazole, as compared to terbinafine and ketoconazole was low, but the difference was not statistically significant (P = 0.8 and 0.09, respectively). Griseofulvin was found to be significantly less effective than fluconazole (P = 0.007) and it was found to be the least effective among all the tested drugs.

Discussion

Morbidities of tinea infection are not only because of its frequent relapses but also due to increasing resistance to antifungal drugs, that has become a major concern of dermatologists and patients. In this study, majority of patients were adults (20–40 years) which is the norm in previous studies too.[9-11] Male:female ratio was 3:1; a male preponderance has been seen in some earlier studies.[12-16] However, others have showed female predominance, with females mainly having tinea pedis and manuum and onychomycosis due to kitchen and household work.[17,18]

Table 1: Results of *in vitro* sensitivity testing of fifty strains of *Trichophyton mentagrophytes* (μg/ml)

Drugs	MIC range	Mode	
		MIC_{50}	MIC_{90}
Fluconazole	0.25->64	<0.25	0.25-16
Terbinafine	0.03->16	<0.06	0.03
Itraconazole	0.03->16	<0.06	0.03
Griseofulvin	0.03-8	2	4
Ketoconazole	0.03->16	<0.06	0.03
Voriconazole	0.03->16	<0.06	0.03

MIC: Minimum inhibitory concentration

A prolonged duration of illness of 6 months and above was found in 53.9% of the patients. The reason behind such chronicity may be due to inadequate doses of anti-fungal medication, irregular treatment and application of topical steroids, which only reduce inflammation and pruritus, but help in proliferation of fungi by modifying their microenvironment. In an earlier study, Kumar *et al*. had found the duration of symptoms to be greater than 3 months in 53.3% of the patients, 1–3 months in 33.7% cases and less than 1 month in 13% of the cases.[12]

A history of fungal infections in family members was elicited in 30.9% of cases, of which 9.4% were conjugal. Transmission by direct contact occurs in tinea infection, explaining the conjugal cases, while transmission in family members might be due to fomites or *de novo* infection.[12,14,15,19,20]

Potassium hydroxide examination for fungal elements was positive in 79.6% of the patients. Previous studies had reported similar findings for potassium hydroxide positivity.[16,21-25] In the present study, culture positivity was 52.4 per cent; previous reports show a variance of this ranging from 24 to 87 per cent.[9,12-16,19,22-29] On the basis of these findings, sensitivity of potassium hydroxide examination, considering culture to be the gold standard, was 94.2% and its specificity was 31.8 per cent Sensitivity and specificity of culture, if one were to consider potassium hydroxide as the gold standard was 68.6% and 77.8%, respectively. Hence, we can say that potassium hydroxide is highly sensitive and less specific and culture is highly specific and less sensitive. Similar results were found in other studies.[30-33]

In studies conducted between 2002 to 2011, *T. rubrum* was the most common isolate. In the present study, the most common species identified was *T. mentagrophytes* (75.9%) followed by *T. rubrum* (11.3%). Similar findings were also observed by Sahai and Mishra and Bhatia and Sharma.[16,34] Ajello, in 1960, said "species not only differ from region to region but may change with the passage of time."[1]

Resistance of dermatophyte infections to all antifungals (except voriconazole) has been reported in literature. Minimum inhibitory concentration of fluconazole in the present study ranged from 0.25 to >64 μg/ml. Similar observations were noticed in other studies, in which the minimum inhibitory concentration range of fluconazole for *T. mentagrophytes* varied from 0.06 to >64 μg/ml.[35-39] In previous studies, resistance to fluconazole in dermatophytoses is well documented.[6,40,41]

Resistance to terbinafine was first reported in 2003, in which minimum inhibitory concentration of terbinafine for *T. rubrum* strains was >4 μg/ml, whereas it was <0.0002 μg/ml for the susceptible reference strains.[3] In the present study, minimum inhibitory concentration of terbinafine ranged from 0.03 to >16 μg/ml. Only two studies had similar minimum inhibitory concentration which ranged from 0.003 to 16 μg/ml.[39,42]

Resistance to griseofulvin was found in 50% of strains, as they had a minimum inhibitory concentration >3 μg/ml which is considered as the limit of effectiveness.[8] Similar findings have been reported previously.[7,35,40,42-44]

In a study conducted by Magagnin *et al*., resistance to itraconazole was observed in 42.3% and resistance to ketoconazole was observed in 53% of the strains.[40] Minimum inhibitory concentration of ketoconazole in the present study was found in the range of 0.03–>16 μg/ml. None of the studies showed such wide range of variation in minimum inhibitory concentration of ketoconazole.

Minimum inhibitory concentration of itraconazole in the present study ranged from 0.03 to >16 μg/ml. Ataides *et al*. (2012) also reported similar results (0.062–15 μg/ml).[45] Most of the other studies had a narrow range of minimum inhibitory concentration (0.01–4 μg/ml).[36,40,42-44,46] Gupta *et al*. observed a wider range of minimum inhibitory concentration (0.06–32 μg/ml).[38,47]

In the present study, 48 strains (96%) showed minimum inhibitory concentration of voriconazole ≤16 μg/ml, only 2 strains have shown <90% inhibition at 16 μg/ml. In previous studies, minimum inhibitory concentration for voriconazole was found in the range of 0.031–16 μg/ml.[48] Resistance of voriconazole in dermatophytoses has not yet been reported.

Thus, itraconazole was found to be the most sensitive drug among the tested antifungals. The second most sensitive drug was found to be ketoconazole, followed by terbinafine and fluconazole. Griseofulvin was the least effective drug among the tested antifungals.

To conclude, treatment of this menacing tinea infection can be helpful with the help of antifungals sensitivity testing.

Limitations

The limitations of the study were: a) a small sample size on which sensitivity testing was done and b) minimum inhibitory concentration ranges of only *T. mentagrophytes* were calculated.

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Conflicts of interest

There are no conflicts of interest

References

1. Ajello L. Geographic distribution and prevalence of the dermatophytes. Ann N Y Acad Sci 1960;89:30-8.
2. Lenhart K. Griseofulvin-resistant mutants in dermatophytes 1. The frequency of spontaneous and UV-induced mutants. Mykosen 1969;12:655-60.
3. Mukherjee PK, Leidich SD, Isham N, Leitner I, Ryder NS, Ghannoum MA. Clinical *Trichophyton rubrum* strain exhibiting primary resistance to terbinafine. Antimicrob Agents Chemother 2003;47:82-6.
4. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. Approved Standard. CLSI Document M38-A2. 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
5. Krakhecke AG, Afonso E, Ferreira JC, Candido RC. *In vitro* susceptibility testing of *Microsporum gypseum* isolated from healthy cattle and soil samples against itraconazole, terbinafine, fluconazole and topical veterinarian drugs. Mycopathologia 2005;159:377-80.
6. Santos DA, Hamdan JS. *In vitro* activities of four antifungal drugs against *Trichophyton rubrum* isolates exhibiting resistance to fluconazole. Mycoses 2007;50:286-9.
7. Ghannoum M, Isham N, Herbert J, Henry W, Yurdakul S. Activity of TDT 067 (terbinafine in Transfersome) against agents of onychomycosis, as determined by minimum inhibitory and fungicidal concentrations. J Clin Microbiol 2011;49:1716-20.
8. Scholz R, Meinhof W. Susceptibility of *Trichophyton rubrum* to griseofulvin. Mycoses 1991;34:411-4.
9. Gopi A, Harindranath D, Kaushik AR. Mycological profile of dermatophytes isolated from clinical samples in KIMS hospital, Bangalore. J Evol Med Dent Sci 2015;4:835-42.
10. Patel P, Mulla S, Patel D, Shrimali G. A study of superficial mycosis in South Gujarat region. Natl J Community Med 2010;1:85-8
11. Sumana V, Singaracharya MA. Dermatophytosis in Khammam (Khammam district, Andhra Pradesh, India). Indian J Pathol Microbiol 2004;47:287-9.
12. Kumar Y, Singh K, Kanodia S, Singh S, Yadav N. Clinico-epidemiological profile of superficial fungal infections in Rajasthan. MedPulse-Int Med J 2015;2:139-43.
13. Kumar S, Mallya PS, Kumari P. Clinico-mycological study of dermatophytosis in a tertiary care hospital. Int J Sci Study 2014;1:27-32.
14. Kamothi MN, Patel BP, Mehta SJ, Kikani KM, Pandhya JM. Prevalence of dermatophyte infection in district Rajkot. Electron J Pharmacol Ther 2010;3:1-3.
15. Bindu V, Pavithran K. Clinico-mycological study of dermatophytosis in Calicut. Indian J Dermatol Venereol Leprol 2002;68:259-61.
16. Sahai S, Mishra D. Change in spectrum of dermatophytes isolated from superficial mycoses cases: First report from Central India. Indian J Dermatol Venereol Leprol 2011;77:335-6.
17. Prabhu SR, Shetty VH, Shetty NJ, Girish PN, Rao BP, Oommen RA, *et al*. Clinico-mycological study of superficial fungal infections in coastal Karnataka, India. J Evol Med Dent Sci 2013;2:8638-46.
18. Asadi MA, Dehghani R, Sharif MR. Epidemiologic study of onychomycosis and tinea pedis in Kashan, Iran. Jundishapur J Microbiol 2009;2:61-4.
19. Madhavi S, Rama Rao MV, Jyothsna K. Mycological study of dermatophytosis in rural population. Ann Biol Res 2011;2:88-93.
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;3:254-6.
21. Bhagra S, Ganju SA, Kanga A, Sharma NL, Guleria RC. Mycological pattern of dermatophytosis in and around shimla hills. Indian J Dermatol 2014;59:268-70.
22. Sarma S, Borthakur AK. A clinico-epidemiological study of dermatophytoses in Northeast India. Indian J Dermatol Venereol Leprol 2007;73:427-8.
23. Gupta S, Gupta BL. Evaluation of the incidences of dermatophillic infection in Rajasthan: Case studies from Rajasthan, India. Int J Med Med Sci 2013;5:229-32.
24. Surekha A, Ramesh Kumar G, Sridevi K, Murty DS, Usha G, Bharathi G. Superficial dermatomycoses: A prospective clinicomycological study. J Clin Sci Res 2015;4:7-15.
25. Jain N, Sharma M, Saxena VN. Clinico-mycological profile of dermatophytosis in Jaipur, Rajasthan. Indian J Dermatol Venereol Leprol 2008;74:274-5.
26. Aggarwal A, Arora U, Khanna S. Clinical and mycological study of superficial mycoses in Amritsar. Indian J Dermatol 2002;47:218-20.
27. Malik A, Fatima N, Khan PA. A clinico-mycological study of superficial mycoses from a tertiary care hospital of a North Indian town. Virol Mycol 2014;3:135.
28. Sen SS, Rasul ES. Dermatophytosis in Assam. Indian J Med Microbiol 2006;24:77-8.
29. Sumana MN, Rajagopal V. A study of dermatophytes and their *in-vitro* antifungal sensitivity. Indian J Pathol Microbiol 2002;45:169-72.
30. Garg J, Tilak R, Garg A, Prakash P, Gulati AK, Nath G. Rapid detection of dermatophytes from skin and hair. BMC Res Notes 2009;2:60.
31. Levitt JO, Levitt BH, Akhavan A, Yanofsky H. The sensitivity and specificity of potassium hydroxide smear and fungal culture relative to clinical assessment in the evaluation of tinea pedis: A pooled analysis.

Dermatol Res Pract 2010;2010:764843.
32. Shenoy MM, Teerthanath S, Karnaker VK, Girisha BS, Krishna Prasad MS, Pinto J. Comparison of potassium hydroxide mount and mycological culture with histopathologic examination using periodic acid-Schiff staining of the nail clippings in the diagnosis of onychomycosis. Indian J Dermatol Venereol Leprol 2008;74:226-9.
33. Ecemis T, Degerli K, Aktas E, Teker A, Ozbakkaloglu B. The necessity of culture for the diagnosis of tinea pedis. Am J Med Sci 2006;331:88-90.
34. Bhatia VK, Sharma PC. Epidemiological studies on Dermatophytosis in human patients in Himachal Pradesh, India. Springerplus 2014;3:134.
35. Silva LB, de Oliveira DB, da Silva BV, de Souza RA, da Silva PR, Ferreira-Paim K, *et al.* Identification and antifungal susceptibility of fungi isolated from dermatomycoses. J Eur Acad Dermatol Venereol 2014;28:633-40.
36. Singh J, Zaman M, Gupta AK. Evaluation of microdilution and disk diffusion methods for antifungal susceptibility testing of dermatophytes. Med Mycol 2007;45:595-602.
37. Barros ME, Santos Dde A, Hamdan JS. *In vitro* methods for antifungal susceptibility testing of *Trichophyton* spp. Mycol Res 2006;110(Pt 11):1355-60.
38. Gupta AK, Kohli Y, Batra R. *In vitro* activities of posaconazole, ravuconazole, terbinafine, itraconazole and fluconazole against dermatophyte, yeast and non-dermatophyte species. Med Mycol 2005;43:179-85.
39. Fernández-Torres B, Carrillo AJ, Martín E, Del Palacio A, Moore MK, Valverde A, *et al. In vitro* activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrob Agents Chemother 2001;45:2524-8.
40. Magagnin CM, Stopiglia CD, Vieira FJ, Heidrich D, Machado M, Vetoratto G, *et al.* Antifungal susceptibility of dermatophytes isolated from patients with chronic renal failure. An Bras Dermatol 2011;86:694-701.
41. Favre B, Hofbauer B, Hildering KS, Ryder NS. Comparison of *in vitro* activities of 17 antifungal drugs against a panel of 20 dermatophytes by using a microdilution assay. J Clin Microbiol 2003;41:4817-9.
42. Adimi P, Hashemi SJ, Mahmoudi M, Mirhendi H, Shidfar MR, Emmami M, *et al. In-vitro* activity of 10 antifungal agents against 320 dermatophyte strains using microdilution method in Tehran. Iran J Pharm Res 2013;12:537-45.
43. Indira G. *In-vitro* antifungal susceptibility testing of 5 antifungal agents against dermatophytic species by CLSI (M38-A) micro dilution method. Clin Microbial 2014;3:145.
44. Galuppi R, Gambarara A, Bonoli C, Ostanello F, Tampieri MP. Antimycotic effectiveness against dermatophytes: Comparison of two *in vitro* tests. Vet Res Commun 2010;34 Suppl 1:S57-61.
45. Ataides FS, Chaul MH, El Essal FE, Costa CR, Souza LK, Fernandes OF, *et al.* Antifungal susceptibility patterns of yeasts and filamentous fungi isolated from nail infection. J Eur Acad Dermatol Venereol 2012;26:1479-85.
46. Yenisehirli G, Tunçoglu E, Yenisehirli A, Bulut Y. *In vitro* activities of antifungal drugs against dermatophytes isolated in Tokat, Turkey. Int J Dermatol 2013;52:1557-60.
47. Gupta AK, Kohli Y. *In vitro* susceptibility testing of ciclopirox, terbinafine, ketoconazole and itraconazole against dermatophytes and nondermatophytes, and *in vitro* evaluation of combination antifungal activity. Br J Dermatol 2003;149:296-305.
48. Deng S, Zhang C, Seyedmousavi S, Zhu S, Tan X, Wen Y, *et al.* Comparison of the *in vitro* activities of newer triazoles and established antifungal agents against *Trichophyton rubrum*. Antimicrob Agents Chemother 2015;59:4312-4.