Cluster Infection Caused by a Terbinafine-resistant Dermatophyte at a Group Home: The First Case Series in Japan

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Accepted Aug 11, 2021; Epub ahead of print xx

Terbinafine, which targets squalene epoxidase (SQLE), has been used to treat dermatophyte infections for approximately 30 years. In 2017, a Swiss study reported that 1% (16/1,644) of *Trichophyton rubrum* and 0.2% (1/412) of *T. interdigitale* were resistant to terbinafine (1). In 2019, we presented the first Japanese case of tinea unguium caused by a terbinafine-resistant *T. rubrum* isolate (Phe397Leu substitution), which was deposited as IFM 65760 (2). Our clinic obtained 3 terbinafine-resistant *T. rubrum* strains (Leu393Phe substitution) from 95 dermatophyte clinical isolates including *T. rubrum* ($n=62$) and *T. interdigitale* ($n=33$) in June 2020 (3). One strain (*T. rubrum* N79) was derived from a group home for individuals with intellectual disabilities. In this study, we examined the residents of this facility using mycological and molecular techniques to detect terbinafine-resistant *T. rubrum* strains.

MATERIALS AND METHODS

The group home accommodates 54 individuals over 18 years of age and provides 24/7 care including bathing, toileting and meals. The facility is in Kumamoto, which has a subtropical climate (Köppen climate classification Cfa) with hot, humid summer at 32° north latitude. All patients diagnosed with dermatophytosis during a 6-month period (June to November 2020) were included in this study ($n=30$ (20 males, 10 females); mean age 54.1 ± 17.7 years). Pathogens were identified in cultures on Sabouraud agar with chloramphenicol and cycloheximide (Mycosel agar; Kyokuto Pharmaceutical Industrial Co. Ltd, Tokyo, Japan) and/or by DNA-based detection for culture-negative onychomycosis in our clinic and Kahotechno Co., Ltd (Fukuoka, Japan). Molecular identifications, sequence analyses and antifungal susceptibility tests were performed at the Department of Veterinary Dermatology, Nihon University College of Bioresource Sciences (Kanagawa, Japan). The homology of the internal transcribed spacer region sequences in the *rRNA* gene of the strains was 100% (688/688 bp) identical to that of the *T. rubrum* reference strain IFM 63288 (GenBank, LC317851). The mutation hotspot of *SQLE* was determined based on the conserved sequence of *T. rubrum SQLE* (GenBank accession number XM_003233797) (3). The following primers were used: SQEL397S (5′- GTTGACTGGTGGCGGTATG; position 1002–1020) and SQEL397R (5′- GCTACGGAGTAAAAATGCCG; position 1315–1334) (Japanese patent application number 2021–2373). The antifungal susceptibility of the isolates to terbinafine, itraconazole, ravuconazole and luliconazole was evaluated by a broth microdilution assay according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 guidelines, with some modification (4).

RESULTS

The 30 patients with dermatophytosis included patients with tinea pedis ($n=10$), tinea unguium ($n=12$), tinea pedis et unguium ($n=6$), and tinea cruris et unguium ($n=2$). Fungal cultures were positive in 17 cases and DNA-based detection of culture-negative onychomycosis was positive in 6 cases. The causative fungi were *T. rubrum* ($n=22$, 95.6%) and *T. interdigitale* ($n=1$, 4.3%). Among these cases, 47% (14/30) were caused by terbinafine-resistant *T. rubrum*. In addition, 60% (8/14) of the patients with terbinafine-resistant dermatophyte had tinea unguium and 5.4 ± 3.2 nails were affected. Regarding the affected nails, 62.5% ($n=10$), 43.8% ($n=7$), 50% ($n=8$), 50% ($n=8$), and 62.5% ($n=10$) of the lesions involved the big, 2nd, 3rd, 4th and 5th toenail, respectively. Most patients (8/14) with terbinafine-resistant dermatophytes received oral terbinafine (125 mg, daily) for a mean duration of 24.4 ± 14.3 months. Five patients (5/14) were treated with topical SQLE inhibitors, either alone or in combination with oral treatment. Topical regimens included terbinafine, bifonazole, butenafine and liranaftate. Five patients (5/14) had no history of oral terbinafine or topical SQLE inhibitor use. The characteristics of patients with terbinafine-resistant isolates are shown in **Table I**. Nucleotide substitution within *SQLE* (^{1177}TTA→TTC) was consequently detected in all 14 terbinafine-resistant strains, leading to Leu393Phe substitution in *T. rubrum* SQLE proteins. These isolates were deposited in Chiba University with IFM numbers. The minimum inhibitory concentrations (MICs) for the mutant strains were >32 mg/l for terbinafine, <0.03–1 mg/l for itraconazole, <0.03 mg/l for luliconazole and <0.03 mg/l for ravuconazole.

DISCUSSION

Terbinafine is the most commonly prescribed oral antifungal medicine that is approved for the treatment of tinea unguium. Meanwhile, terbinafine-resistant tinea corporis due to prolonged terbinafine therapy has been reported in a 62-year-old man with Darier disease (5). In our study, cases 5 and 12 received oral terbinafine for 3 years. In particular, case 12, who had hyperkeratotic-type tinea pedis and severe tinea unguium, might have been an index

doi: 10.2340/00015555-3926
Acta Derm Venereol 2021; 101: adv00563

Table I. Characteristics of patients with terbinafine-resistant *Trichophyton rubrum* isolates

Pat. No.	Strain	Age, years/ sex	Type of dermatophytosis	Affected toenail	Amino acid substitution	MICs (mg/l)				Treatment history		Treatment
						TBF	ITCZ	RVCZ	LLCZ	Oral TBF	Topical SQLE inhibitor	
1	N79[3)]	39/F	*T. pedis et unguium*	Lt. V, Rt. V	Leu393Phe	>32	<0.03	<0.03	<0.03	9Mo	+	F-RVCZ
2	IFM 66684	75/M	*T. unguium*	Rt. I–V, Lt. I, II	Leu393Phe	>32	<0.03	<0.03	<0.03	–	–	F-RVCZ
3	IFM 66728	54/M	*T. cruris et unguium*	Rt. I, III–IV, Lt. I–V	Leu393Phe	>32	0.125	<0.03	<0.03	1Mo	–	F-RVCZ
4	IFM 66729	78/M	*T. unguium*	Rt. I–V, Lt. IV, V	Leu393Phe	>32	0.125	<0.03	<0.03	5Mo	–	F-RVCZ
5	IFM 66863	32/F	*T. pedis*	–	Leu393Phe	>32	0.125	<0.03	<0.03	36Mo	+	LLCZ
6	IFM 66864	36/M	*T. pedis*	–	Leu393Phe	>32	0.25	<0.03	<0.03	12Mo	–	LLCZ
7	IFM 66865	48/M	*T. pedis*	–	Leu393Phe	>32	0.5	<0.03	<0.03	–	+	LLCZ
8	IFM 66866	28/M	*T. pedis*	–	Leu393Phe	>32	1	<0.03	<0.03	–	–	LLCZ
9	IFM 66867	70/F	*T. unguium*	Rt. I	Leu393Phe	>32	0.5	<0.03	<0.03	–	–	EFCZ
10	IFM 66868	61/M	*T. pedis et unguium*	Rt. I–III, V, Lt. I–V	Leu393Phe	>32	0.125	<0.03	<0.03	16Mo	–	F-RVCZ
11	IFM 66869	35/M	*T. pedis*	–	Leu393Phe	>32	0.25	<0.03	<0.03	–	–	LLCZ
12	IFM 66870	38/M	*T. pedis et unguium*	Rt. I–IV, Lt. I, III, IV	Leu393Phe	>32	0.25	<0.03	<0.03	36Mo	+	F-RVCZ
13	IFM 66730*	50/M	*T. unguium*	Rt. V, Lt. V	Leu393Phe	–	–	–	–	29Mo	+	F-RVCZ
14	IFM 66731*	19/M	*T. pedis*	–	Leu393Phe	–	–	–	–	–	–	LLCZ

T.; tinea; TBF; terbinafine; ITCZ; itraconazole; RVCZ; ravuconazole; LLCZ; luliconazole; SQLE; squalene epoxidase; EFCZ; efinaconazole; F-RVCZ; fosravuconazole; Rt.; right; Lt.; left; F: female; M: male.
*Sensitivity test failed due to contamination.

patient. Because Japanese people remove their shoes and walk barefoot in tatami rooms, the infection may spread even in a living room (6).

Itraconazole resistance in *T. rubrum* depends not on mutation of the target enzyme (lanoconazole 14-α-demethylase), but on the overexpression of the *TruNDR2* gene, which encodes multidrug transporters of the ABC family (7). Itraconazole-resistant *T. rubrum* (TIMM20092) was isolated from a tinea pedis patient in Switzerland with a minimum inhibitory concentration (MIC) of 0.5 mg/l for itraconazole (7, 8). In our study, the MIC for itraconazole ranged from <0.03 to 1 mg/l. The MICs for ravuconazole and luliconazole were <0.03 mg/l, suggesting separated mechanisms of resistance.

Fosravuconazole, which was approved for tinea unguium treatment by the Japanese government in 2018 (9), is a novel triazole antifungal drug developed as a water-soluble prodrug for ravuconazole (10). Its excellent oral absorbability and systemic bioavailability have resulted in high serum drug concentrations and a long half-life (10). We treated 7 severe cases of tinea unguium with oral fosravuconazole and 1 mild case with efinaconazole 10% solution (11). Six patients with tinea pedis were treated with luliconazole 1% cream. As of July 2021, among the 7 fosravuconazole-treated patients with tinea unguium, 3 had been successfully cured, while 2 were showing a positive improvement. In addition, one patient treated with efinaconazole was also found to be improving. Among the 6 luliconazole-treated patients with tinea pedis, 5 had been successfully cured, while the other patient with a hyperkeratotic type had begun a treatment regimen with oral fosravuconazole.

A questionnaire survey of antifungal-resistant dermatophytes in representatives from European countries revealed that 85% of all countries (17/20) observed clinical and/or mycological confirmed resistance to terbinafine in 64% (61/96) and to itraconazole in 41% (39/96), while also observing resistance to fluconazole in 16% (15/96) (12). The 3 prevalent species were *T. rubrum* (33/95), *Microsporum canis* (23/95), and *T. mentagrophytes* (17/95) (12). Dermatologists should focus on the increasing numbers of terbinafine-resistant dermatophytes.

ACKNOWLEDGEMENTS

This work was partly supported by the Japan Agency for Medical Research and Development, AMED under Grant Number JP21fk0108094. The authors thank Dr. Yoshiharu Ohsato (Kahotechno Co., Ltd., Fukuoka, Japan) for the genetic diagnosis of onychomycosis.

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