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Evaluation of Antifungal Susceptibility Testing Methods for Dermatophytes

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ABSTRACT: Standardized broth microdilution is a laborious, intensive method, and therefore, is not readily applicable in routine laboratories with high workloads. Agar-based disk diffusion methods are easier, and could be more feasible options for AST (antifungal susceptibility testing) of dermatophytes. Disk diffusion method for yeasts has been standardized by CLSI (Clinical Laboratory Standards Institute) in 2004, and further modified in 2018, as document M44 (1). However, no standardized CLSI guidelines exist for disk diffusion AST of dermatophytes. To compare disk diffusion methods; one using plain Mueller-Hinton medium (MH), and another using Dermasel agar medium (DA), with CLSI-approved broth microdilution method under document M38. Microbroth dilution (CLSI-M38), disk diffusion on MHA (CLSI-M44), and on DA, were performed on 52 dermatophyte strains, for fluconazole, griseofulvin, itraconazole and terbinafine. Disk diffusion results were compared with the reference microbroth dilution. The results from disk diffusion on MH, as well as on DA, for fluconazole against dermatophytes, yielded moderate agreement with the results of microdilution format. AST results for griseofulvin on disk diffusion on MH were in moderate agreement with microdilution format, whereas with DA, there was substantial agreement. When compared with reference microbroth dilution, there was no difference in results procured by either of the disk diffusions for itraconazole and terbinafine, giving perfect agreement, respectively.This study suggests that disk diffusion techniques with either MH or DA, provide an alternative for AST of dermatophytes, especially in low-resource and routine clinical laboratory settings.

KEYWORDS:broth microdilution, disk diffusion, Clinical Laboratory Standards Institute, antifungal susceptibility testing, dermatophytes

I. INTRODUCTION

The incidence of superficial fungal infections has increased in recent years, with dermatophytosis being the most common, affecting 20-25% of the world's population. (1,2). India has proven to show a noticeably significant rise in number of dermatophytosis cases with chronic recalcitrant diseases, atypical presentations, frequent relapse and with some cases presenting as refractory to antifungal therapyleading to treatment failures(3).Commonly encountered dermatophytes' genera are Trichophyton, Microsporum and Epidermophyton(4).CLSI (Clinical and Laboratory Standard Institute) M38-A2 AST (antifungal susceptibility testing) for molds, introduced in 2008, has undergone recent modifications and has been replaced with M38Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi(5).Despite the availability of a standard method for AST for not specifically dermatophytes, but for filamentous fungi as a whole, including non-dermatophyte molds as well, the process of performing, interpreting and drawing inference using this method seems rather complicated.Agar-based methods, such as disk diffusion, are quick and easy, and could be good options for AST (antifungal susceptibility testing). Disk diffusion method for yeasts has been standardized by CLSI in 2004, under M44-A, and further modified recently, as document M44 (5). There is a modified document, M38 of CLSI, for broth microdilution (AST of filamentous fungi in general, but not specific to dermatophytes). However, no standardized CLSI guidelines exist for disk diffusion testing of dermatophytes. The need to carry out researches, pertaining to investigating correlations between disk diffusion testing and CLSI broth dilutions for dermatophytes, is vastly overlooked (6)A limited number of investigations are being carried out to develop a correlation between disk diffusion testing and CLSI broth dilution method (6). There is an inevitable need to develop a reproducible and standard technique, especially for high workload setups, for these important fungi, to lead to protocols for proper antifungal therapy.The present study describes the use of agar-based disk diffusion (ABDD) assays, for in vitro susceptibility of dermatophytes, against four antifungal agents:

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fluconazole, griseofulvin, itraconazole and terbinafine; obtained ABDD results have been compared with the results procured by broth microdilution (BMD) assays performed according to CLSI-M38.

II. MATERIALS AND METHODS

52 dermatophytestrains were isolated and identified (via appropriate collection, plating onto Sabouraud's Dextrose agar, incubation, microscopy and relevant identification tests) from patient's dermatophytic lesions. Three methods of antifungal susceptibility testing were performed on each isolated dermatophyte (n=52).:

a. The broth microdilution method was performed as described by the CLSI-approved document, M38. The medium used was the Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich), buffered at pH 7.0± 0.1 at room temperature (25˚C), with 0.165M Morpholinepropanesulfinic acid(7).
b. Disk diffusion method was performed following the method, M44 of CLSI (but with dermatophytes, instead of yeasts), using the medium Mueller-Hinton agar (MHA), at pH 7.0 ± 0.1 at room temperature (25˚C)(5).
c. A second disk diffusion method was performed, following the guidelines of CLSI-M44, but with the medium, Dermasel agar (containing chloramphenicol and cycloheximide agents), at pH 7.0 ± 0.1 at room temperature (25˚C).

BrothMicrodilution Method (M38 CLSI): Following the instructions in CLSI-M38, the antifungal agent stock solutions were prepared for the purpose of pipetting two-fold dilutions. The antifungal powders used were: fluconazole (Sigma-Aldrich); griseofulvin (Sigma-Aldrich); itraconazole (Sigma-Aldrich); terbinafine (Sigma-Aldrich). Inoculum suspensions of our dermatophyte isolates, were prepared from potato dextrose agar (PDA) slants (which had been incubated for 8 to 10 days at 30˚C). The inoculum suspensions were set to achieve optical densities of 0.5 McFarland and microtitre plates with the respective dilutions were set up. Incubation of the titre plates was done at 25˚C, and read after 5-7 days of incubation. The MICs were read and noted, as defined in the CLSI-M38.

Disk Diffusion Method using Mueller-Hinton Agar (M44 CLSI):We followed the CLSI M44 guidelines (disk diffusion assay for yeasts), using MHA, as advised by the document, with some adjustments for dermatophytes. Inoculum suspensions were made as instructed for broth microdilution, which we optimized to 0.5 MacFarland.Commercially available, preloaded, 9 mm paper disks for fluconazole (25 µg/disk) and itraconazole (10 µg/disk) were used (Oxoid). We used blank 6mm paper disks (Becton Dickinson) for loading stock solutions prepared from griseofulvin and terbinafine powders (Sigma-Aldrich) to obtain 10 µg and 2 µg per disk, respectively.MHA plates, supplemented with 2% glucose, were inoculated by streaking with a sterile swab dipped in the respective suspensions.

Disk Diffusion Method using Dermasel agar: Similar techniques were followed as with disk diffusion on Mueller-Hinton (MH) and as in CLSI-M44, as aforementioned; except for the media, for which Dermal agar base (Thermo Fisher Scientific)was employed.

Statistical Analysis: The ranges were noted; for the minimum inhibitory concentrations (MICs) by microdilution method, and for the inhibition zone diameters (IZDs) by disk diffusion methods on MHA and Dermasel (DA) plates, respectively. The agreements between BMD and disk diffusion assays (with MHA and DA, respectively), were calculated by means of Cohen's kappa coefficient k value of 1 was considered as perfect agreement with classic microdilution.

III. RESULTS

The susceptibility patterns and range of MICs obtained from microdilution assays for 4 antifungals are summarized in Table 1. With all isolated strains considered together, itraconazole and terbinafine produced lowest MIC ranges; 0.03-1 µg/ml and 0.03-4 µg/ml, respectively.The susceptibility patterns and range of IZDs obtained from disk diffusion studies on MH agar for 4 antifungals are summarized in Table 2. Itraconazole (10µg) disks and terbinafine (2µg) disks produced the largest IZDs. Small inhibition zone diameters were observed with griseofulvin (10µg) and fluconazole (25µg) disks respectively.Thesusceptibility patterns and range of IZDs obtained from the disk diffusion studies on DA for the 4 antifungals are summarized in Table 2. As with the DD assays on MHA, terbinafine (2µg) disks and itraconazole (10µg) disks produced the largest IZDs. Small inhibition zone diameters were observed with the griseofulvin (10µg) and fluconazole (25µg) disks respectively.

Table 3 presents the agreement between antifungal susceptibility results for dermatophytes, obtained by disk diffusion on MHA (CLSI-M44),

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against those obtained by the recognized BMD method for molds (CLSI-M38) as determined using Cohen's kappa coefficient analysis for inter-rater reliability.

Table 4demonstrates a comparison of the antifungal susceptibility results for dermatophytes obtained by disk diffusion on Dermasel agar (DA), against those obtained by the recognized BMD method for molds (CLSI-M38).

Table 1. Antifungal Susceptibility Patterns of Antifungal Agents for 52 Dermatophyte Isolates, by Microbroth Dilution Method (CLSI-M38)

Fungal Isolates	Fluconazole (µg/ml)		MIC Range	Griseofulvin (µg/ml)		MIC Range	Itraconazole (µg/ml)		MIC Range	Terbinafine (µg/ml)		MIC Range
	Sensitive* (≤ 4)	Resistant (>4)		Sensitive* (≤0.5)	Resistant (> 0.5)		Sensitive* (≤ 0.25)	Resistant (>0.25)		Sensitive* (≤0.25)	Resistant (>0.25)	
C. parapsilosis ATCC 22019	1 (100%)	-	4	1 (100%)	-	0.12	1 (100%)	-	0.12	1 (100%)	-	0.12
T. interdigitale (23)	22 (95.7%)	1 (4.3%)	1 – 8.	22 (95.7%)	1 (4.3%)	.06 – 8.	22 (95.7%)	1 (4.3%)	.03 – 1.	21 (91.3%)	2 (8.7%)	.03 – 1.
T. rubrum (20)	20 (100%)	-	1 – 4.	17 (85%)	3 (15%)	.25 – 4.	20 (100%)	-	.03 – .25	18 (90%)	2 (10%)	.03 – 4.
T. verrucosum (01)	1 (100%)	-	4.	-	1 (100%)	1.	1 (100%)	-	.03	1 (100%)	-	.06
E. floccosum (05)	5 (100%)	-	1 – 4.	5 (100%)	-	.25 – 0.5	5 (100%)	-	.03 – .25	5 (100%)	-	.06
M. canis (01)	1 (100%)	-	2.	1 (100%)	-	.06	1 (100%)	-	.03	1 (100%)	-	.03
M. gypseum (02)	2 (100%)	-	4.	2 (100%)	-	.06 - .12	2 (100%)	-	.03 - .06	2 (100%)	-	.03 - .12
TOTAL (52)	**51 (98.1%)**	**1 (1.9%)**	**1 – 8.**	**47 (90.4%)**	**5 (9.6%)**	**.06 – 8.**	**51 (98.1%)**	**1 (1.9%)**	**.03 - 1.**	**48 (92.3%)**	**4 (7.7%)**	**.03 – 4.**

*****MIC cut-off; in accordance with "CLSI M61:Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi"1st ed., 2017

*****MIC cut-off; in accordance with "CLSI M61:Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi"1st ed., 2017

Table 2. Antifungal Susceptibility Patterns of 52 Dermatophyte Isolates by Agar-based Disk Diffusion Me

Dermatophyte spp.	by DD on Mueller Hinton Agar												by DD on Dermasel Agar							
	Fluconazole [25µg]			Griseofulvin [10µg]			Itraconazole [10µg]			Terbinafine[2µg]			Fluconazole [25µg]			Griseofulvin [10µg]			Itraconaz le [10µg]	
	Sensitive	Resistant	IZD Range	Sensitive	Resistant	IZD Range	Sensitive	Resistant	IZD Range	Sensitive	Resistant	IZD Range	Range	Resistant	IZD Range	Sensitive	Resistant	IZD Range	Sensitive	Resistant
T. interdigitale (23)	22 (95.%	1 (4.3 %	0,22 0-2	22 (95.%	1 (4.3 %	0,22 5-2	22 (95.%	1 (4.3 %	0,22 7-3	21 (91.%	2 (8.7 %	0,22 0-3	22 (95.%	1 (4.3 %	0,22 0-2	22 (95.%	1 (4.3 %	0,22 5-2	22 (95.%	1 (4.3 %

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	7%)	)	7	7%)	)	9	7%)	)	0	3%)	)	5	7%)	)	4	7%)	)	8	7%)	)	0	3%)	)	4
T. rubrum (20)	18 (90%)	2 (10%)	15-23	19 (95%)	1 (5%)	17-27	20 (100%)	0	25-36	19 (95%)	1 (5%)	0,25-32	18 (90%)	2 (10%)	11-20	19 (95%)	1 (5%)	19-27	20 (100%)	0	25-29	18 (90%)	2 (10%)	0,22-32
T. verrucosum (01)	1 (100%)	0	20	1 (100%)	0	25	1 (100%)	0	36	1 (100%)	0	31	1 (100%)	0	19	0	1 (100%)	21	1 (100%)	0	25	1 (100%)	0	31
E. floccosum (05)	5 (100%)	0	19-23	5 (100%)	0	26-29	5 (100%)	0	22-34	5 (100%)	0	31-32	5 (100%)	0	20-23	5 (100%)	0	26-29	5 (100%)	0	22-30	5 (100%)	0	30-32
M. canis (01)	1 (100%)	0	21	1 (100%)	0	31	1 (100%)	0	35	1 (100%)	0	33	1 (100%)	0	23	1 (100%)	0	29	1 (100%)	0	28	1 (100%)	0	34
M. gypseum (02)	2 (100%)	0	20	2 (100%)	0	30-31	2 (100%)	0	33-34	2 (100%)	0	32-34	2 (100%)	0	19-20	2 (100%)	0	27-28	2 (100%)	0	28-30	2 (100%)	0	33
TOTAL (52)	**49 (94.2%)**	**3 (5.8%)**	0,15-27	**50 (96.2%)**	**2 (3.8%)**	0,17-31	**51 (98.1%)**	**1 (1.9%)**	0,22-36	**49 (94.2%)**	**3 (5.8%)**	0,20-35	**49 (94.2%)**	**3 (5.8%)**	0,11-24	**49 (94.2%)**	**3 (5.8%)**	0,19-21	**51 (98.1%)**	**1 (1.9%)**	0,22-30	**48 (92.3%)**	**4 (7.7%)**	0,20-34

Note: IZDs > 19 mm (FLU); >25mm (GRI); >21 mm (ITR); > 25 mm (TER) are taken as 'sensitive',with exceptions of Epidermophyton and Microsporum species: >26 mm (GRI) and >22 mm (ITR), are 'sensitive'.

Table 3. Comparison of Antifungal Susceptibility Testing Methods by Disk Diffusion on Mueller Hinton Against Microbroth Dilution Method (CLSI-M38) for Dermatophytes (n=52)

Drugs	**Antifungal Susceptibility Testing Methods**				**Sensitivity**	**Specificity**	**Cohen's kappa (k)**
	Microdilution format		**Disk Diffusion with Mueller-Hinton**				
	S	**R**	**S**	**R**			
Fluconazole	51 (98.1%)	1 (1.9%)	49 (94.2%)	3 (5.8%)	100%	96.08%	0.49 (Moderate agreement)

Griseofulvin	47 (90.4%)	5 (9.6%)	50 (96.2%)	2 (3.8%)	40%	100%	0.55 (Moderate agreement)
Itraconazole	51 (98.1%)	1 (1.9%)	51 (98.1%)	1 (1.9%)	100%	100%	1 (Perfect agreement)
Terbinafine	48 (92.7%)	4 (7.7%)	49 (94.2%)	3 (5.8%)	75%	100%	0.85 (Almost Perfect agreement)

Table 4. Comparison of Antifungal Susceptibility Testing Methods by Disk Diffusion on Dermasel Agar against Microbroth Dilution Method (CLSI-M38) for Dermatophytes (n=52)

Drugs	**Antifungal Susceptibility Testing Methods**				**Sensitivity**	**Specificity**	**Cohen's kappa (k)**
	Microdilution format		**Disk Diffusion with Dermasel**				
	S	**R**	**S**	**R**			
Fluconazole	51 (98.1%)	1 (1.9%)	49 (94.2%)	3 (5.8%)	100%	96.08%	0.49 (Moderate agreement)
Griseofulvin	47 (90.4%)	5 (9.6%)	49 (94.2%)	3 (5.8%)	60%	100%	0.73 (Substantial agreement)
Itraconazole	51 (98.1%)	1 (1.9%)	51 (98.1%)	1 (1.9%)	100%	100%	1 (Perfect agreement)
Terbinafine	48 (92.7%)	4 (7.7%)	48 (92.7%)	4 (7.7%)	100%	100%	1 (Perfect agreement)

IV. DISCUSSION

In our study, antifungal susceptibility testing (AST), using broth microdilution (CLSI-M38), for six different species, namely, Trichophyton mentagrophytes, T. rubrum, T. verrucosum, Epidermophyton floccosum, Microsporum canis, and M. gypseum, were done. The MIC ranges for fluconazole (Flu), griseofulvin (Gri), itraconazole (Itr), and terbinafine (Ter), were determined and compared results from disk diffusion on MHA (CLSI-M44) and on DA.

Overall, we observed good IZDs on Dermasel agar (DA)with same disk strengths for antifungals, as were used for disk diffusion (DD) on Mueller-Hinton. There is a lack of availability for in-depth researches employing disk diffusion on DAfor AST of dermatophytes. However, Singh J et

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al., (11) conducted an evaluation of DD on Dermasel for AST of dermatophytes and concluded with results which were as satisfactory as those done by BMD technique. The IZD ranges noted by his study and ours, were varied, which makes sense, considering the diversity of dermatophytes based on various factors, such as prior antifungal exposure, or even geographical distribution. Also, Aneke et al.2018, like us, employed 25 µg Flu disks, with DD on MHA, but all their strains, showed nil IZD, whereas, only 1 of our strain gave a 'zero' inhibition zone.

Another important factor which can affect zones of inhibition, is the inoculum preparation; many workers recommend the use of microconidia for inoculum preparation(8). However, we have used both, conidia, and hyphae, perhaps being the reason for getting moderate inhibition zone diameters.

Agarwal R. et al.,(2015) also employed DD withidentical disk strengths(i.e., Flu 25µg, Gri 10µg, Itr 10µg, Ter 2µg), and procured zone diameters varying from 10 to 32 mm, 21 to 49 mm, 17 to 36 mm, to 0 to 44 mm(8). These IZD ranges were similar to ours, except for griseofulvin and terbinafine, for which their maximum zones were larger. Our maximum diameters for these drugs, were measured as 32, and 35 mm, likewise. By Agarwal R et al, a 'nil' zone of inhibition was seen in 5 T.rubrum strains, against terbinafine. In the present study, against terbinafine, nil IZDs were seen in 1 T.mentagrophytes, and 1 T.rubrum strain. Perhaps these strains were intrinsically resistant to terbinafine. However, all their 5 strains were fully susceptible to other antifungal agents tested, suggesting that cross resistance to azoles & griseofulvin does not exist.

This activity possibly demonstrates that some T.rubrum strains may show primary resistance to terbinafine, as reported in this study as well others (8).T. rubrum has proven in many researches to have a tendency to develop resistance to azoles and allylamines, after prolonged exposure to sub-inhibitory concentrations of these drugs, consequently leading to treatment failures, as well as persistence and chronicity of dermatophytoses (9,10).It should also be noted, that the otherT.mentagrophytes strain was found to be resistant to all the other tested antifungals though (Figure 1).

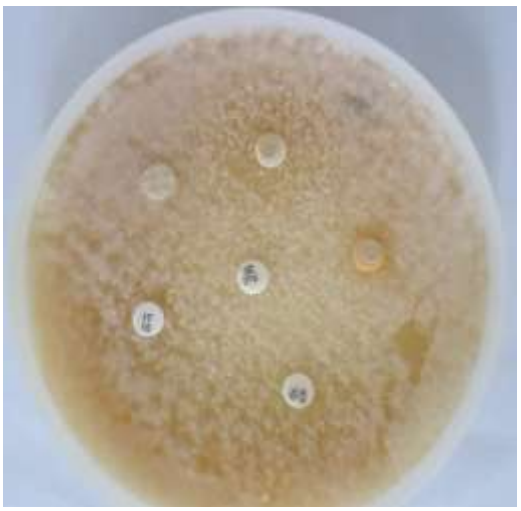

Figure 1. Disk diffusion on Dermasel Agar, showing a resistant strain of T. interdigitale.

This study suggested that disk diffusion is a reproducible method, which shows agreement with the reference method for micro-dilution AST. Dogra et al., also compared these two antifungal susceptibility testing methods, with all three genera of dermatophytes against many antifungal agents, including fluconazole, griseofulvin, itraconazole, and terbinafine (11). Comparable results were procured from disk diffusion method when equated with the micro-broth dilution methods in our study and in Dograet al.'s2019 study. So, the disk diffusion technique may be considered as an alternate option to the standard dilution method. Development of a standardized disk diffusion-based assay for determining the antifungal susceptibility of dermatophytes is desirable and provides a number of advantages.

In the present study, the in vitro susceptibility evaluation showed that the antifungal drugs tested showed good activity against the dermatophytes, except for fluconazole which showed slightly lower activities, with lower IZDs, as well as just moderate agreement when compared with the BMD method.

Standard disk diffusion assays may be adopted for assessing dermatophyte resistance against antifungal agents. Some studies, such as those conducted by Singh J, et al., and Khadka, propose DD to be a reliable, reproducible method, with good correlation to reference BMD for antifungal susceptibility testing (12,13).

Agreement surveys for MIC values of antifungals, by standard reference CLSI micro-dilution, versus results obtained with ABDD methods, may be of use, to find out whether agar-based diffusion could be an alternative for BMD, for use in clinical laboratory settings. High agreement levels have

been identified for yeasts and some filamentous fungi, but for dermatophytes, this needs yet to be determined with more precision (16).

In our study, disk diffusion on MHA, for fluconazole, against dermatophytes, was calculated to be 100% sensitive and 96.08% specific (taking BMD as standard). Furthermore, it was found to yield just moderate agreement with the microdilution results (k 0.49). For griseofulvin (DD on MH), sensitivity and specificity were 40% and 100%respectively (moderate agreement withBMD;Cohen's k= 0.55). For itraconazole, there was no difference in the results procured by both DD methods (100% sensitivity, 100% specificity; perfect agreement, k=1).For terbinafine, DDon MH results were in almost perfect agreement with BMD (75% sensitivity, 100% specificity; k = 0.85).DD on DA for fluconazole was 100% sensitive and 96.08% specific, with moderate agreement to BMD (k = 0.49). The DD results on DAfor griseofulvin were in substantial agreement with the BMD(sensitivity: 60%, specificity: 100%; k= 0.73). The results procured by both methods for itraconazole and for terbinafine, against the dermatophyte isolates, viz., BMD versus DD on DA, were identical (100% sensitivity and specificity; k = 1; perfect agreement).Only a very limited number of studies have been published on this issue, and a few results have shown that between these methods, the level of agreement may be drug dependent. Itoi et al., have observed low levels of agreement when using fluconazole and griseofulvin (17). Surprisingly enough, we too found only moderate agreement with these drugs, with the exception of griseofulvin disk diffusion on DA, for which there was a substantial level of agreement with CLSI BMD.

It is well known that MICs generated using agar-based techniques tend to be much higher than those produced by broth assays. This was seen in our fluconazole disk diffusion assays on MH and DA. Both their sensitivities (when compared against reference CLSI microdilution) were 100%, but specificities were 96%. These data advise caution in interpreting the MICs of fluconazole, which may either falsely indicate resistance by ABDD, or falsely imply susceptibility by broth microdilution. The better of these methods which may be more predictive to yield successful outcomes, needs best to be investigated further, by extensive evaluations on clinical efficacy of the drug and full susceptibility profiles of the dermatophyte strains responsible for the infection.

Singh et al., tested the reproducibility of DD on Dermasel agar and broth dilution methods, and produced findings, which, to some extent, may be considered similar to ours, in terms of correlation between MICs and IZDs for the four antifungal drugs, using identical drug densities (12). Their results showed fluconazole to be less active, as was the case with our strains as well. Both our broth dilutions yielded high mean MIC values for fluconazole. However, in Singh's study their MIC mean was very inflated, at 24.30 µg/ml, as opposed to ours of 2.29 µg/ml. Our mean IZD was calculated as 17.5 mm, which is low, but of course their IZD mean was even lower, at 3.37mm.

Singh and his colleagues yielded in vitro inhibitory activities of griseofulvin and itraconazole which did not correlate in BMD and DD assays (12). Their MICs for griseofulvin were high as would be expected (0.85 µg/ml), but their mean IZD (44.9 mm) was unexpectedly larger, despite the high MIC. Comparatively speaking, our MIC and IZDs were better matched, yielding a mean MIC of 0.59, with IZD of 24.5mm.

Another finding in Singh et al.'s data was the observation of small inhibition zone diameters, with itraconazole (mean IZD, 21.7 mm), which was surprisingly smaller than that with griseofulvin (44.9 mm). Their DD assay results showed itraconazole to be relatively less active, but with the present study, itraconazole gave large IZDs, with a mean of 29 mm, larger than our IZDs for griseofulvin (24.5 mm). Moreover, as would be probable with itraconazole in vitro, we had a rightfully low mean MIC of 0.14 µg/ml. Singh and co-workers also gave a somewhat low MIC of 0.52 µg/ml for itraconazole, but for some reason their IZD did not quite match; their strains paradoxically gave IZDs which were smaller than their diameters for griseofulvin.

Like us, Singh et al., observed very good inhibition zone diameters for terbinafine, on DA agar, which correlated well with MIC values obtained from microdilution assays (12). Both our studies gave low mean MIC values, viz., 0.007 µg/ml, by Singh et al., and 0.11 µg/ml, by us. Accordingly, both observed large IZDs, although our studies gave a mean diameter of 27 mm, which in relation to Singh and colleagues (72.8 mm), was astonishingly lesser. Nevertheless, in terms of correlation between the 2 methods, we were able to show very good agreement not only for terbinafine, but also for itraconazole.

Even though our data is in general agreement with reports in other studies, who have employed these two methods, there is variability in MIC values and IZDs. These differences may be because we followed the endpoint criterion in accordance with MIC reading in CLSI M38-A, viz., growth inhibition of 50% for fluconazole and

complete growth inhibition for other antifungals. Irrespective of data variations, it is obvious that the gold standard drug, terbinafine, for dermatophyte infections, produced the highest in vitro activities against the tested dermatophytes.In a nutshell, we have shown terbinafine to be the most potent antifungal drug against the dermatophytes tested in our study, as shown using both BMD, and disk diffusion assays. Also, itraconazole demonstrated similar activity, with low MICs and large inhibition zone diameters. However, griseofulvin also produced well defined IZDs on DA. Compared to the other drugs, fluconazole showed less in vitro activity in both techniques. The activities of fluconazole in disk diffusion assay were minimal as they could not produce a well-defined inhibition zone with 20 mg/disk and 25 mg/disk, respectively.

V. CONCLUSION

The in vitro antifungal susceptibilities of dermatophytes should be evaluated by different types of in vitro assays. We have found significant agreement between microdilution and disk diffusion methods, notably for itraconazole and terbinafine. But there is a need for more researches from diverse study groups and populations, so there may eventually be enough correlation data to standardize the disk diffusion method for dermatophytes.

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Data Availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Statement: The study was cleared through Institutional Ethical Committee (D.No.239/FM).

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