




MIC and Upper Limit of Wild-Type Distribution for 13 Antifungal Agents against a *Trichophyton mentagrophytes-Trichophyton interdigitale* Complex of Indian Origin

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ABSTRACT Dermatophytosis due to the *Trichophyton mentagrophytes-Trichophyton interdigitale* complex is being increasingly reported across India. Reports of therapeutic failure have surfaced recently, but there are no clinical break points (CBP) or epidemiological cutoffs (ECVs) available to guide the treatment of dermatophytosis. In this study, a total of 498 isolates of the *T. mentagrophytes-interdigitale* complex were collected from six medical centers over a period of five years (2014 to 2018). Antifungal susceptibility testing of the isolates was carried out for itraconazole, fluconazole, ketoconazole, voriconazole, luliconazole, sertaconazole, miconazole, clotrimazole, terbinafine, amorolfine, naftifine, ciclopirox olamine, and griseofulvin. The MICs (in mg/liter) comprising >95% of the modeled populations were as follows: 0.06 for miconazole, luliconazole, and amorolfine; 0.25 for voriconazole; 0.5 for itraconazole, ketoconazole, and ciclopirox olamine; 1 for clotrimazole and sertaconazole; 8 for terbinafine; 16 for naftifine; 32 for fluconazole; and 64 for griseofulvin. A high percentage of isolates above the upper limit of the wild-type MIC (UL-WT) were observed for miconazole (29%), luliconazole (13.9%), terbinafine (11.4%), naftifine (5.2%), and voriconazole (4.8%), while they were low for itraconazole (0.2%). Since the MICs of itraconazole were low against the *T. mentagrophytes-interdigitale* complex, this could be considered the choice of first-line treatment. The F397L mutation in the squalene epoxidase (SE) gene was observed in 77.1% of isolates with a terbinafine MIC of ≥ 1 mg/liter, but no mutation was detected in isolates with a terbinafine MIC of < 1 mg/liter. In the absence of CBPs, evaluation of the UL-WT may be beneficial for managing dermatophytosis and monitoring the emergence of isolates with reduced susceptibility.

KEYWORDS MIC, UL-WT, antifungal resistance, dermatophytes, *Trichophyton mentagrophyte*

An indisputable rise in the prevalence of dermatophytosis with an alarming rise in chronic, recalcitrant, relapse, and recurrent cases has been seen in India over the past few years (1–3). The main etiological agents implicated in these infections are members of three different genera, namely *Trichophyton*, *Microsporum*, and *Epidermophyton*, with various species within each genus (4). In contrast to the past, wherein *T.*

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rubrum was the most predominant among the *Trichophyton* species, a notable rise in the incidence of *T. mentagrophytes-interdigitale* complex-associated dermatophytosis has been observed in India in recent times (1, 5). Apart from the changing pathogen epidemiology, several atypical clinical presentations are being reported, perhaps due to the rampant use of corticosteroids and poor compliance to therapy, which results in significant disease-related morbidity in affected patients (4, 6).

In the current clinical scenario, experience-driven consensus guidelines for diagnosing and managing dermatophytosis are needed. Recently, the Expert Consensus on the management of Dermatophytosis (ECTODERM) was published in India to bridge the large void in research related to disease management (3). The current treatment recommendations include the use of terbinafine, itraconazole, sertaconazole, ciclopirox olamine, ketoconazole, naftifine, and newer drugs such as amorolfine and luliconazole, which have shown potent fungicidal activity against *Trichophyton* species (7). However, recent reports of resistance to terbinafine resulting in treatment failure in dermatophytosis caused by species belonging to the *T. mentagrophytes-interdigitale* complex is of great concern to the treating physicians (1, 2, 8). Apart from allylamine resistance, triazole resistance, especially to fluconazole, is high (35.4%) in the Indian isolates, which is also associated with treatment failure (1).

To guide the treatment choice of an appropriate antifungal agent, the Clinical and Laboratory Standards Institute (CLSI) has introduced guidelines for the antifungal susceptibility testing (AFST) for dermatophytes (9, 10). Clinical breakpoints (CBPs), which are based on information derived from clinical studies, help in predicting response to treatment. However, they depend on several factors such as drug MIC, pharmacokinetic (PK)/pharmacodynamic (PD) parameters, treatment outcome in clinical studies, and post-marketing susceptibility data. In view of the lack of CBPs, either the epidemiological cutoff values (ECVs or ECOFFs) or the upper limit of wild-type MIC (UL-WT) may be useful to differentiate between wild type (WT) and non-wild-type (NWT) isolates, as these depend only on the MIC (11). Unfortunately, to date there is no CBP, ECV, or UL-WT established for any of the *Trichophyton* species. Available guidelines for the determination of ECV focus on *Candida*, *Cryptococcus*, and *Aspergillus* species (12). In view of the veritable country-wide epidemic of dermatophytosis predominantly by a single species, the *T. mentagrophytes-interdigitale* complex, and the lack of CBPs, there is an urgent need to define either ECVs or the UL-WT to guide therapy.

In this study, a large collection of *T. mentagrophytes-interdigitale* complex isolates collected from six medical centers in India was tested against 13 antifungal drugs to elucidate the MIC distribution and to define the India-specific UL-WT for each antifungal agent.

RESULTS

The antifungal susceptibility profiles and UL-WT of 13 antifungal drugs against *T. mentagrophytes-interdigitale* complex are depicted in Table 1. The antifungal susceptibility testing (AFST) of all 498 isolates was performed against nine antifungals (fluconazole, itraconazole, voriconazole, clotrimazole, sertaconazole, terbinafine, griseofulvin, amorolfine, and ciclopirox olamine). For AFST to sertaconazole, luliconazole, ketoconazole, and naftifine, a total of 327, 415, 463, and 462 isolates were used, respectively. Among the azoles, itraconazole (geometric mean [GM] 0.08 mg/liter) and miconazole (GM 0.06 mg/liter) exhibited potent activity against the *T. mentagrophytes-interdigitale* complex, whereas among allylamines, terbinafine (GM 0.09 mg/liter) was more potent than naftifine (GM 0.133 mg/liter). Higher MIC₉₀ values were observed for fluconazole (16 mg/liter), terbinafine (8 mg/liter), naftifine (8 mg/liter), and griseofulvin (32 mg/liter). The MIC₉₀ of ketoconazole, clotrimazole, sertaconazole, and ciclopirox olamine were 0.5 mg/liter. Amorolfine, luliconazole, miconazole, itraconazole, and voriconazole had low MIC₉₀ values of 0.06, 0.125, 0.25, 0.25, and 0.25 mg/liter, respectively.

The MIC range, MIC₅₀, MIC₉₀, and GM values for all 13 antifungal drugs against *T. mentagrophytes-interdigitale* complex are summarized in Table 2. Both the 95% and 97.5% MICs were calculated to determine the UL-WT. For the antifungals which showed

TABLE 1 MIC and UL-WT distribution of *T. mentagrophytes-interdigitale* complex isolates against 13 antifungal drugs tested using the CLSI M38-A2 broth microdilution method^a

Antifungal agent	Total no of isolates	No. of isolates with MIC (mg/liter) of:														UL-WT 95%	UL-WT 97.5%	% NWT 95%	% NWT 97.5%	
		0.0078	0.015	0.03	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64					128
Miconazole	327			179	53	39	33	19	1	2	1						0.06	0.06	29	29
Luliconazole	415			227	130	34	13	10	1								0.06	0.125	13.9	5.7
Fluconazole	498		1		2	26	8	24	17	119	139	92	37	32	1		32	64	0.2	0
Itraconazole	498	31	47	28	158	109	81	42	2								0.5	1	0.2	0
Voriconazole	498	61	17	83	185	85	43	18	4	1	1						0.25	0.5	4.8	1.2
Ketoconazole	463	24		34	113	112	116	59	3	2							0.5	1	1	0.4
Clotrimazole	498	2	3	34	32	51	215	142	16	3							1	1	0.6	0.6
Sertaconazole	498	46		104	58	94	113	64	18	1							1	2	0.2	0
Naftifine	462			247	55	29	12	18	12	13	24	28	24				16*	16*	5.2	5.2
Terbinafine	498		209	88	24	19	17	39	8	18	19	49	6	2			8*	8*	11.4	11.4
Griseofulvin	498						70	9	15	19	29	44	79	198	11	24	64*	128*	ND	ND
Amorolfine	498	59	231	137	48	8	11	3			1						0.06	0.06	4.6	4.6
Ciclopirox olamine	498			19	2	5	381	41	20	30							0.5	0.5	10	10

^aMost frequently obtained MIC or mode is indicated in boldface type. NWT, non-wild-type; ND, not determined; UL-WT, upper limit of wild-type MIC. The UL-WT for naftifine, terbinafine, and griseofulvin are indicated by * and were calculated based on MIC₉₅ and MIC₉₇ values.

multimodal, nonsymmetrical, or truncated MIC frequency distribution, both the MIC₉₅ and MIC₉₇ were documented as UL-WT. The highest 95% UL-WTs were observed for griseofulvin (64 mg/liter) and fluconazole (32 mg/liter), whereas the lowest value of 0.06 mg/liter was found for two azoles (miconazole and luliconazole) and morpholine (amorolfine). High percentages of isolates above the UL-WT were observed for miconazole (29%), luliconazole (13.9%), terbinafine (11.4%), naftifine (5.2%), and voriconazole (4.8%), but low percentages for itraconazole (0.2%), sertaconazole (0.2%), and fluconazole (0.2%).

The phylogenetic analysis by internal transcribed spacer (ITS) dendrogram revealed that all our isolates belong to *T. mentagrophytes* type VIII. Therefore, genotype-based differences in MIC distribution could not be analyzed. Molecular screening for the mechanism of allylamine resistance was performed by sequencing the squalene epoxidase (SE) gene of all isolates with terbinafine at a MIC of ≥1 mg/liter and representative isolates with <1 mg/liter. Of 133 isolates sequenced, the F397L mutation was seen in 74 isolates (55.6%). The MIC range of the isolates with this mutation were between 1 and 32 mg/liter for terbinafine and 0.06 to 16 mg/liter for naftifine. Distribution of the F397L mutation in relation to the terbinafine MICs is provided in Fig. 1. Among 57 isolates with a high terbinafine MIC (≥8 mg/liter), the mutation was observed in 43 isolates (75.4%). Of the 39 isolates with terbinafine MICs between 1 and 4 mg/liter, 31 isolates (79.5%) had the F397L mutation. No mutation was observed in the 37 isolates with MIC values of <1 mg/liter (Fig. 1). While calculating the UL-WT based on the mutation analysis, a lower UL-WT was observed for terbinafine (1 mg/liter versus 8 mg/liter) and naftifine (0.06 mg/liter versus 16 mg/liter) compared to broth microbroth dilution-based results. Thus, the percentage of isolates above the UL-WT was higher both for naftifine (46.5% versus 5.2%) and terbinafine (20.5% versus 11.4%) on mutation analysis versus microbroth dilution method.

DISCUSSION

The testing for susceptibility to any drug is extremely valuable for defining the likely response of infection by a particular organism. CBPs enable us to predict therapeutic outcomes and are based on clinical trial data, global surveys of susceptibility, and PK/PD parameters (13). Either the ECV or the UL-WT offers an alternative for guiding the choice of optimum treatment by virtue of the MIC data. Since neither the CBPs nor the ECVs or UL-WT for *T. mentagrophytes-interdigitale* complex have been defined, we conducted this study to determine the UL-WT to antifungal agents commonly used in the management of dermatophytosis. To the best of our knowledge, this is the first

TABLE 2 Various studies representing the MIC (mg/liter) distribution of *Trichophyton mentagrophytes-interdigitale* complex isolates against antifungal drugs tested using the CLSI M38-A2 broth microdilution method^a

Reference	Year	Total no. of drugs tested	No. of isolates	Drug	Range	MIC ₅₀	MIC ₉₀	GM
This study	2020	13	498 (Tm/Ti complex)	Miconazole ^b	0.03–4	0.03	0.25	0.06
				Luliconazole ^c	0.03–1	0.03	0.125	0.05
				Fluconazole	0.0625–64	4	16	3.28
				Itraconazole	0.0078–1	0.06	0.25	0.08
				Voriconazole	0.0078–4	0.06	0.25	0.05
				Ketoconazole ^d	0.0078–2	0.125	0.5	0.120
				Clotrimazole	0.0078–2	0.25	0.5	0.231
				Sertaconazole	0.0078–2	0.125	0.5	0.108
				Naftifine ^e	0.03–16	0.03	8	0.133
				Terbinafine	0.015–32	0.03	8	0.096
				Griseofulvin	0.25–128	16	32	9.27
				Amorolfine	0.0078–4	0.015	0.06	0.024
				Ciclopirox olamine	0.03–2	0.25	0.5	0.289
Rudramurthy et al. (1)	2018	12	88 (Ti)	Fluconazole	2–32	4	16	5.03
				Ketoconazole	0.0625–2	0.125	0.5	0.17
				Sertaconazole	0.03–1	0.125	0.5	0.13
				Clotrimazole	0.125–2	0.25	0.5	0.36
				Voriconazole	0.0312–2	0.125	0.5	0.12
				Itraconazole	0.15–8	0.125	0.5	0.13
				Terbinafine	0.015–32	0.0312	4	0.06
				Naftifine	0.0312–16	0.0312	8	0.1
				Amorolfine	0.007–4	0.0156	0.0625	0.02
				Ciclopirox olamine	0.25–0.5	0.25	0.25	0.25
				Griseofulvin	2–128	32	64	26.31
				Luliconazole	0.0312–0.25	0.0312	0.125	0.05139
				Pathania et al. (15)	2018	4	36 (Tm)	Griseofulvin
Fluconazole	0.12–32	4	16					
Terbinafine	0.015–8	0.125	8					
Itraconazole	0.015–1	0.063	0.5					
Khurana et al. (8)	2018	11	64 (Ti)	Terbinafine	0.25–≥32	1	32	2.813
				Itraconazole	0.06–≥16	0.25	1	0.287
				Fluconazole	0.5–≥64	16	32	13.205
				Voriconazole	0.06–2	0.25	0.5	0.202
				Ketoconazole	0.25–≥32	0.5	0.5	0.666
				Amphotericin B	0.25–1	0.5	0.5	0.38
				Griseofulvin	0.5–≥8	2	4	2.874
				Miconazole	0.25–≥16	2	4	1.978
				Econazole	0.5–8	2	4	1.836
				Luliconazole	0.0035–0.125	0.007	0.007	0.005
Sertaconazole	0.25–≥16	1	2	0.842				
Rezaei-Matehkolaei et al. (34)	2018	6	66 (Ti)	Efinaconazole	0.002–0.006	0.008	0.016	0.008
				Lanoconazole	0.001–0.008	0.002	0.004	0.002
				Luliconazole	0.0002–0.004	0.0005	0.001	0.0006
				Fluconazole	4–64	8	16	11.07
				Itraconazole	0.03–0.5	0.12	0.25	0.111
Terbinafine	0.004–0.12	0.015	0.03	0.013				
Salehi et al. (19)	2018	8	27 (Ti)	Terbinafine	0.003–0.125	0.01	0.06	0.01
				Griseofulvin	0.03–64	0.12	35.2	0.41
				Itraconazole	0.01–4	0.06	1.3	0.07
				Voriconazole	0.01–16	0.37	8.8	0.41
				Ketoconazole	0.03–4	0.25	2.2	0.32
				Econazole	0.03–0.5	0.06	0.5	0.08
				Lanoconazole	0.03–0.5	0.06	0.5	0.09
Butenafine	0.03–0.5	0.06	0.5	0.09				

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TABLE 2 (Continued)

Reference	Year	Total no. of drugs tested	No. of isolates	Drug	Range	MIC ₅₀	MIC ₉₀	GM
Singh et al. (2)	2018	11	63 (Ti)	Terbinafine	0.06–32	1	32	2.7
				Itraconazole	0.06–16	0.5	2	0.51
				Voriconazole	0.06–16	0.25	2	0.32
				Fluconazole	0.5–64	32	64	16.7
				Luliconazole	0.003–0.5	0.015	0.06	0.014
				Sertaconazole	0.125–16	2	16	2.3
				Miconazole	0.5–16	2	8	2.44
				Ketoconazole	0.125–64	1	32	1.37
				Clotrimazole	0.008–32	4	4	2.83
				Amphotericin B	0.25–8	0.5	1	0.56
Griseofulvin	1–32	4	8	4.09				
Sadeghi-Nejad et al. (20)	2017	2	3 (Tm)	Ketoconazole	0.78–6.25	1.56	6.25	3.52
				Griseofulvin	12.5–100	25	100	56.25
Baghi et al. (22)	2016	12	52 (Ti)	Luliconazole	0.016–0.032	0.016	0.032	0.02
				Itraconazole	0.031–0.5	0.25	0.5	0.18
				Micafungin	0.5–8	4	8	3.31
				Fluconazole	2–64	32	64	20.8
				Griseofulvin	0.5–4	1	2	0.98
				Terbinafine	0.008–0.125	0.063	0.25	0.071
				Laniconazole	0.063–0.5	0.25	0.25	0.17
				Econazole	0.031–0.5	0.5	0.5	0.3
				butenafine	0.031–1	0.25	1	0.26
				Caspofungin	0.008–0.032	0.016	0.032	0.016
				Anidulafungin	0.008–0.016	0.008	0.016	0.01
Tolnaftate	0.008–0.125	0.063	0.25	0.076				
Ansari et al. (35)	2015	4	156 (Ti)	Fluconazole	4–128	32	64	27.47
				Itraconazole	0.008–0.25	0.063	0.125	0.062
				Terbinafine	0.004–0.25	0.016	0.125	0.017
				Griseofulvin	0.25–4	1	4	0.93
Badali et al. (36)	2015		13 (Tm)	Amphotericin B	0.125–1	1	2	0.68
				Fluconazole	16–64	32	64	41.77
				Itraconazole	0.5–16	2	16	2.11
				Voriconazole	0.25–2	1	2	1.41
				Posaconazole	0.5–1	1	1	0.85
				Isavuconazole	0.25–2	1	2	0.94
				Caspofungin	0.5–2	1	2	1.05
				Anidulafungin	0.008–0.063	0.031	0.063	0.03
Terbinafine	0.016–0.31	0.031	0.031	0.03				
Adimi et al. (30)	2013	10	136 (Tm)	Terbinafine	0.0156–16	0.0312	16	0.093
				Griseofulvin	0.0312–56	2	256	2.31
				Itraconazole	0.0009–4	0.0625	0.5	0.035
				Ketoconazole	0.0312–32	2	8	0.43
				Fluconazole	0.0625–256	64	256	10.44
				Voriconazole	0.0156–8	0.5	4	0.174
				Clotrimazole	0.0312–32	0.125	2	0.33
				Ciclopirox olamine	0.0312–32	2	16	1.81
				Amorolfine	0.0078–32	0.25	8	0.245
Naftifine	0.0625–6	0.25	12	0.326				
Yenişehirli et al. (37)	2013	6	49 (Tm)	Terbinafine	0.015–0.125	0.06	0.125	0.06
				Amphotericin B	0.03–2	0.125	0.5	0.14
				Miconazole	0.03–2	0.125	2	0.16
				Itraconazole	0.03–0.5	0.25	0.5	0.13
				Ketoconazole	0.03–4	0.25	1	0.2
				Griseofulvin	0.03–16	0.5	2	0.49

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TABLE 2 (Continued)

Reference	Year	Total no. of drugs tested	No. of isolates	Drug	Range	MIC ₅₀	MIC ₉₀	GM
Carrillo-Muñoz et al. (21)	2013	10	19 (Ti)	Amorolfine		0.015	0.062	
				Bifonazole		0.015	16	
				Clotrimazole		0.015	1	
				Econazole		0.015	1	
				Ketoconazole	0.015	4		
				Miconazole		0.015	4	
				Oxiconazole		0.015	4	
				Sertaconazole	0.015	1		
				Ticonazole		0.015	8	
			Terbinafine		0.015	0.5		
			26 (Tm)	Amorolfine		0.015	0.031	
				Bifonazole		0.061	16	
				Clotrimazole		0.015	1	
				Econazole		0.015	2	
				Ketoconazole	0.015	0.5		
				Miconazole		0.015	8	
				Oxiconazole		0.031	2	
				Sertaconazole	0.031	16		
				Ticonazole		0.015	0.5	
Terbinafine		0.015		0.015				
Silva et al. (38)	2014	6	24 (Ti)	Ketoconazole	0.031–16	0.25	2	0.46
				Fluconazole	2–64	8	64	5.9
				Griseofulvin	0.125–64	2	32	1.6
				Itraconazole	0.0312–2	0.125	0.5	0.19
				Terbinafine	0.031–2	0.0312	0.0312	0.03
				Voriconazole	0.0312–1	0.062	0.5	0.24
Ataides et al. (39)	2012	4	7 (Tm)	Itraconazole	0.125–16	0.25	16	0.41
				Ketoconazole	0.5–4	0.5	4	1.48
				Griseofulvin	1–8	1	8	0.61
				Terbinafine	0.015–0.062	0.015	0.062	0.01
Zalacain et al. (40)	2011	5	29 (Tm)	Ciclopirox olamine	0.032–0.500	0.25	0.5	0.18
				Fluconazole	32	-	-	ND
				Itraconazole	0.032–1.000	0.5	1	0.35
				Terbinafine	0.016–0.500	0.125	0.5	0.082
				Eberconazole	0.016–1.000	0.25	1	0.18
Bueno et al. (41)	2010	4	18 (Tm)	Itraconazole	0.03–0.5	0.25	0.5	0.2
				Fluconazole	16–64	64	64	50.37
				Terbinafine	0.007–0.06	0.015	0.03	0.014
				Voriconazole	0.03–0.5	0.125	0.5	0.19
Mota et al. (42)	2009	5	14 (Tm)	Fluconazole	4–16	16	16	
				Itraconazole	0.03–0.25	0.125	0.25	
				Ketoconazole	0.03–1	0.125	0.25	
				Terbinafine	0.03–0.5	0.06	0.25	
				Griseofulvin	0.25–1	0.5	0.5	
Valverde et al. (43)	2008	3	30 (Tm)	Itraconazole		0.25	0.5	
				Fluconazole		16	64	
				Voriconazole		0.12	0.25	

^aGM, geometric mean; Ti, *Trichophyton interdigitale*; Tm, *Trichophyton mentagrophytes*.

^b327 isolates screened.

^c415 isolates screened.

^d463 isolates screened.

^e462 isolates screened.

multicenter study to propose the UL-WT for the *T. mentagrophytes-interdigitale* complex isolates of Indian origin.

An alarming rise in prevalence and incidence of dermatophytosis has been observed across India (14). Recent reports indicate an epidemic-like situation, with the chief

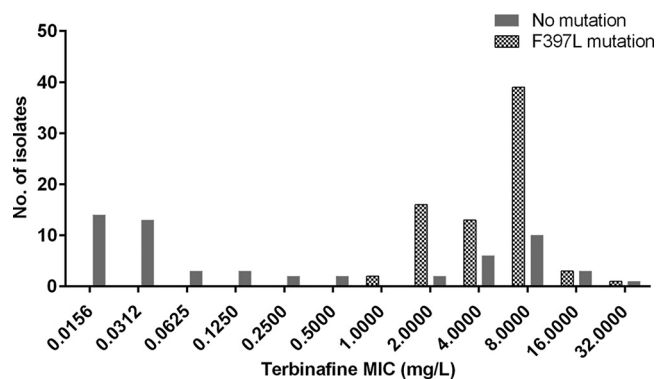


FIG 1 Relationship between mutation in the squalene epoxidase gene and the MIC for terbinafine in 133 *T. mentagrophytes-interdigitale* complex isolates.

etiological agent being *T. mentagrophytes* followed by *T. rubrum* (1, 2, 15). *T. mentagrophytes* is a complex group containing different species. The taxonomy of this species is still not well delineated. Based on the ITS sequences, two major studies from India report that the *T. mentagrophytes* complex causing the epidemic in India belongs to *T. interdigitale* (1, 2). Later, on the basis of multigene sequences like ITS, beta tubulin, translation elongation factor, and calmodulin gene, all the *T. mentagrophytes* complex strains of India were found to be distinct from other clades and were described as *T. mentagrophytes* type VIII. The whole-genome sequence of Indian isolates shares similarity with the recent common ancestor, *T. mentagrophytes-interdigitale* complex, and these could not be conclusively differentiated as two species (16). Thus, we characterized all of our isolates as *T. mentagrophytes-interdigitale* complex to preclude any misidentification (1, 16–18). Experts recommend that accurate identification of the implicated fungal pathogen and its AFST may be necessary for the management of the recurrent, relapse, or chronic cases (3). Six medical centers from both North India and South India participated in the present study and a large number of *T. mentagrophytes-interdigitale* complex isolates were evaluated against 13 antifungal agents. The MICs obtained in this study compared with the results of previous studies are provided in Table 2. Previously reported MIC₅₀ ranges for *T. mentagrophytes-interdigitale* of various antifungal drugs were similar to those observed in the present study (8, 19–22). The MIC distributions of itraconazole, miconazole, luliconazole, voriconazole, ketoconazole, sertaconazole, naftifine, ciclopirox olamine, and clotrimazole show good susceptibility of *T. mentagrophytes-interdigitale* complex compared to terbinafine, fluconazole, and griseofulvin. Recent reports in Indian literature have revealed the high MIC of terbinafine for *Trichophyton* spp. (1, 2). Clinical evidence of relapse and incomplete mycological cure after standard (250 mg, twice daily for 2 weeks) oral terbinafine therapy have also been reported (23). Since the pharmacodynamic properties of terbinafine remain incompletely described, a cumulative percentage (%T_{>MIC}) of 100% was used as a conservative approach to describe its dosing interval in an animal study (24). According to that study, the use of 250 mg of terbinafine twice daily was appropriate for treatment of dermatophytic infections caused by *T. mentagrophytes* with a MIC of 0.01 mg/liter (24). However, since the tissues infected by dermatophytes are avascular components of the skin and adenexa, the time to attain therapeutic concentrations in them may differ greatly from plasma (24). In the present study, we observed that >40% of the *T. mentagrophytes-interdigitale* complex isolates had a terbinafine MIC of >0.01 mg/liter. A higher dose with a multidosing strategy may be required to treat infections by *T. mentagrophytes-interdigitale* complex isolates with higher MICs. Unfortunately, this may not be clinically practical due to the possibility of drug-related side effects. These findings emphasize that the use of terbinafine as the first line drug against dermatophytic infections is largely unsubstantiated. In view of the contemporary disease epidemiology, it may have lost clinical efficacy against Indian *T.*

mentagrophytes-interdigitale complex and requires further evaluation. However, although serum levels of terbinafine follow a predictive PK/PD, these levels may not parallel the site-specific drug levels. In fact, some studies show that the skin levels of terbinafine exceed trough plasma levels by nearly 10 to 40 times the MIC, indicating little rationale for higher doses (25).

In clinical practice, CBPs are needed to optimize appropriate antifungal treatment. However, CBPs are not defined for any antifungal agent against dermatophytes. In such a scenario, ECVs may help in identifying isolates with antifungal resistance, as has been seen for other molds such as *Aspergillus* spp., Mucorales, and *Sporothrix* spp. (13, 26–28). According to CLSI guidelines, the evaluation of ECVs should follow certain criteria, i.e., (i) the MIC should be determined as per standard guidelines, either by following the protocol of CLSI or the European Committee on Antimicrobial Susceptibility Testing (EUCAST); (ii) large numbers of isolates ($n \geq 100$) should be included; (iii) the MIC of isolates from different laboratories (≥ 3) should be used; and (iv) the AFST should be performed by many investigators (≥ 3) (13). Our study fulfils the above criteria (except for partial fulfillment of the last criteria), and thus we propose the UL-WT instead of an ECV for the *T. mentagrophytes-interdigitale* complex of Indian origin against the antifungal agents in Table 1. For UL-WT determination, isolate identification to the species level is important according to CLSI guidelines (12). In our study, three antifungal agents (terbinafine, naftifine, and griseofulvin) had nonsymmetric, multimodal, or truncated MIC distribution due to which the ECOFF could not be determined using the software. Thus, the UL-WT (in mg/liter) calculated based on the MIC₉₅ (27) for terbinafine, naftifine, and griseofulvin was 8, 16, and 64, respectively. Interestingly, the mutation analysis of the SE gene revealed that even isolates with MICs at less than 1 mg/liter for terbinafine and 0.06 mg/liter for naftifine (both allylamines) exhibited the F397L mutation that was absent in all isolates with MICs of <1 mg/liter for terbinafine and 0.03 mg/liter for naftifine. This finding suggests that the UL-WT of these drugs is probably lower than that estimated based on MIC₉₅ and MIC₉₇. These findings substantiate our interpretation that the use of terbinafine for first-line management of *T. mentagrophytes-interdigitale* complex infections needs further evaluation. Overall, we noted low MIC and UL-WT values for miconazole, luliconazole, itraconazole, voriconazole, and ketoconazole. Itraconazole has also been found to have a lower MIC than terbinafine against a majority of *T. mentagrophytes* complex isolates and the use of itraconazole as a first line drug in the management of dermatophytosis may be beneficial (29).

The major limitation of this study was that the majority of the isolates were from a single center, the Postgraduate Institute of Medical Education and Research (PGIMER). Also, AFST was performed at a single institute (PGIMER) independently by laboratory persons belonging to all 6 centers. Another concern is that being a tertiary care center, most patients presenting to us might have prior exposure to antifungals, antibiotics, and/or steroids. This could be responsible for the high MIC values to some antifungals (terbinafine, griseofulvin, and fluconazole) and the UL-WT may artificially encompass resistant (non-wild-type) isolates. Additionally, using the Indian *T. mentagrophytes-interdigitale* complex to determine the UL-WT may have limited global applicability, as some antifungals have been shown to have high UL-WT values compared to global data. Thus, future multicentric studies involving isolates with more geographical diversity must be designed to further define the ECVs or UL-WT. The inclusion of isolates belonging to different genotypes from diverse geographic locations will enable the analysis of genotype/clade-related differences in MIC distribution, which could not be performed in the present study. The lack of facilities for AFST of dermatophytes at most Indian institutes limited the testing of isolates at more than one center in the present study. Regular training programs and capacity building at multiple centers could enable the determination of ECVs and CBPs in future studies.

In conclusion, UL-WT values are an important tool for distinguishing WT from NWT isolates and provide a preliminary idea to the treating physician in optimizing antifungal therapy. However, the UL-WT values are based solely on the *in vitro* data of isolates

collected from various centers and are not an interpretative breakpoint. Nonetheless, since breakpoints are not available for dermatophyte infections, these values can assist the clinician to predict whether an isolate is likely to respond to a specific antifungal. Future studies assessing the clinical response to treatment and monitoring the PK and PD of antifungal therapy could be beneficial in establishing CBPs.

MATERIALS AND METHODS

Isolates. A total of 498 clinical isolates of *T. mentagrophytes-interdigitale* complex were collected over a period of 5 years (2014 to 2018) from six tertiary care hospitals in India. All clinical isolates were recovered from skin ($n = 481$), hair ($n = 13$), and nail ($n = 4$) samples at the following medical centers: Postgraduate Institute of Medical Education and Research, Chandigarh ($n = 354$), Government Medical College Haldwani, Nainital ($n = 44$), Jawaharlal Nehru Medical College, Belagavi, Karnataka ($n = 41$), Father Muller Medical College, Mangalore ($n = 32$), Bhasker Medical College & General Hospital, Telangana ($n = 15$), and St. John's Medical College & Hospital, Bangalore ($n = 12$). In cases of multiple isolates from the same patient, only unique clinical isolates were used. The identification of all strains as *T. mentagrophytes-interdigitale* complex was based on culture morphology and microscopic characteristics and confirmed by sequencing of the internal transcribed spacer (ITS) region and 28S region of ribosomal DNA, and beta tubulin (1, 16, 17).

Antifungal susceptibility testing. The AFST of all the isolates was performed at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, and the tests were performed independently by laboratory personnel of respective institutes contributing the isolates. The strains were subcultured on potato dextrose agar (PDA) medium prior to AFST. The broth microdilution technique was performed according to Clinical and Laboratory Standards Institute (CLSI) M38 A2 protocol with minor modifications, as previously described (1, 10, 30). Inoculum suspension with approximately 10^6 CFU/ml conidia was collected from 7 to 14 days culture grown on PDA and quantified microscopically by hemocytometer. The suspension was then diluted to 1:100 according to primary concentration. Double the final concentration (10^3 CFU/ml) of the conidia was adjusted before adding to the drug plates. An initial inoculum corresponding to 65 to 70% transmittance at 530 nm in a spectrophotometer was used. Inoculated plates were incubated at 28°C for 4 days prior to interpretation. In all the isolates, the MICs of azoles, griseofulvin, and amorolfine were documented as the concentration showing prominent inhibition of growth (approximately 80%) compared to that in growth-control wells. For terbinafine, naftifine, luliconazole, ciclopirox olamine, and miconazole, a 100% growth inhibition was documented, as described by Rudramurthy et al. (1). To check the purity of the inoculated plates, 10 μ l of the growth from the growth-control well was inoculated onto Sabouraud dextrose agar (10). Panels of 13 topical or systemically applied antifungals commonly used for the treatment of dermatophytosis were tested. The panel included azoles (fluconazole, voriconazole, itraconazole, ketoconazole, sertaconazole, luliconazole, clotrimazole, and miconazole), allylamines (terbinafine and naftifine), a morpholine (amorolfine), an oxaborole/hydroxamic acid (ciclopirox olamine), and an antifungal antibiotic (griseofulvin), all purchased from Sigma-Aldrich, Bengaluru, India. The drugs were dissolved in dimethyl sulfoxide and the final concentrations (in mg/liter) ranged from 0.0625 to 32 for fluconazole; 0.0078 to 4 for voriconazole, itraconazole, and amorolfine; 0.0312 to 16 for ketoconazole, luliconazole, sertaconazole, clotrimazole, ciclopirox olamine, and naftifine; 0.0156 to 64 for terbinafine; 0.0156 to 8 for sertaconazole; and 0.25 to 128 for griseofulvin. *T. mentagrophytes-interdigitale* complex (NCCPF 800035), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), and *Aspergillus flavus* (ATCC 204304) strains were used as quality control measures. The AFST was performed in triplicate for each isolate to ensure quality and reproducibility.

Distribution of MIC and UL-WT determination. The data analysis was performed using an Excel 2018 spreadsheet. The MIC₅₀ and MIC₉₀ were obtained by a descriptive statistics analysis. In statistical analysis, the modeled population was established based on fitting a normal distribution at the lower end of the MIC and the mean and standard deviation of the normal distribution were calculated to determine the MIC capturing at least 95%, 97.5%, and 99% of the modeled WT population. The Microsoft Excel spreadsheet calculator (EcoFinder program version XL2000+, http://www.eucast.org/mic_distributions_and_ecoffs/) was used for statistical determination of the UL-WT for the 13 antifungal drugs (26, 31, 32).

The isolate with no mechanisms of acquired resistance or mutational resistance for the antifungal agent tested is defined as the WT. The NWT isolates are those with presumed or known mechanisms of resistance for the antifungal agent being tested. The isolates were classified as WT or NWT depending on whether the MIC was \leq UL-WT or $>$ UL-WT for the above-mentioned antifungals (26, 31, 32).

Phylogenetic analysis. Phylogenetic analysis was performed using the ITS sequences of representative isolates of the present study and sequences of all genotypes described previously (sequences retrieved from NCBI database) (33). *T. quinckeanum* was used as the outgroup due to its high divergence. Sequences were aligned using the multiple sequence alignment mode in ClustalX2 software. The aligned sequences were exported to Molecular Evolutionary Genetics Analysis software version 7 (MEGA 7) and a neighbor joining tree was constructed with 1,000 bootstrapping replicates by using Kimura 2 parameter model.

Mutation analysis for terbinafine. A total of 133 isolates were screened for mutation in the squalene epoxidase gene. The complete gene was sequenced by using the primer pairs SE1aF-5'-CAG AGATAATGCAGCCATCG-3' and SE1aR-5'-CCGATTGATGTTCTAGGT-3'; SE2aF-5'-CCACCAGCGCGAAT ATAGA-3' and SE2aR-5'-AGTCCAGTGCCAGACTGATG-3'; and SE3aF-5'-AGTCTGGCACTGGACTCCA-3' and SE3aR-5'-ATGATGCAGCGACGGTGACA-3' (Sigma) as described earlier (1). Bionumerics software

(Applied Maths, Ghent, Belgium) was used for consensus and concatenation of the sequences. The sequences and amino acid sequences depicted by using the ExPASy online tool (<https://web.expasy.org/translate/>) were aligned.

Data availability. Sequences of the representative isolates have been deposited in GenBank under accession numbers [MH517546](#) to [MH517560](#), [MN822738](#) to [MN822771](#), [MN824042](#) to [MN824085](#), [MN830960](#) to [MN831002](#), [MN830945](#) to [MN830959](#), and [MN831003](#) to [MN831103](#) for the ITS region of ribosomal DNA; [MK967531](#) to [MK967551](#) for the 28S region of ribosomal DNA; [MK982906](#) to [MK982926](#) for BT; and [MN836335](#) to [MN836372](#) for SE.

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