Antifungal Drug Susceptibility Testing of Dermatophytes: Laboratory Findings to Clinical Implications

Dermatophytes are the most commonly encountered fungi in humans and other vertebrates that spread through direct contact with infected humans, animals, and soil.[1] Infections due to these agents are usually restricted to the stratum corneum and are generally referred as 'tinea' or *'ringworm'* (tinea capitis; tinea barbae; tinea corporis; tinea cruris; tinea manuum; tinea pedis and tinea unguium).[2,3] Dermatophytes belong to 3 closely related genera- *Trichophyton, Microsporum* and *Epidermophyton*.[4] Worldwide, several studies have documented a varied prevalence rate of dermatophytosis ranging from 14-26.8% in North America, East Asia and Europe, and 5-31.6% in Africa (Ethiopia, Kenya, Nigeria, and Tanzania).[5-7] The regional variations are mainly due to differences in the lifestyle, socioeconomic conditions, underlying risk factors, and environmental factors of different geographic areas.[1] Epidemics of dermatophytosis have also been reported in the area of overcrowding and poor hygienic conditions.[4,8-10] In 2005, World Health Organization (WHO) reported a prevalence of up to 19.7% for tinea capitis in the general population of developing countries.[11] High prevalence rates of tinea pedis and onychomycosis have been recognized in certain occupational groups like a marathon runner (22-31%), miners (21-72.9%), and soldiers (16.4-58%).[12,13] *Trichophyton* species are the major causative agents responsible for dermatophytosis with a prevalence rate of 70-90% for onychomycosis and 53-86% for rest of the tinea infections.[14,15] Of these, *Trichophyton rubrum* is the key etiological agent followed by *T. mentagrophytes* complex*, Microsporum canis,* and *M. gypseum*.[16-18] In India, we are presently noticing a significant rise in number of dermatophytosis cases with chronic recalcitrant disease, atypical presentations, frequent relapses, and treatment failures.[19-22] Though the reason for this phenomenon is not yet clear, it is assumed that unchecked availability of cheap and irrational fixed-dose corticosteroid–antifungal–antibacterial combinations sold over the counter in India and *in-vitro* resistance to common antifungals (to some extent) is playing a pivotal role. Due to recent increase in the reports of antifungal drug resistance in dermatophytes, many groups have suggested to perform the antifungal drug susceptibility testing especially for the dermatophytes isolated from chronic/recurrent/recalcitrant cases or those with atypical presentations. Clinical successful treatment does not always correlate with the MIC (minimum inhibitory concentration) value of antifungals (*in-vitro*) [Table 1]. The discordance between the *in-vivo* and *in-vitro* resistance in fungi has been illustrated by the "90–60 rule," which states that infections due to susceptible strains respond to appropriate therapy in 90% of cases, whereas infections due to resistant strains respond in approximately 60% of patients.[23] The clinical breakpoints (CBP) for different antifungals against dermatophytes, has not been defined due to lack of clinical correlation and pharmacokinetic/pharmacodynamic (PK/PD) studies [Table 1]. This manuscript provides a comprehensive update on the antifungal drugs susceptibility testing and its application in treating dermatophytosis.

Resistance in Dermatophytes

There is not much data available regarding *in-vitro* drug resistance to dermatophytes, but recently many reports suggest that resistance is on rise.[24,25] Though, few reports suggest a good correlation between *in-vitro* resistance and treatment failure, there is no conclusive evidence to implicate *in-vitro* resistance with therapeutic failure in dermatophyte infections.[26] But relapse/recalcitrant infection after completion of recommended therapy in different presentations of dermatophyte infections is now well known. Resistance/recurrence after griseofulvin therapy in patients with *T. rubrum* and *T. tonsurans* is known since 1960's.[27,28] A study from North India also showed that there were non-responders to griseofulvin therapy among the tinea capitis patients.[17] With the emergence of treatment failure with griseofulvin, allylamines became the preferred choice of treatment.[29] Mukherjee *et al.*, in 2003 first reported *T. rubrum* strain exhibiting primary resistance to terbinafine and later Osborne *et al.*, showed single missense amino acid substitution at L393F and F398L leading to terbinafine resistance.[30-32] This missense substitution in *T. rubrum* also contributes to cross-resistance to the other antifungals in this class (allylamines). Recently, Yamada *et al.*, showed the presence of amino acid substitution at one of the four positions (Leu 393, Phe 397, Phe 415, His 440) of the squalene epoxidase protein in 17 isolates with a higher MIC to terbinafine.[33] Rudramurthy *et al.*, in 2018 from India, reported high terbinafine resistance in 17% of *T. interdigitale* and 14.3% of *T. rubrum* isolates, with few strains exhibiting F397L mutation.[25] Another recent study from India also reported L393F and F397L mutations with higher terbinafine MIC's in isolates.[24]

It has been reported that repeated exposure to azole antifungals may be responsible for the development of azole resistance in dermatophytes.[34] *T. rubrum* can develop resistance to azoles, amorolfine and terbinafine after prolonged exposure to sub-inhibitory concentrations of these drugs leading to treatment failures and consequently contributing to persistence and chronicity

Table 1: Common Definitions used in Antifungal Drug Susceptibility Methods[59]

Terms	Definition
Minimum Inhibitory Concentration (MIC)	The MIC is defined as the lowest concentration of the drug which clearly inhibits the growth of the micro-organisms
	After the MIC values of large number of isolates are read, further interpretation of the MIC's at 50% (MIC_{50}) and 90% (MIC_{90}) of the total isolates are carried out
	Basically, the MIC_{50} or MIC_{90} values are used to understand the epidemiological pattern of the susceptibilities of any given species that may guide to choose the most effective drug for management
Minimum Fungicidal Concentrations (MFC)	MFCs were defined as the lowest drug dilution that yields <3 colonies (approximately 99 to 99.5% killing activity) on culture after exposing the fungus to given antifungal agent during antifungal susceptibility testing
Epidemiological Cutoff Value (ECV)	The definition of an epidemiological cutoff value is the MIC or MEC that separates a given population of isolates into those with and without acquired/mutational resistance based on their phenotypic MIC value
Clinical Breakpoint (CBP)	CBP is a chosen concentration of antifungal that defines whether the fungus is resistant or susceptible to that antifungal. This can be used as a predictor of the clinical success of a particular antifungal-fungus combination. In creating a breakpoint, the MIC distribution, pharmacokinetic/pharmacodynamic (PK/PD) data of the antifungal are important, but perhaps most critical is the addition of outcome data, especially from a clinical trial

of the infections.[35,36] Azole resistance in dermatophytes has been reported as high as 19% worldwide.[37] Azambuja *et al.*, found high MIC values for fluconazole and itraconazole (66.7% and 25% respectively) in 100 isolates of *T. rubrum* obtained from the patients with onychomycosis.[38]

Off late, there is an alarming trend of recalcitrant dermatophyte infection[22] in India, which could be related to inadequate treatment regimen or discontinuation of medication, difficulties in eliminating predisposing factors and sources of re-infection. However, very few reports have addressed the issue of resistant mechanisms operating in dermatophytes.[39] *In vitro* antifungal drugs susceptibility testing of dermatophytes may help significantly in the management of the patients, especially cases presenting with therapy failure,[40] when the disease has failed to respond to an empiric regimen and whenever prolong therapy is required.[23] Antifungal susceptibility testing also helps to understand the epidemiological pattern of drug resistance in any given region, and thus may help to choose more efficacious antifungal agents for standard treatment. Antifungal susceptibility data may also help in future to determine the clinical breakpoints or epidemiological cutoff (ECV)/ECOFF values that may assist in effective management. At present, the correlation between clinical outcome; and *in-vitro* dermatophytes' drug susceptibility patterns and MICs is not clearly understood.[41] Moreover, all species of dermatophytes may not have the same pattern of drug susceptibility.

Antifungal Susceptibility Testing (AFST) Methods

Antifungal drug susceptibility testing is not routinely performed with dermatophytes due technical difficulties, expertise required and suboptimal reproducibility. However, the recent epidemic like scenario particularly in Indian subcontinent and emerging isolated case reports of recalcitrant tinea from Europe have greatly enhanced interest among microbiologists and dermatologists.

Different techniques have been evaluated to test the antifungal susceptibility of dermatophytes such as agar disc diffusion, agar dilution and macro- and micro broth dilution methods.[40] The standard guidelines by Clinical Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST) and British Society of Antimicrobial Chemotherapy (BSAC) are available for testing antifungal susceptibility of yeasts and molds [Table 2]. In 2008, CLSI included a method for antifungal susceptibility testing for dermatophytes[42] in their document on AFST of molds. Many modifications of this standard guideline has been proposed to improve the results.[43,44] Standardization of antifungal susceptibility testing for dermatophytes is generally difficult as there are many variable critical parameters that needs to be considered while performing the test such as inoculum size (*i.e.,* number of conidia/spores), incubation temperature and duration, media to be used, and time and percentage of growth inhibition for end point detection.[45,46]

Standardization of media

Norris *et al.*, evaluated four different culture media with 18 clinical dermatophyte isolates of 3 different species (*T. mentagrophytes*, *T. rubrum*, and *T. tonsurans*).[45] RPMI 1640 (chemically defined media) and Sabouraud's dextrose agar (chemically non-defined media) supported the growth of all the isolates but antibiotic medium #3 (Penassay; Difco Laboratories, Michigan) (chemically non-defined media), yeast nitrogen base (chemically defined media) with 0.5% dextrose showed a consistent growth of the isolates tested in the experiment. This study recommended RPMI 1640 as a superior media as its chemical composition is defined. Thus this study formed the basis for the establishment of future development of antifungal susceptibility testing[45] Though Sabouraud's dextrose broth and RPMI 1640 shows a similar type of efficiency for the growth of different species of dermatophytes, very few studies have been done to evaluate antifungal susceptibility testing against dermatophytes. McVeigh and Morton (MVM) is also a

Table 2: Antifungal susceptibility testing techniques

Standard guideline for Antifungal Drug Susceptibility Testing for fungi

  CLSI (Clinical Laboratory Standards Institute)- USA

  EUCAST (European Committee on Antimicrobial Susceptibility Testing)- Europe

  BSAC (British Society for Antimicrobial Chemotherapy)- London

Role of Antifungal Drug Susceptibility Tests

  Provide a reliable measure of the relative activities of antifungal agents.

  Possibly correlates with *in vivo* activity and predict the likely outcome of therapy.

  Reliable technique to monitor the development of resistance among a normally susceptible population of fungi.

  Predict the therapeutic potential of newly discovered investigational antifungal agents.

  Helps to determine the clinical breakpoint or epidemiological cutoff value

Methods of Antifungal Drug Susceptibility Tests

  Macro and Micro-broth dilution

  Agar dilution

  Disc diffusion method

Technique	Advantages	Disadvantages
Macro and Micro-broth dilution	Accurate and reproducible results	Technically laborious
	Gold standard technique according to standard guideline, but till now only CLSI technique has been more evaluated against dermatophytes	Costly
Agar dilution method	Easy to perform	Not recommended to test dermatophytes using standard guideline
	Relatively cheaper	
Disc diffusion method	Easy to perform	Not recommended to test dermatophytes using standard guideline
	Relatively cheaper	

chemically defined medium but the interpretation of the results becomes difficult due to the non-transparency of media.[47] Thus various studies concluded that RPMI 1640 should be used as a standard media for a determination of antifungal susceptibility testing of dermatophytes.[44,46,47]

Standardization of Inoculums Size, Incubation Time and Temperature

The initial inoculum size required to start the susceptibility testing is a critical factor while determining the minimum inhibitory concentrations (MIC). Any variation in the inoculum size leads to variable result during interpretation. Thus, an intra and interlaboratory comparison becomes important to validate the inoculums size.[44] Apart from standardization of culture media, Norris and co-researchers also tested 3 different inoculums size: 10^3, 10^4 and 10^5 conidia/ml. Four antifungal drugs such as griseofulvin, itraconazole, terbinafine and fluconazole against 18 dermatophytes isolates tested revealed 10^3 conidia/ml as an optimum inoculum for antifungal susceptibility testing. There was no difference in the MICs of itraconazole and terbinafine with higher inoculums size but higher MICs were observed for fluconazole and griseofulvin.[45] Effect of temperature and incubation time for antifungal susceptibility testing varies according to studies. Norris *et al.,* checked two different temperature conditions *i.e.* 30°C and 35°C in which no significant difference in their growth was noticed.[45] The studies reported that micro broth dilution method requires incubation at 35°C

and should be read at the end of 72-96 hour.[48-50] Though the incubation period of 3-4 days is generally accepted to read the results, the time duration may exceed for a slow growing dermatophyte. Hence, final reading should be read on the basis of presence of growth in the control well.[51] A multi-center study tested 60 dermatophyte isolates against 3 antifungals (clotrimazole, itraconazole and terbinafine) with different incubation time (3, 7, 14 days) and temperature (28°C and 37°C). Significantly better and reproducible results were obtained after 7 days at 28°C.[52] In contrast, Perea *et al.*, determined that sufficient growth of dermatophytes while performing antifungal susceptibility testing not only depends on the incubation time but also on nature of the solvent used to dissolve the drugs. The shorter time duration (48 to 72 h) was required when water was used as a solvent whereas the incubation time increased to 10-14 days' when polyethylene glycol was used as solvent.[53]

Reliability of In-vitro Antifungal Susceptibility Testing

In 2008, antifungal susceptibility testing protocol for dermatophytes was approved for the first time by CLSI, which was further modified in 2010.[44] Reproducibility of the endpoint should be considered as a principal factor for the detection of any resistance. The experts recommend multicenter studies to develop, and validate accurate optimal conditions for performing antifungal susceptibility testing of dermatophytes.[44,51] Ghannoum and co-researchers

in 2004 conducted a multicenter study including six laboratories to evaluate the reproducibility of antifungal susceptibility testing results. In this study, the activities of seven antifungal agents (ciclopirox- olamine, fluconazole, griseofulvin, itraconazole, posaconazole, terbinafine and voriconazole) were examined against 5 different species of dermatophytes (*T. rubrum, T. mentagrophytes, T. tonsurans, E. floccosum* and *M. canis*). MIC for all the isolates was determined by microbroth dilution method according to CLSI (previously known as NCCLS) M38-A standard. The MIC for all the isolates were determined using the endpoint of 50% and 80% inhibition of growth compared to control. The MIC data generated at different laboratories for all the dermatophytes isolates were analyzed. MIC values read at 50% inhibition compared with control growths showed an agreement of 92-100% whereas for 80% growth inhibition the agreement was 88 to 99%. Thus, the study concluded that CLSI M38-A standard guidelines for testing antifungal susceptibility of dermatophytes gave reproducible results.[54] Another collaborative study was conducted to define the specific inoculum sizes, incubation temperatures and other procedural end points for performing antifungal susceptibility testing by broth microdilution test against dermatophytes for clotrimazole, itraconazole, and terbinafine. A total of 60 isolates of six different species of dermatophytes including *T. mentagrophytes*, *T. rubrum*, *T. tonsurans*, *M. gypseum*, *M. canis* and *E. floccosum* were evaluated. The study concluded that the optimal condition for *in- vitro* antifungal susceptibility of dermatophytes requires incubation at 28°C for 7 days with 10^4 CFU/ml inoculum density. The MIC of all the drugs should be determined by 100% growth inhibition.[52] Other than terbinafine, clotrimazole, and itraconazole, newly introduced antifungal drugs including sertaconazole, luliconazole and lanoconazole, amorolfine, bifonazole, and miconazole have also been evaluated by *in- vitro* antifungal testing against dermatophytes.[55-57] ME1111 is a newer antifungal agent mainly used as a topical agent for the treatment of onychomycosis. Ghannoum and co-workers evaluated and standardized the activity of the ME 1111 antifungal agent by performing CLSI M38- A2 methodology against three isolates (*T. mentagrophytes*, *T. rubrum*, and *E. floccosum*) along with ATCC strain of *T. rubrum* and *T. mentagrophytes* as quality control. Evaluation of their results showed the interlaboratory agreement of more than 90% for the MIC's read with 80% inhibition as end point and it reduced to 76.2% when 100% inhibition was taken as criteria to read endpoint. At least on the basis of the above-mentioned studies it is clear that broth microdilution test of CLSI as per the M38-A2 protocol is the standard guideline for performing antifungal susceptibility testing against dermatophytes [Table 3]. Although methods are available for the performance of susceptibility testing, clinical interpretation of the MIC values or the breakpoints to consider whether the agent tested is susceptible or resistant clinically is yet to

be defined clearly.[58] In the clinical setting, for better management of patients, clinical breakpoint (CBP) play an important role.[59] CBP depends upon several factors like the MIC distribution, pharmacokinetic/pharmacodynamic (PK/PD) data of the antifungals, and the most importantly, the outcome of disease.[59] Due to paucity of data on clinical outcome with the antifungal susceptibility data, it is hard to decide the CBP for a particular species. Therefore, in such situations, epidemiological cutoff value (ECV) may be determined for any given species and antifungal agent. This is the MIC value that is provisionally used to differentiate the wild-type isolates (generally considered as susceptible) from non-wild-types (generally considered as resistant isolates). This provisional value may help to choose appropriate antifungals while treating the infection. However, it is pertinent to mention that ECV's are not determinant of successful treatment, it only predicts and separate the population into two categories- wildtype or non- wildtype.[59]

Alternative Susceptibility Testing Methods

Disk diffusion method

Macro broth dilution and micro broth dilution methods are generally laborious and need expertise to perform in laboratories compared to the antimicrobial susceptibility testing by disc diffusion and 'E' test method. According to the standard guideline of CLSI, disc diffusion test and E-test are not recommended for dermatophytes antifungal susceptibility testing.[44] But, studies are available comparing disc diffusion with broth microdilution methods.[60,61] Niewerth *et al*., in 1998, compared two methods of antifungal susceptibility testing to test four different species against five topical antifungal agents (griseofulvin, itraconazole, sertaconazole, terbinafine, and ciclopirox olamine) and found discrepancy in the result obtained from these two methods. The agar dilution method yielded higher MIC value than the broth dilution method.[62] In contrast, Macura *et al*., reported that disk diffusion technique for antifungals susceptibility for dermatophytes was much simpler and easy to perform in routine clinical settings and provided as consistent results as broth dilution method.[63] Karaca *et al*., compared these two antifungal susceptibility testing technique using four species of dermatophytes against a large number of antifungal agents (itraconazole, fluconazole, ketoconazole, miconazole, sulconazole, oxiconazole, bifonazole, griseofulvin, ciclopirox olamine and terbinafine). Similar results were obtained from disk diffusion method when compared with the micro- broth dilution methods. So disk diffusion method may be considered as an alternative to gold standard dilution method.[64] E- Test methods are mainly based on agar diffusion method and are used to determine the MIC of fastidious, slow growing or nutritionally deficient microorganisms. Castro Mendez and co-researchers compared the two agar based methods;

Table 3: Review of antifungal susceptibility testing techniques (CLSI) for dermatophytes

Reference	Technique	Total no. of antifungals	Antifungal agents	Dermatophytes tested	Total no isolates	Inoculum size	Incubation temperature	Duration of incubation	End point criteria	Result/Remarks
Rudramurthy et al., 2018[25]	Micro broth dilution#	12	F, K, Cl, Ci, L, N, V, A, I, T, S, G	Ti, Tr, Tt	127	$1\text{-}3 \times 10^3$ CFU/ml	28°C	4-5 days	80% inhibition compared to growth control	20 isolates showed higher MIC to T; 45 isolates showed higher MIC to F
Baghi et al., 2016[48]	Micro broth dilution#	12	F, I, T, G, L, La, To, Ec, M, Cas, Ani, Bu	Ti, Tr, Tt, Ef, Mc	100	$0.5\text{-}3 \times 10^3$ CFU/ml	35°C	96 h&	Read visually to determine MICs and MECs value by comparison with growth control	MIC values of all dermatophyte isolates showed susceptibility to antifungal agents, except for fluconazole
Ansari et al., 2016[66]	Micro broth dilution#	4	F, I, T, G	Ti, Tr, Tt, Ef, Mc, Ab	316	$1\text{-}3 \times 10^3$ CFU/ml	35°C	48-72 h&	Read visually to determine MICs and MECs value by comparison with growth control	T had the highest in vitro activity against all strains except Ab
Adimi et al., 2013[67]	Micro broth dilution#	10	F, I, T, G, K, Cl, V, A, N, Ci	Tr, Tt, Ts, Te, Ter, Tm, Tv, Tve, Ef, Ab, Mc, Mg, Mf, Mfe, Mr	370	$1\text{-}3 \times 10^3$ CFU/ml	28°C	7 days	For F and G 50% inhibition; other antifungals 100% inhibition	I and T showed lowest and F showed highest MIC value
Silva et al., 2014[68]	Micro broth dilution#	6	F, I, T, G, K, V	Ti, Tr, Tt, Ts, Tve, Mc, Mg, Ef	70	$2\text{-}4 \times 10^4$ CFU/ml	28°C	4-7 days	For azoles 50%, G 80% and T 100% inhibition	T is most potent followed by V, I; F and G least active drug
Zalacain et al., 2011[69]	Micro broth dilution#	5	F, I, T, Ci, Eb	Tr, Tm, Ef, Mc	70	$0.5 \times 10^5\text{-}5 \times 10^5$ spores/ml	30°C	3-5 days	50% inhibition	The activity of both T and Eb significantly higher than other drugs
Barros et al., 2007[70]	Micro broth dilution#	4	F, I, T, G	Tr, Tm	100	$0.5 \times 10^6\text{-}5 \times 10^6$	28°C	7 days	F, I, G 80% inhibition and T- 100% inhibition	Activities of T and I higher than F and G
Chadeganipour et al., 2004[71]	Micro broth dilution#	1	G	Tm, Tve, Ef, Mc	50	0.5 McFarland	35°C	14 days	100% inhibition compared to growth control	12% - higher MIC values for G
Eba et al., 2016[72]	Disk diffusion method@	5	I, G, K, M, Amp	Tr, Tt, Tm	58	0.5 McFarland	27°C	24-48 h	Zones of inhibition measured	Isolates were most sensitive to M, Amp, K and least sensitive to G and I
Nweze et al., 2010[73]	Micro broth dilution# and Disk diffusion$	8	F, I, T, G, K, M, V, Ci	Tr, Tt, Tm, Ef, Mc	47	1.0×10^6 conidia/ml	30°C	4-7 days	Zones of inhibition measured	Disk diffusion results similar to microbroth dilution methods
Aktas et al., 2014[40]	E-test%	5	F, I, K, Cas, Amp	Tr, Tt, Tm, Tv, Ef, Mc	66	$10^5\text{-}10^6$ CFU/mL-1	28°C	72-69 hour	Border of the elliptical inhibition zone intercepted the MIC scale on the E-test strip	Most active agent were Cas, I and F least active

Contd...

Table 3: Contd...

Reference	Technique	Total no. of antifungals	Antifungal agents	Dermatophytes tested	Total no isolates	Inoculum size	Incubation temperature	Duration of incubation	End point criteria	Result/Remarks
Moti *et al.*, 2009[60]	Micro broth dilution# and disk diffusion%	5	F, I, T, G, K	Tr, Tm, Mc	60	$0.4\text{-}5 \times 10^4$ cells/ml.	28°C	72-120 h	Terbinafine 100% inhibition rest all 80%; reading made at every 24 hour until growth in growth control	100% agreement for Tm isolates evaluated with K and G
Singh *et al.*, 2007[61]	Micro broth dilution# and disk diffusion!	6	I, T, G, Po, Ra, Ci	Tr, Tt, Tm, Tv, Ef, Mc	63	$0.5 \times 10^4\text{-}4 \times 10^4$	30°C	4 days	For fluconazole 50% inhibition and rest all 100% 1, measure zone of diameter 2	MICs obtained by the microdilution method did not correlate disk diffusion assays
Esteban *et al.*, 2005[74]	Micro broth dilution# and disk diffusion@	3	I, T, Cl	Tr, Tt, Tm, Ef, Mc, Mg	59	$1 \times 10^3\text{-}10^4$	28°C	3-7 days	Clotrimazole 50% inhibition and rest two 100% inhibition 1, measure zone of diameter 2	Both methods detect T as a most potent antifungal
Méndez *et al.*, 2008[65]	Micro broth dilution#, disk diffusion% and E-Test%	3	F, I, V	Tr, Tm, Mg	46	$0.5 \times 10^4\text{-}0.5 \times 10^5$	35°C	42-72 h 1 and 48 h 2	100% inhibition 1 and measure zone of diameter 2	Agreement between E-test and microbroth was 45.6% for F, 19.5% for I and 52.1% for V but low correlation with disk diffusion

Fluconazole- F, Ketoconazole- K, Clotrimazole- Cl, Ciclopirox olamine- Ci, Luliconazole- L, Naftifine- N, Voriconazole-V, Amorolfine-A, Itraconazole-I, Terbinafine-T, Sertaconazole-S, Griseofulvin- G, Lanoconazole-La, Tolnaftate- To, Econazole- Ec, Miconazole- M, Caspofungin-Cas, Anidulafungin-Ani, Butenafine-Bu, Eberconazole- Eb, Amphotericin B- Amp, Posaconazole-Po, Ravuconazole- Ra, *T. interdigitale*-Ti, *T. rubrum*- Tr, *T. tonsurans*- Tt, *T. schoenleinii*- Ts, *T. erinacei*- Te, *T. eriotrephon*- Ter, *T. mentagrophytes*- Tm, *T. violaceum* - Tv, *T. verrucosum*- Tve, *E. floccosum*- Ef, *M. canis*- Mc, *M. gypseum*- Mg, *M. fulvum*- Mf, *M. ferrugineum*- Mfe, *M. racemosum*- Mr, *A. benhamiae* - Ab, CFU- colony forming units, &- plates with insufficient growth were incubated for 120 h, # RPMI-1640 medium, @- Sabouraud's dextrose agar, %- RPMI-1640 agar medium, $- Mueller Hinton agar, !- Dermasel agar, 1- Micro broth dilution, 2- Disk diffusion method and E test

E test and disk diffusion method and CLSI broth dilution method (CLSI M38-A) against three antifungal drugs (fluconazole, itraconazole, voriconazole). The results obtained with this disk diffusion method had low correlation with the results obtained from CLSI broth microdilution method for azoles. E- test and broth dilution methods showed agreement of 45.6% for fluconazole, 19.5% for itraconazole and 52.1% for voriconazole.[65] Although, an agar-based method is much simpler and easier to perform than broth dilution method, further research is essential before incorporating this technique in routine laboratory practice to test dermatophytes susceptibility[44,51] [Table 3].

In conclusion, various techniques are available for antifungal susceptibility testing of dermatophytes but only broth microdilution technique is currently accepted to determine *in-vitro* susceptibility of dermatophytes. As this technique is laborious and need expertise, only few mycology laboratories can perform this test. In the present scenario of increasing resistance to the dermatophytes, there is a need to perform antifungal drug susceptibility tests at least in cases with chronic/recurrent dermatophytosis or treatment failure/relapse. As there is no CBP defined as of yet, there is urgent need to establish ECV for dermatophytes and this value may guide the clinician while managing recalcitrant/resistant dermatophytosis.

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