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

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Letter

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Alicia Moreno-Sabater[1], Camille Cordier[2,3]*, Anne Cécile Normand[4]*, Anne Laure Bidaud[5], Geneviève Cremer[6], Jean Philippe Bouchara[7], Antoine Huguenin[8,9], Sébastien Imbert[10], Isabelle challende[11], Cécile Brin[12], Françoise Foulet[13], Boualem Sendid[2,3], Illan Laloum[5], Denis Magne[14], Christophe Hennequin[15], Michel Monod[16], Guillaume Desoubeaux[17], Éric Dannaoui[5,18].

*Authors equally contributed.

[1] Sorbonne Université, Inserm, Centre d'Immunologie et Maladies infectieuses, CIMI-PARIS, AP-HP, Hôpital Saint-Antoine, Service de Parasitologie-Mycologie, F-75012 Paris, France.

[2] Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire de Lille, Lille, France.

[3] INSERM U1285, Unité de Glycobiologie Structurale et Fonctionnelle (CNRS UMR 8576), Université de Lille, Lille, France.

[4] Service de Parasitologie-Mycologie, Hôpital La Pitié-Salpêtrière, AP-HP, Paris 75013, France.

[5] Unité de Parasitologie-Mycologie, Service de Microbiologie Hôpital Necker Enfants Malades, France.

[6] Laboratoire Bioclinic Madeleine. Groupe Inovie. Paris, France.

[7] IRF (Infections Respiratoires Fongiques), SFR ICAT 4208, Université Angers, Université Brest, Angers, France.

[8] Laboratoire de Parasitologie-Mycologie, Pôle de Biologie et de Pathologie, CHU de Reims, Reims, France.

[9] Université de Reims Champagne Ardenne, ESCAPE EA7510, Reims, France.

[10] Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire de Bordeaux, 33000 Bordeaux, France.

[11] Cabinet liberal, Chambéry, France.

[12] Service de Dermatologie, Centre Hospitalier Métropole Savoie, 73000 Chambéry, France.

[13] Service de Parasitologie-Mycologie, Hôpitaux Universitaires Henri Mondor, AP-HP, Créteil 94000, France.

[14] Service de Parasitologie-Mycologie, Hôpital Saint Antoine, AP-HP, Paris, 7012, France.

[15] Sorbonne Université, Inserm, Centre de Recherche Saint-Antoine, CRSA, AP-HP, Hôpital Saint-Antoine, Service de Parasitologie-Mycologie, F-75012 Paris, France.

[16]Department of Dermatology, Lausanne University Hospital (CHUV), Lausanne, Switzerland; Faculty of Biology and Medicine (FBM), University of Lausanne, Lausanne, Switzerland.

[17] Service de Parasitologie – Mycologie – Médecine tropicale, Hôpital Bretonneau, Tours 37044, France.

[18] Université Paris Cité, Paris, France.

Corresponding author: Alicia Moreno-Sabater. Service de Parasitologie-Mycologie. 184 Rue du Faubourg Saint Antoine, 75012, Paris, France. Alicia.morenoysabater@aphp.fr.

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To the editor,

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

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

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

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

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

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

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

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

Autochthonous *T. rubrum* resistant to terbinafine occurs in France. We recommend different AFST methods to evaluate the antifungal susceptibility profile of TBRi, guiding clinicians to propose an antifungal susceptibility-based treatment.

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

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

ID	Gender	Age (yr)	Clinical Lesions	2^{en} 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	0.25	0.03	0.016	0.016	0.008	0.002	0.06
06	Female	42	ONY (foot)	FLU (Pp)	L393S F397L	>0.125	0.25	0.03	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

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

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