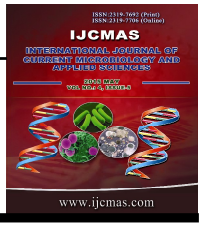
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 5 (2015) pp. 24-33**
http://www.ijcmas.com



Original Research Article

Comparison of Broth Micro Dilution and Disk Diffusion Method for Susceptibility Testing of Dermatophytes

Shalini Gupta[1], Rajiv Kumar Agarwal[1]*, Garima Mittal[1], Samarjit Roy[2], Fauzia Khan[1] and Ankit Agarwal[3]

[1]Department of Microbiology, Himalayan Institute of Medical Sciences, Dehradun, India
[2]Department of Dermatology, Himalayan Institute of Medical Sciences, Dehradun, India
[3]Department of Anaesthesiology, AIIMS, Rishikesh, India
**Corresponding author*

Keywords

Disk diffusion, Broth micro dilution, Dermatophytes, Antifungal susceptibility

A B S T R A C T

Dermatophytosis is an important public health problem especially in India where hot and humid climate predisposes to such infections. In spite of therapy, relapse may be seen and disease may not respond to therapy in fair number of cases. A number of newer antifungal drugs have been made available for clinical use which in turn has led to acquired antifungal resistance among previously susceptible strains or species. The in-vitro antifungal susceptibility testing has been limited by a lack of availability of a standardized, simple and reproducible method. In this study disk diffusion method was compared with broth micro dilution against four drugs viz. fluconazole, itraconazole, terbinafine, and griseofulvin employing 58 strains of Dermatophytes. A good correlation was seen between the two methods. Disk diffusion was found to be simple and promising.

Introduction

Though fungi are widely distributed, many fungal infections go unrecognised as fungal diseases are not notifiable like some viral, parasitic and bacterial diseases. In recent years, there has been a remarkable increase in the number of fungal infections specially in those people whose immune system is compromised by aging, HIV infection, organ transplantation or cancer therapy (Kannan *et al.,* 2006). This is more so in India due to hot and humid climate which favours fungal growth. Dermatophytes are responsible for the majority of the fungal infections involving skin, hair and nails.

Concurrent with the increase in fungal infections combined with increased use of antifungal drugs often for prolonged periods has led to development of resistance to antifungal drugs. The availability of therapeutic options has led to the demand for in vitro antifungal susceptibility testing. However, unlike antibacterial susceptibility testing, antifungal susceptibility testing is still in its infancy (Jain *et al.,* 2008; Koneman, 2006) inspite of the fact that a reference method for dermatophytes is available (CLSI-M 38A2, 2008). We have earlier reported a disk diffusion method for

dermatophytes and the results were encouraging. The present study was therefore undertaken to compare disk diffusion and broth micro dilution methods & to determine in-vitro activity of two azole derivatives Fluconazole (FLC) & Itraconazole (ITR), Griseofulvin (GRI) & Terbinafine (TER) that are most commonly used to treat dermatophyte infection against commonly isolated species of dermatophytes.

Materials and Methods

Fifty eight clinical isolates of dermatophytes were tested along with *Trichophyton rubrum* ATCC28188 and *Trichophyton mentagrophytes* ATCC 9533 as controls. Dermatophytes were subcultured on Potato Dextrose Agar (PDA) & incubated at 28°C for 7 days to enhance sporulation. The growth was harvested in sterile saline & the conidial and hyphal suspension was adjusted to $1x10^6$/ml using a haemocytometer.

Broth micro dilution (BMD) method

The test was performed in micro titre plates with RPMI-1640 without bicarbonate and buffered to pH 7.0 with 3-[N-morpholino] propane sulfonic acid (MOPS). For each drug six dilutions were used. One hundred µl of two fold drug dilutions were placed in wells with a multichannel pipette to yield twice the final strength required for the test i.e. 4–128 µg/ml for FLC, 0.25–8 µg/ml for GRI, 0.125–4.0 µg/ml for ITR and 0.015–0.50 µg/ml for TER. The plate was then inoculated with 100 µl of the diluted inoculum suspension to contain $0.5x10^4$–$5.0x10^4$ spores/hyphae per ml thus bringing the final dilution of drugs to 2.0-64.0 µg/ml for FLC, 0.125–4.0 µg/ml for GRI, 0.062–2.0 µg/ml for ITR and 0.015–0.5 µg/ml for TER respectively. Growth and sterility controls along with 1:100 DMSO were included for each assay. The plates were incubated at 28°C for 5 days and read visually. MIC for FLC, ITR and GRI were lowest drug concentration that showed approximately 90% growth inhibition. For TER, the MIC was the lowest drug concentration that showed 100% inhibition.

Disk diffusion (DD) method

The ABDD was performed as described by Nweze *et al.* (2010). Plates of Mueller Hinton Agar (MHA) were inoculated using a swab dipped in the inoculums suspension. The inoculated plates were then dried before applying the disks. Fluconazole (25µg) & Itraconazole (10 µg) disks were available commercially (HIMEDIA), Griseofulvin (10 µg) & Terbinafine (2µg/disk) were prepared in lab by dissolving the pure powders in DMSO & then diluting it to give a final concentration of 1mg/ml & 200 µg/ml for Griseofulvin & Terbinafine respectively & then delivering 10 µl onto each sterile disk. Sterile disks were also impregnated with 10 µl of 1:100 dilution of DMSO to serve as control. The above 5 disks were applied to each inoculated & dried plate & then incubated at 28°C for up to 5 days. When growth took place, the size of zones of inhibition was measured for each antifungal agent (Pakshir *et al.*, 2009).

Results and Discussion

A total of 58 strains of dermatophytes were tested for antifungal susceptibility by DD & BMD methods. Isolates belonged to 2 genera and 6 species of dermatophytes as shown in table 1.

Figure 1 shows the results of broth micro dilution method. The wells in row H contains only RPMI 1640 and serves as negative control. The wells in row G contain RPMI 1640 with 1:100 DMSO together with

isolates and well A through F contain doubling dilutions of the drugs. Wells in column 11 were inoculated with *T. mentagrophytes* ATCC 9533 and column 12 with *T. rubrum* ATCC 28188.

In case of fluconazole (FLC), the dilutions of the drug tested ranged from 64.0 μg to 2.0 μg/ml. Majority of the strains were inhibited at 8 μg and 16 μg/ml. However a few strains showed a MIC of 32 or even 64 μg/ml. Both the ATCC strains of *T. mentagrophytes* and *T. rubrum* had a MIC of ≤2.0 μg/ml.

Itraconazole (ITR) was tested at dilutions ranging from 2.0 μg /ml to 0.0625 μg/ml and most of the strains of *T. mentagrophytes*, *T. rubrum* and others had a MIC value of 0.125 or 0.250 μg/ml and none had a MIC higher than 0.5 μg/ml.

The ATCC strains of *T. mentagrophytes* and *T. rubrum* had a MIC of 0.125 and ≤0.0625 μg/ml respectively.

The terbinafine (TER) was tested at dilutions ranging from 0.5 to 0.015 μg/ml. The MIC for terbinafine ranged from 0.125 μg/ml to ≤ 0.015 μg/ml in case of *T. mentagrophytes* and others. It was found to be > 0.5 to ≤ 0.015 μg/ml in case of *T. rubrum* while in case of ATCC strains of both *T. mentagrophytes* and *T. rubrum* it was <0.015 μg/ml. Most of the strains had MIC of up to 0.062 μg/ml. Only five strains of *T. rubrum* had a MIC value of >0.50 μg/ml.

The dilutions of griseofulvin (GRI) tested ranged from 4.0 μg/ml to 0.125 μg/ml. Majority of the strains of *T. mentagrophytes*, *T. rubrum* and others had a MIC of 0.25 μg/ml to 0.50 μg/ml. Seven strains of *T. mentagrophytes* and six of *T. rubrum* had a MIC of 1.0 μg/ml. The MIC of ATCC strains was found to be 0.25 μg/ml and 0.125 μg/ml for *T. mentagrophytes* and *T. rubrum* respectively.

Except 5 strains of *T. rubrum* all the strains show zones of inhibition around them (Fig. 2). The zone of inhibition varied from 10–32 mm for Fluconazole, 17–36 mm for Itraconazole, nil-44 mm for Terbinafine and 21–49 mm for Griseofulvin with mean ± SD of 22.6 ± 4.2, 27.3 ± 6.2, 32.1 ± 6.1 and 35.9 ± 4.9 respectively. No zone of inhibition was seen with disk containing DMSO against any of the species tested.

The results of zone of inhibition for all the drugs for each group of fungi are summarised in table 2.

An attempt was made to correlate MICs obtained in broth micro dilution method with zone of inhibitions obtained in disk diffusion method. Correlation between MICs and IZDs with fluconazole, itraconazole, terbinafine and griseofulvin has been shown in table 3 to 6 respectively.

There was a steady increase in IZD with lowering of MIC in most of the strains. Thus, MICs and IZDs are inversely proportional to each other i.e. when the MIC for the drug is more; the IZD is smaller and vice versa.

Successful treatment of fungal infections depends on the ability of a given antimycotic agent to eradicate the fungus from the tissue (Santos *et al.*, 2001). Though some in-vitro antifungal susceptibility tests are now available (Fernandez Torres *et al.*, 2001; Karaca and Koç, 2004; Santos *et al.*, 2001) including CLSI document regarding filamentous fungi (CLSI, 2008), no simple reference method has been standardised for testing the drug susceptibility of dermatophytes. We tested 58 strains of dermatophytes against 4 commonly used

antifungal agents viz. Fluconazole, Itraconazole, Terbinafine and Griseofulvin.

Fluconazole was found to be the least effective drug. Its MIC_{90} ranged from 2 to 64 μg/ml. Though majority of the strains had a MIC of 8 and 16 μg/ml in both the cases of *T. mentagrophytes* and *T. rubrum*, a few strains had a MIC value of 32 and 64 μg/ml or higher. A MIC value of 32 or 64 may not be desirable for treatment with fluconazole. Favre *et al.* (2003) and Barros *et al.* (2007) have also reported a very high MIC values for FLC. Problems with interactions of fluconazole with particular media or problems with dilutions in higher concentration have also been suggested as being responsible for its high MIC values by Korting *et al.* (1995).

The MIC_{90} for Itraconazole was found to lie between 0.0625 and 0.5 μg/ml with majority of the strains showing a value of 0.125 μg. A more or less similar MIC_{90} have been reported by Barros *et al.* (2007), Fernandez Torres *et al.* (2002) and Serrano-Martino *et al.* (2003). However, Gupta and Kohli (2003) have found MIC as high as up to 32 μg/ml also both in *T. mentagrophytes* and *T. rubrum*.

The MIC_{90} for GRI ranged from 0.0625 to 0.5 μg/ml both *T. mentagrophytes* and *T. rubrum* and none of the strain had a MIC higher than 0.5 μg/ml. Similar MIC have also been reported by Favre *et al.* (2003), Barros *et al.* (2007) and Araújo Mota *et al.* 2009. However, Ghannoum *et al.* (2000) have found MIC values up to 64 μg/ml also in *T. mentagrophytes* as well as *T. rubrum*. Mukherjee *et al.* (2003) also found MIC to GRI up to 64 μg/ml in case of *T. rubrum* making the treatment ineffective.

The lowest MIC values were obtained for terbinafine (TER). Most of the strains had a MIC_{100} to be 0.063. Five strains of *T. rubrum* had a MIC value >0.5 μg/ml indicating intrinsic resistance to TER. Resistant strains of *T. rubrum* having MIC 4 μg/ml have also been reported by Nweze *et al.* (2010) and Mukherjee *et al.* (2003). But generally speaking TER is the most effective drug with lowest MIC as has also been reported by Favre *et al.* (2003); Serrano-Martino *et al.* (2003), Tong *et al.* (2007) and Indira (2014).

For disk diffusion method, the strength of each disk was 25 μg, 10 μg, 2 μg & 10 μg respectively for Fluconazole, Itraconazole, terbinafine & Griseofulvin. The zone sizes varied from10-32 mm, 17-36 mm, nil-44 mm & 21-49 mm for Fluconazole, Itraconazole, Terbinafine & Griseofulvin with an average of 22.6 ± 4.2, 27.3 ± 6.2, 32.1 ± 6.1 & 35.9 ± 4.9 respectively.

No zone of inhibition was seen in 5 strains of *T.rubrum* against terbinafine. Perhaps these strains were intrinsically resistant to terbinafine. However, all these 5 strains were fully sensitive to other antifungal agents tested indicating that cross resistance to azoles & griseofulvin does not exists. Strains of *T.rubrum* showing primary resistance to terbinafine have also been reported by other workers also (Nweze *et al.*, 2010 & Mukherjee *et al.*, 2003).

The strains were classified into sensitive, intermediate sensitive or resistant on the basis of their mean IZD ± SD (Table-7) and following the above criterion only 6 strains were found resistant to FLC and 5 to TER and 5, 4 and 3 strains were found intermediate sensitive to FLC, ITR and GRI respectively. All other strains were sensitive to all the 4 antimycotic agents.

The factors that may affect the results of BMD or DD are type and size of inoculum,

composition of the media, temperature and duration of incubation and disk strength (Santos *et al.*, 2001 & Fernandez Torres *et al.*, 2002).

Table.1 Number of dermatophytes tested

Fungi	No. of strains
Trichophyton mentagrophytes	23
T. rubrum	27
T. tonsurans	02
Microsporum audonii	01
M. gypseum	04
M. ferrugineum	01

Table.2 IZDs obtained with different dermatophytes

Fungi	No. of strains	Drugs	Range	Arithmetic Mean
T. mentagrophytes	23	ITC FLU TRB GRI	20-35 13-30 30-41 30-49	29.3 25 33.6 37.6
T. rubrum	27	ITC FLU TRB GRI	17-33 10-32 Nil-44 32-43	24.7 20.9 28.5 35.5
T. tonsurans	02	ITC FLU TRB GRI	31-36 16-23 40-49 21-38	33.5 19.5 44.5 29.5
M. audonnii	01	ITC FLU TRB GRI	25 19 37 31	25 19 37 31
M. gypseum	04	ITC FLU TRB GRI	23-36 20-28 38-44 35-40	28.3 22 40.5 36.5
M. ferrugineum	01	ITC FLU TRB GRI	19 20 40 37	19 20 40 37

Table.3 Correlation between MICs and IZDs for fluconazole

	Trichophyton mentagrophytes	***Trichophyton rubrum***	**Others**
MICs(µg/ml)	**IZDs(mean) in mm**	**IZDs(mean) in mm**	**IZDs(mean) in mm**
64	17.5	11.33	-
32	13	14.33	-
16	21	21.5	18.7
8	22.28	23.77	23.4
4	25.75	23.66	-
2	26.66	30.66	-

Table.4 Correlation between MICs and IZDs for itraconazole

	Trichophyton mentagrophytes	***Trichophyton rubrum***	**Others**
MICs(µg/ml)	**IZDs (mean) in mm**	**IZDs(mean) in mm**	**IZDs(mean) in mm**
2	-	-	-
1	-	-	-
0.5	22.25	16.75	-
0.25	24.83	24.33	25.4
0.125	27.37	23.5	29.3
0.0625	31	29.5	35.0

Table.5 Correlation between MICs and IZDs for terbinafine

	Trichophyton mentagrophytes	***Trichophyton rubrum***	**Others**
MICs(µg/ml)	**IZDs(mean) in mm**	**IZDs(mean) in mm**	**IZDs(mean) in mm**
0.5	-	nil	-
0.25	-	-	-
0.125	33	30	33.1
0.062	35.3	32.11	37.5
0.031	38.62	34.62	39.2
0.015	38	41.66	49

Fig.1 MICs for terbinafine

Photograph 11 :MICs for Terbinafine
Column 1 through 10: Different isolates
Column 11: ATCC 9533 (Trichophyton mentagrophytes)
Column 12: ATCC 28188 (Trichophyton rubrum)
Row A: 0.50µg/ml
Row B: 0.250 µg/ml
Row C: 0.125µg/ml
Row D: 0.062µg/ml
Row E: 0.031µg/ml
Row F: 0.015µg/ml
Row G: RPMI +DMSO +Culture (Growth control)
Row H: RPMI only (Media control)

Fig.2 Disk Diffusion testing of *Trichophyton mentagrophytes* showing susceptibility to all the four antimycotic agents

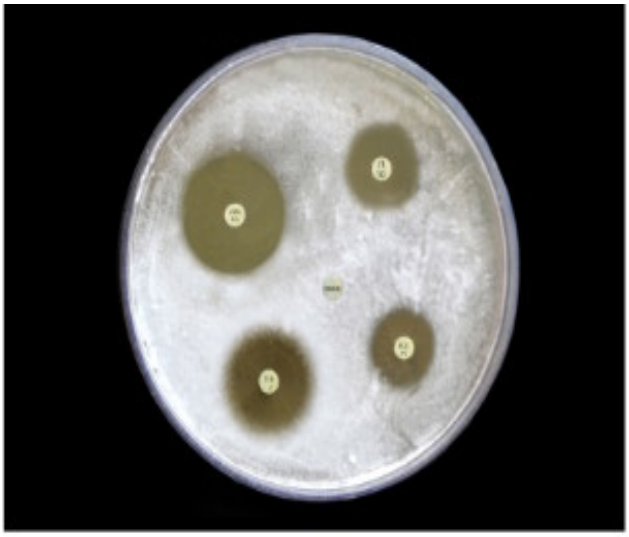


Table.6 Correlation between MICs and IZDs for griseofulvin

	Trichophyton mentagrophytes	***Trichophyton rubrum***	**Others**
MICs(µg/ml)	**IZDs(mean) in mm**	**IZDs(mean) in mm**	**IZDs(mean) in mm**
4	-	-	-
2	-	-	-
1	35	33.33	31
0.5	42.7	34.25	32.8
0.25	44	39.22	37.3
0.125	-	-	-

Table.7 Cut off values for IZDs for each of the four drugs

Drugs	Inhibition Zone Diameters			
	Mean ± SD	Sensitive Mean ± 1 SD	Intermediate Sensitive Mean-1 SD to Mean-2 SD	Resistant <Mean -2 SD
FLC	22.6±4.2	>19	14-19	<14
ITR	27.3±6.2	>21	15-21	<15
TER	32.1±6.1	>26	20-26	<20
GRI	35.9±4.9	>31	26-31	<26

In the present study a good correlation was generally seen between MICs and IZDs of all the four drugs. It was seen that if MIC was low for a particular isolate a larger zone of inhibition was seen. On the other hand if MIC was high a smaller zone of inhibition was observed and with terbinafine was even absent in 5 isolates of *T. rubrum* which had a MIC value of >0.5µg/ml. Similarly in case of FLC a small IZD of average 17.5 mm and 13 mm were seen when the MIC was found to be 64 and 32 µg/ml. Pakshir *et al.* (2009), Nweze *et al.* (2010) and Venugopal and Venugopal (1995) have also reported good correlation between MIC and IZD. Contrary results have also been reported by Singh *et al.* (2007) and Mendez *et al.* (2008) with poor or no correlation at all between broth micro dilution and disk diffusion.

In spite of contradictory reports on correlation between MIC and IZD, disc diffusion method appears to be simple, reproducible, cheap and easily adaptable and will become a useful and necessary procedure for selection of appropriate antifungal agent once the conditions are properly standardised by studying a large number of strains.

Though Broth Micro Dilution (BMD) is the standard method for testing antifungal susceptibility of dermatophytes, it is costly and cumbersome. The disk diffusion (DD) susceptibility testing methods is simple, inexpensive & does not require specialised equipment & can be adapted for routine assessment of dermatophyte resistance to antifungal agents. Further studies are

necessary to properly standardize antimycotic sensitivity testing by disk diffusion method to make it useful & necessary procedure for selection of appropriate drug in a routine mycology laboratory.

References

Araújo Mota, C.R., Miranda, K.C., Lemos, J.A., Costa, C.R., Souza, L.K.H., Passos, X.S., *et al.* 2009. Comparison of in vitro activity of five antifungal agents against dermatophytes, using the agar dilution and broth micro dilution methods. *Revista da Socieda de Brasileira de Medicina Tropical,* 42(3): 250–254.

Barros, M.E., Santos, D., Hamdan, J. 2007. Evaluation of susceptibility of *Trichophyton mentagrophytes* and *Trichophyton rubrum* clinical isolates to antifungal drugs using a modified CLSI micro dilution method (M38-A). *J. Med. Microbiol.*, 56: 514–518.

Clinical and Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, 2nd ed. Approved standard. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA. 11.

Favre, B., Hofbauer, B., Hildering, K., Ryder, N.S. 2003. Comparison of in-vitro activities of 17 antifungal drugs against a panel of 20 dermatophytes by using a micro dilution assay. *J. Clin. Microbiol.*, 41: 4817–4819.

Fernández-Torres, B., Carrillo, A.J., Martín, E., Del Palacio, A., Moore, M.K., Valverde, A., Serrano, M., Guarro, J. 2001. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrob. Agents Chemother.*, 45: 2524–2528.

Fernández-Torres, B., Cabañes, F.J., Carrillo-Munõz, A.J., Esteban, A., Inza, I., Abarca, L., Guarro, J. 2002. Collaborative evaluation of optimal antifungal susceptibility testing condition for dermatophytes. *J. Clin. Microbiol.*, 40: 3999–4003.

Ghannoum, M.A., Hajjeh, R.A., Scher, R., Konnikov, N., Gupta, A.K., Summerbell, R., Sullivan, S., Daniel, R., Krusinski, P., Fleckman, P., Rich, P., Odom, R., Aly, R., Pariser, D., Zaiac, M., Rebell, G., Lesher, J., Gerlach, B., Leon, G.F., Ghannoum, A., Warner, J., Isham, N., Elewski, B. 2000. 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.*, 43: 641–648.

Gupta. A.K., Kohli, Y. 2003. In vitro susceptibility testing of ciclopirox, terbinafine, ketoconazole and itraconazole against dermatophytes and non dermatophytes and in vitro evaluation of combination antifungal activity. *Br. J. Dermatol.*, 149: 296–305.

Indira, G. 2014. In vitro antifungal susceptibility testing of 5 antifungal agents against dermatophytic species by CLSI (M38-A) micro dilution method. *Clin. Microbial.*, 3: 1–5.

Jain, N., Sharma, M., Saxena, V.N. 2008. Identification and antifungal susceptibility testing of fungal infections in clinical samples of suspected superficial fungal infections. *Indian J. Dermatol. Venerol. Leprol.*, 74(3): 274–75.

Kannan, P., Janaki, C., Selvi, G.S. 2006. Identification and antifungal susceptibility testing of fungal infections in clinical samples of suspected superficial fungal

infections. *Indian J. Med. Microbiol.*, 24(3): 212–15.

Karaca, N., Koç, A. 2004. In- vitro susceptibility testing of dermatophytes: comparison of disk diffusion and reference broth dilution methods. *Diagn. Microbiol. Infect. Dis.*, 48: 259–264.

Koneman, E.W. 2006. Identification and antifungal susceptibility testing of fungal infections in clinical samples of suspected superficial fungal infections. Color Atlas and Textbook of Diagnostic Microbiology, 6th edn., Pp. 1002–1043.

Korting, H.C., Ollert, M., Abeck, D. 1995. The German collaborative dermatophyte drug susceptibility study group results of German multicenter study of antimicrobial susceptibilities of *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains causing tinea unguium. *Antimicrob. Agents Chemother.*, 39: 1206–1208.

Mendez, C.C., Serrano, M.C., Valverde, A., Peman, J., Almeida, C., Martin-Manzuelos, E. 2008. Comparison of E-test disk diffusion and a modified CLSI broth micro dilution (M 38-A) method for in-vitro testing of itraconazole, fluconazole and voriconazole against dermatophytes. *Med. Mycol.*, 46(2): 119–23.

Mukherjee, P.K., Leidich, S.D., Isham, N., Leitner, I., Ryder, N.S., Ghannoum, M.A. 2003. Clinical *Trichophyton rubrum* strain exhibiting primary resistance to terbinafine. *Antimicrob. Agents Chemother.*, 47: 82–86.

National Committee for Clinical Laboratory Standards, 2004. Method for antifungal disk diffusion susceptibility testing of yeasts. Approved guidelineM44-A. National Committee for Clinical Laboratory Standards, Wayne, PA.

Nweze, E.I., Mukherjee, P.K., Ghannoum, M.A. 2010. Agar-based disk diffusion assay for susceptibility testing of dermatophytes. *J. Clin. Microbiol.*, 48(10): 3750–3752.

Pakshir, K., Bahaedinie, L., Rezaei, Z., Sodaifi, M., Zomorodian, K. 2009. In-vitro activity of six antifungal drugs against clinically important dermatophytes. *Jundishapur J. Microbiol.*, 2(4): 158–163.

Santos, J.J., Paula, C.R., Viani, F.C., Gambale, W. 2001. Susceptibility testing of *Trichophyton rubrum* and *Microsporum canis* to three azoles by E-test. *J. de Mycologie Medicale*, 11: 42–43.

Serrano-Martino, M., del, C., Cha´ vez-Caballero, Valverde-Conde, M., Claro, A., Pema´n, R.M.J., Martı´n-Mazuelos, E. 2003. In vitro activity of voriconazole and three other antifungal agents against dermatophytes. *Enferm Infect. Microbiol. Clin.*, 21: 484–487.

Singh, J., Zaman, M., Gupta, A.K. 2007. Evaluation of micro dilution and disk diffusion methods for antifungal susceptibility testing of dermatophytes. *Med. Mycol.*, 45: 595–602.

Tong, Z., Widmer, F., Sorrell, T.C., Guse, Z., Jolliffe, K.A., Halliday, C., Lee, O.C., Kong, F., Wright, L.C., Chen, S.C.A. 2007. In-vitro activities of Miltefosine and two novel antifungal biscationic salts against a panel of 77 dermatophytes. *Antimicrob. Agents Chemother.*, 51(6): 2219–2222.

Venugopal, P.V., Venugopal, T.V. 1995. Disk diffusion susceptibility testing of dermatophytes with imidazoles. *Indian J. Pathol. Microbiol.*, 1995: 369–374.