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## **Autochthonous *Trichophyton rubrum* terbinafine resistance in France: Assessment of antifungal susceptibility tests**

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1 **Letter**

2 **Title: Autochthonous *Trichophyton rubrum* terbinafine resistance in France: Assessment of**  
3 **antifungal susceptibility tests**

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42 **To the editor,**

43 Terbinafine resistance is now a serious issue in dermatophytosis treatment due to the  
44 worldwide expansion of *Trichophyton indotineae*. Incidence of terbinafine treatment failure is  
45 increasingly reported in patients with *T. rubrum* dermatophytosis [1, 2]. This trend has raised  
46 concerns among healthcare professionals, emphasizing the importance of performing  
47 antifungal susceptibility tests (AFST) to identify *T. rubrum* terbinafine resistant isolates (TRBi).

48

49 Over a two-year period, eleven isolates from patients with *T. rubrum* dermatophytosis failing  
50 terbinafine treatment (250 mg/d; >6 months) were referred to our institution (Table 1).  
51 Patients were mainly men (82%) and mean age was 43.4 years. Onychomycosis was mainly  
52 observed (72.2%), with lesions affecting feet (87.5%) and hands (25%). Three patients were  
53 diagnosed with *Tinea corporis* (27.3%). *T. pedis* and foot intertrigo were detected in one  
54 patient each (9.1%). Only 1 out of 11 patients reported a travel history in India, suggesting  
55 that terbinafine resistance development likely originated in France.

56

57 Itraconazole, griseofulvin, and fluconazole were prescribed as a second line treatment.  
58 Itraconazole (100-200 mg/day) successfully cleared the infection in 6 out of 7 patients. One  
59 patient experienced recurrence of clinical lesions after two months of treatment and was  
60 successfully treated with voriconazole cream (1%) for two and a half months. Griseofulvin  
61 treatment (500 mg/day) was ineffective in one patient, who was subsequently successfully  
62 treated with itraconazole (200 mg/day). Fluconazole treatment (150 mg/once weekly) cleared  
63 the infection in one patient but only prevented the progression of the lesions in two patients.

64

65 Molecular identification confirmed that all isolates belonged to the *T. rubrum* species.  
66 Squalene epoxidase *SQLE* gene Sanger sequencing revealed mutations implicated in  
67 terbinafine resistance in all isolates [3]. F397L substitution was found in four isolates  
68 individually (36.4%) or in combination with the F415S substitution (9.1%). A double mutation  
69 L393S/F397L was observed in two isolates (18.2%), while the L393F and H440Y mutations  
70 were each observed in one patient. One isolate each carried the F397I substitution alone  
71 (9.1%) or associated to F415S (9.1%). Terbinafine containing agar method (TCAM) [3], also  
72 confirmed terbinafine resistance as all isolates grew at a terbinafine concentration of 0.125  
73 mg/L.

74

75 Standardized inoculums were prepared using culture conditions previously described with  
76 minors modifications [3]. Using the EUCAST method, terbinafine Minimum Inhibitory  
77 Concentration (MIC) values ranged from 0.25 to >8 mg/L. As there are currently no clinical  
78 breakpoints for *T. rubrum*, ECOFF determined by EUCAST (<http://www.eucast.org>) were used  
79 for isolate categorization. All TBRI were susceptible to itraconazole (range: 0.016-0.25 mg/L),  
80 voriconazole (range: 0.008-0.125 mg/L) and amorolfine (range: 0.008-0.25 mg/L). The ability  
81 of the GT to determine *T. rubrum* susceptibility to terbinafine (HiMedia®), itraconazole and  
82 voriconazole (BioMerieux®) was also evaluated and MIC values were compared with those  
83 determined using the EUCAST method (Table 1). MIC values from both methods were similar  
84 for itraconazole and voriconazole whereas MIC values for terbinafine differed between  
85 methods for two isolates.

86

87 We document here the occurrence in France of terbinafine resistant dermatophytosis due to  
88 *T. rubrum*. Emergence of these autochthonous TBRI is likely related to terbinafine pressure for

89 several years since it is the first line treatment when topical treatments fail. Itraconazole is  
90 often proposed after terbinafine treatment failure. Although itraconazole resistance is rare in  
91 *T. rubrum* [4], failures to itraconazole treatment have been described, likely due to  
92 inappropriate serum levels. Successful voriconazole cream treatment presented in this study  
93 suggests that this formulation holds promise for recalcitrant dermatophytosis [5]. Griseofulvin  
94 and fluconazole treatments have shown a lower efficiency and must probably be proposed  
95 when comorbidities restrict the use of itraconazole.

96

97 The present study reinforces the importance of prioritizing *T. rubrum* TBRI detection in a  
98 context of treatment failure. Identification of substitutions allows the detection of resistant  
99 isolates but remains a method restricted to specialized laboratories. SQLE substitutions  
100 detected have been previously described (Supplementary references) but to our knowledge,  
101 this is the first study that detects the double substitution L393S/F397L. TCAM can also be  
102 proposed to confirm *T. rubrum* terbinafine resistance in non-expert routine diagnostic  
103 laboratories. However, both methods fail to consider susceptibility to other effective  
104 antifungal alternatives. EUCAST method can confirm the *in vitro* terbinafine resistance of  
105 isolates and provide the isolate susceptibility profile to itraconazole, voriconazole and  
106 amorolfine. Nevertheless, the lack of commercialization of the EUCAST method restricts its  
107 use to specialized laboratories. The availability of GT offers the opportunity to carry out  
108 antifungal susceptibility using a more simple and accessible method to medical biology  
109 laboratories than the EUCAST method. Our study reveals a good concordance between results  
110 obtained with EUCAST method and the GT for itraconazole and voriconazole. For terbinafine,  
111 discrepancies between MIC values from EUCAST method and GT were observed, suggesting  
112 that *in vitro* results must be compared with patient treatment available information.

113

114 Autochthonous *T. rubrum* resistant to terbinafine occurs in France. We recommend different

115 AFST methods to evaluate the antifungal susceptibility profile of TBRi, guiding clinicians to

116 propose an antifungal susceptibility-based treatment.

117

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122 **Access to data:** The data presented in this study are available on request from the  
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124 **Contribution:** CC, GC, JPB, AH, SI, IC, CB, FF, BS, GD, provided clinical isolates and the  
125 associated medical records. AMS, ACN, ALB, IL, DM, CH, MM, ED, participated to the  
126 conceptualization, data curation, formal analysis, investigation, and methodology. AMS, ACN,  
127 GD, ED reviewed and edited the first version of the manuscript. Authors approved the final  
128 version before submission.

129 **Statement on research ethics:** The study was conducted using patient's information and  
130 isolates collected during routine clinical practice. All data analyzed in this study (patient  
131 treatment and fungal identifiable information) were collected by medical personnel and  
132 anonymized.



**Table 1. Clinical characteristics and antifungal susceptibility profile of autochthonous *T. rubrum* terbinafine resistant isolates**

ID	Gender	Age (yr)	Clinical Lesions	2 <sup>en</sup> Treatment (Fail;Pp;Suc)	SQLEs	TCAM	TER MIC		ITR MIC		VOR MIC		AMO MIC
							EUCAST	GT	EUCAST	GT	EUCAST	GT	EUCAST
01	Male	71	TC	ITR & VORc (Fail & Suc)	F397L	>0.125	8	>32	0.06	0.002	0.008	0.002	0.016
02	Male	72	TC	ITR (Suc)	F397I	>0.125	LS	LS	LS	LS	LS	LS	LS
03	Male	17	TP & ONY (Hand/foot)	FLU (Suc)	F397L	>0.125	2	1.5	0.06	0.03	0.016	0.002	0.016
04	Male	29	II (foot)	ITR (Suc)	F397L F415S	>0.125	LS	LS	LS	LS	LS	LS	LS
05	Male	51	ONY (foot)	NA	F397I F415S	>0.125	<u>0.25</u>	<u>0.03</u>	0.016	0.016	0.008	0.002	0.06
06	Female	42	ONY (foot)	FLU (Pp)	L393S F397L	>0.125	<u>0.25</u>	<u>0.03</u>	0.016	0.03	0.016	0.002	0.016
07	Male	45	ONY (foot)	FLU (Pp)	L393S F397L	>0.125	0.5	0.5	0.125	0.03	0.03	0.002	0.016
08	Female	45	ONY (hand)	ITR (Suc)	F397L	>0.125	>8	>32	0.06	0.016	0.03	0.002	0.016
09	Male	25	ONY (foot)	ITR (Suc)	F397L	>0.125	4	0.5	0.06	0.03	0.125	0.002	0.03
10	Male	44	TC & ONY (foot)	ITR (Suc)	L393F	>0.125	NA	NA	NA	NA	NA	NA	NA
11	Male	37	ONY (foot)	GRI & ITR (Fail & Suc)	H440Y	>0.125	NA	NA	NA	NA	NA	NA	NA

134 AMO: Amorolfine; Fail: treatment failure; FLU: Fluconazole; GRI: Griseofulvin; GT: Gradient trips; II: Interdigital intertrigo; ITR: Itraconazole; MIC:  
135 Minimum Inhibitory Concentration (mg/L); ONY: Onychomycosis; Pp: Treatment preventing disease progression; SQLEs: Squalene epoxidase  
136 amino acid substitution; Suc: treatment success; TC: Tinea corporis; TCAM: Terbinafine containing agar method (mg/L); TER: Terbinafine; TP:  
137 Tinea pedis; VOR: Voriconazole; VORc: Voriconazole cream; LS: Lack of isolate sporulation; NA: Data not available; Underlined values: MIC values  
138 are inconsistent between methods.

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