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Article *in* Mycoses · February 2024

DOI: 10.1111/myc.13708

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Received: 21 December 2023 | Revised: 8 February 2024 | Accepted: 9 February 2024

DOI: 10.1111/myc.13708

ORIGINAL ARTICLE

mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases

WILEY

Terbinafine resistance in *Trichophyton mentagrophytes* and *Trichophyton rubrum* in the Czech Republic: A prospective multicentric study

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Funding information
Charles University Grant Agency (GAUK 379821); project Strategie AV21"VP33 Houby kolem nás i v nás" of the Czech Academy of Sciences; Czech Academy of Sciences Long-term Research Development Project (RVO: 61388971); Czech Ministry of Health (grant NU21-05-00681)

Abstract

Background: Terbinafine, an allylamine antifungal, is crucial for treating dermatophytosis by inhibiting squalene epoxidase (SQLE) in the ergosterol biosynthetic pathway. However, resistance is emerging, particularly in India and Southeast Asia, but reports of resistance spread worldwide. Despite this, comprehensive studies on terbinafine resistance in *Trichophyton* are still limited.

Objectives: This research aimed to determine the prevalence of terbinafine resistance in the Czech Republic, with a focus on *Trichophyton rubrum* and *Trichophyton mentagrophytes*, and investigate the underlying molecular mechanisms.

Patients/Methods: A total of 514 clinical strains of *T. rubrum* and 240 *T. mentagrophytes* collected from four Czech clinical institutions were screened for terbinafine resistance. Molecular investigations included DNA sequencing, specifically the ITS rDNA region and SQLE gene, as well as antifungal susceptibility testing following EUCAST guidelines.

Results: While no resistance was observed in *T. rubrum*, 2.5% of *T. mentagrophytes* strains exhibited resistance, marked by the F397L mutation in SQLE. Notably, resistance surged from 1.2% in 2019 to 9.3% in 2020 but reverted to 0% in 2021. All resistant strains were identified as *T. mentagrophytes* var. *indotineae*. Resistant strains exhibited high MICs for terbinafine (≥4 mg L^{-1}) but low MICs to the other seven antifungals tested except for fluconazole.

Conclusions: This study highlights the emergence of terbinafine-resistant *T. mentagrophytes* strains in the Czech Republic, with the F397L mutation being pivotal. Due to the relatively low resistance level, the current guidelines for dermatomycosis treatment in the Czech Republic remain effective, but ongoing surveillance is essential for timely adaptations if resistance patterns change.

KEYWORDS
allylamines, anthropophilic dermatophytes, antifungal resistance, dermatomycosis, squalene epoxidase, zoophilic dermatophytes

1 | INTRODUCTION

Dermatophytoses are treated with different classes of antifungals, with terbinafine being a first-choice treatment. Terbinafine is an antifungal from the class of allylamines that targets the cell membrane. It specifically inhibits the enzyme squalene epoxidase (SQLE), which disrupts the synthesis of squalene epoxide, a precursor of ergosterol. Ergosterol is the main sterol found in fungal plasmatic membranes and plays a role in maintaining membrane fluidity and permeability.[1] By disrupting the ergosterol biosynthetic pathway, terbinafine depletes ergosterol and causes the accumulation of squalene, leading to increased cell membrane permeability and cell death.[2]

The increase in the incidence of resistance, particularly in *Trichophyton mentagrophytes* and *T. rubrum* species, is currently reported especially from India and South-East Asia. The problem with dermatomycoses treatment in this region began as early as 2010 and has significantly worsened in subsequent years.[3] Resistant isolates often have reduced affinity for terbinafine due to point mutations in the SQLE gene resulting in the substitution of one or more amino acids, altering the conformation of the substrate binding site and significantly reducing the affinity of the drug for the enzyme.[4,5] This results in a resistant phenotype of the strains and the minimum inhibitory concentration (MIC) of the antifungal increases. In South-East Asia, inappropriate combinations of antibiotics, antifungals and corticosteroid creams are commonly used to treat dermatophytoses. Patients often self-treat with these combination products because they are readily available without a prescription and at a relatively low cost.[6] Other forms of inadequate treatment include failure to complete the entire treatment cycle, low dosage, and non-adherence to dose spacing. These factors contribute to treatment failure and the persistence of the causative agent, which leads to the selection of less susceptible strains.[7]

The prevalence of terbinafine resistance in India based on various studies ranges from 0% to 76%, and it is influenced by factors such as the number of strains tested, the patient group studied, and geographical variation.[8,9] Terbinafine resistance in the genus *Trichophyton* is not only restricted to South-East Asia region but is increasingly reported worldwide. One possible reason for the high incidence of resistance may be the spread of resistant strains by travellers from India, Bangladesh, Pakistan, Saudi Arabia or Thailand,[10] however, a large proportion of infections is reported in residents, suggesting community spread.[11] Individual case reports or series of cases of resistant *T. rubrum* or *T. mentagrophytes* have been documented in various European and non-European countries, including Belgium,[12] Canada,[13] Denmark,[14] France,[10] Germany,[11] Greece,[8] Iran,[15,16] Italy,[17] Japan,[18,19] Poland,[20] Russia,[21] Turkey,[22] and the USA.[23,24] However, only a few studies have investigated the prevalence of terbinafine resistance in a significant number of *T. rubrum* or *T. mentagrophytes* strains over time. Specifically, studies conducted in Switzerland investigated samples from patients with suspected dermatophyte infections in a single dermatology department between 2013 and 2016, and between 2013 and 2021, respectively. These studies found an overall prevalence of 0.92%–0.97% for *T. rubrum* and 0.24%–0.57% for *T. mentagrophytes* during the mentioned periods.[25,26] A prospective multicenter study was conducted in France, involving seven diagnostic laboratories in the Paris area, between January and September 2021. The study found a prevalence of resistance of 0.23% for *T. rubrum* and 0.69% for *T. mentagrophytes* among patients with superficial mycosis.[27] A study conducted in Greece from 2010 to 2019 examined all dermatophytes collected in a single laboratory. It found that none of the tested *T. rubrum* isolates were resistant. However, 25% of the *T. mentagrophytes* isolates showed resistance. Specifically, of the 36 *T. mentagrophytes* isolates tested, none of the 12 *T. mentagrophytes* var. *interdigitale* or 15 *T. mentagrophytes* var. *mentagrophytes* were resistant, while all 9 *T. mentagrophytes* var. *indotineae* isolates showed resistance to terbinafine.[8] Another study focused on toenail samples from patients with suspected tinea unguium across the USA. This study reported a prevalence of resistance of 3.6% for *T. rubrum* and 4.3% for *T. mentagrophytes*.[28] Similarly, another study conducted between 2021 and 2022, which investigated all dermatophyte isolates received by a single reference laboratory from institutions across North America reported a high rate of resistance of 18% for *T. rubrum* (21 resistant isolates out of 117) and 56% for *T. mentagrophytes* (23 resistant isolates out of 41: 1/15 *T. mentagrophytes* var. *mentagrophytes*, 1/5 *T. mentagrophytes* var. *interdigitale* and 21/21 *T. mentagrophytes* var. *indotineae*) (pers. comm. with N. Wiederhold).[29] These high resistance rates may overestimate the real prevalence of terbinafine resistance because specialised clinical laboratories often receive strains with suspected resistance.[30] The prevalence of terbinafine resistance in the Czech Republic has not been studied yet.

The objective of our study was to evaluate the prevalence of terbinafine resistance in the Czech Republic in *T. rubrum* and *T.*

mentagrophytes, the species most commonly associated with resistance. Additionally, we sought to determine the molecular mechanism underlying this resistance. Given the growing global trend of resistance, our research is significant in documenting its initial stages and gaining insight into its future dynamics.

2 | MATERIALS AND METHODS

2.1 | Clinical isolates

Clinical isolates of *T. mentagrophytes* were collected prospectively from 2018 to 2021 and *T. rubrum* isolates were collected from 2020 to 2021 from four Czech clinical institutes: Dermatovenerology Clinic of the General University Hospital and the First Faculty of Medicine, Charles University, Prague; Public Health Institute in Ústí nad Labem, Prague; Public Health Institute in Ostrava, Ostrava; and Hospital České Budějovice, České Budějovice. The isolates were obtained from patients with various types of dermatomycoses, primarily tinea unguium, tinea pedis and tinea corporis (including the tinea faciei and tinea cruris subtypes). All the samples were submitted for initial diagnosis without a history of antifungal therapy.

The isolates were initially identified by clinicians or laboratory personnel based on the macromorphology of colonies and micromorphological characters as either *T. rubrum* or *T. mentagrophytes*/*T. interdigitale*. The identification of all *T. mentagrophytes*/*T. interdigitale* isolates was confirmed through DNA sequencing of the ITS rDNA region (see below). Molecular identification of *T. rubrum* isolates was conducted solely for morphologically atypical strains, while strains with typical morphology were considered to be *T. rubrum* (characterised by relatively slow growing, fluffy to cottony colonies, that are initially white but may turn cream or rose with age, with a reverse colour of wine-red, brown, olive, or yellow; microconidia are usually clavate or pyriform, and macroconidia are usually absent).[31,32] This identification approach to *T. rubrum* is based on our previous work where the same institutions identified morphologically typical *T. rubrum* strains by morphology and atypical strains by molecular methods. When molecular methods were used to confirm morphological identification, the error rate was <1%.[33]

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the research in this article related to microorganisms.

2.2 | Molecular studies

The DNA was isolated from colonies growing on malt extract agar (MEA; malt extract from Oxoid, Basingstoke, UK) incubated for 10 days at 25°C. A commercial kit ZR Fungal/Bacterial DNA Kit™ (ZYMO RESEARCH, Irvine, California, USA) was used and the instructions from manual were followed. The quality of the DNA was verified using the NanoDrop Spectrophotometer. The ITS and partial LSU rDNA region was amplified using forward primer ITS1 (TCCGTAGGTGAACCTGCGG) and reverse primer NL4 (GGTCCGTGTTTCAAGACGG). The PCR protocol by Hubka et al. was followed.[34] PCR amplicons were purified using EXOSAP PP-218 L (Jena Bioscience, Jena, Germany) and sequenced using both terminal primers by Sanger sequencing Bioanalyzer 2100 (Agilent, Santa Clara, USA) at BIOCEV, Czech Republic. The sequences were inspected and assembled using Bioedit v. 7.2.5 (https://bioedit.software.informer.com/7.2/). Identification was achieved by comparing the obtained ITS sequences with sequences originating from ex-type strains of the respective taxa (*T. rubrum*: CBS 392.58, GenBank: MH857821.1; *Trichopyton mentagrophytes* var. *interdigitale*: CBS 428.63, OM283536; *T. mentagrophytes* var. *mentagrophytes*: IHEM 4268, OM283542; *T. mentagrophytes* var. *indotineae*: CCF 6597 = NUBS 19006, OM283543). A similarity of more than 99% was considered sufficient for correct identification of *T. rubrum*. Identification of strains with lower similarity (occurring only in *T. mentagrophytes* complex) was achieved by phylogenetic analysis that included different ITS genotypes of *T. mentagrophytes*.[35] The taxonomical recommendations proposed by Švarcová et al. [36] regarding the *T. mentagrophytes* complex were followed. The inclusive definition of *T. mentagrophytes*, which includes *T. interdigitale* and *T. indotineae* were adopted. When referring to specific subpopulations, the variety rank, *T. mentagrophytes* var. *mentagrophytes*, *T. mentagrophytes* var. *interdigitale* and *T. mentagrophytes* var. *indotineae*, was used.

The ITS sequences of the *T. mentagrophytes* complex obtained in this study were utilised to create the phylogenetic tree. The dataset was supplemented with additional sequences representing various ITS genotypes.[35,37] The alignment was performed using the MAFFT7 program[38] and the FFT-NS-2 strategy. The maximum likelihood (ML) method was employed to construct the phylogenetic tree in IQ-TREE v. 2.1.2.[39] Node supports were determined through ultrafast bootstrapping (BS) with 100,000 iterations. The tree was rooted with *Trichophyton quinckeanum*.

The SQLE gene was sequenced in the resistant isolates (including all remaining isolates classified as *T. mentagrophytes* var. *indotineae*) to identify potential mutations responsible for resistance. Amplification was performed using SQLE-fw1 and SQLE-rv1 primers and sequencing using SQLE-fw1, SQLE-fw2, SQLE-fw3, SQLE-rv1, SQLE-rv2 and SQLE-rv3 primers.[40] The following PCR thermal cycle profile was used: 95°C–1 min; 35 cycles of 92°C–20 s, 55°C–30 s, 72°C–60 s; and a final extension 72°C–10 min. PCR amplicons were purified and sequenced as described above. The resulting amino acid sequences of SQLE were compared with those of susceptible strain (WT) of *T. mentagrophytes* TIMM2789 (GenBank accession number: KU242352).

2.3 | Antifungal susceptibility testing

A total of 514 isolates identified as *T. rubrum* and 240 isolates identified as *T. mentagrophytes* were screened for resistance to terbinafine. The testing was conducted using a screening plate with 24 wells (Nunc 24-well multidishes, Thermo Fisher Scientific, Waltham, USA) which was designed to test 12 strains. In the experiment, the antifungal terbinafine (TERB) was added to the agar medium in 12 wells at a concentration of 0.125 μg mL^{-1}. The remaining 12 wells were filled with agar medium without the antifungal drug and served as a control.

Agar medium with 0.125 μg mL^{-1} of terbinafine (Merck, Sigma Aldrich, St. Luis, USA) was prepared as follows: one litre of the medium contained 900 mL of distilled water, 10.4 g of RPMI 1640 (Merck, Sigma Aldrich, St. Luis, USA), 18 g of glucose, 20 g of Bacto agar (Merck, Sigma Aldrich, St. Luis, USA), 34.53 g of MOPS (Merck, Sigma Aldrich, St. Louis, USA), 0.8 mL of chloramphenicol (final concentration 0.04 mgL^{-1}) and 5 mL of cycloheximide (final concentration 0.5 mgL^{-1}) (all Sigma Aldrich, St. Louis, USA). The pH was adjusted to 7.0 with 10 M NaOH, and then distilled water was added to reach a final volume of 990 mL. The solution was autoclaved at 121°C for 15 min and then poured into two Erlenmeyer flasks (500 mL each). The agar was cooled down to 45°C. Five millilitres of the antifungal working solution with terbinafine (100-fold final concentration) was added to one of the Erlenmeyer flasks. The remaining antifungal-free flask was used as the growth control. Agar was aliquoted from each flask into the respective wells of the multi-dish plate (1 mL per well). The plates were stored at 4°C for up to 3 months.

A suspension of conidia was prepared by covering the colonies with 5 mL of sterile distilled water supplemented with 0.1% Tween 20 (Thermo Fisher Scientific, Waltham, USA). The colonies were disrupted using a stick with a cotton swab, creating a dense suspension of conidia and distilled water. This mixture was added to distilled water and mixed until an even turbidity was achieved. To adjust the density of the suspension to 0.5 McFarland (concentration of $2–5 \times 10^6$ conidia mL^{-1}), distilled water was added and the resulting density was checked using the densitometer DEN-1B (BIOSAN, Riga, Latvia). The screening plate was inoculated with 25 μL of the suspension (each isolate was inoculated on one well with medium containing terbinafine and one control medium without terbinafine). The screening plate was incubated at 25°C for 7 days and then the growth of colonies was evaluated visually. If any growth was detected, the strain was considered terbinafine-resistant and subsequently, an exact MIC was determined (see below). *Aspergillus flavus* (ATCC 204304) was used as a quality control (QC) strain.

All terbinafine-resistant strains identified in this study were deposited into the Culture Collection of Fungi (CCF) in Prague, Czech Republic (Charles University, Faculty of Science, Department of Botany).

2.4 | MIC determination (EUCAST)

The broth microdilution method was performed in accordance with the EUCAST document E.Def 11.0.[41] The antifungals and their respective concentration ranges (mg L^{-1}) were as follows: fluconazole (FLU; 0.06–64), terbinafine (TERB; 0.004–4), itraconazole (ITR; 0.008–8), ketoconazole (KET; 0.008–8), clotrimazole (CLO; 0.008–8), amorolfine (AMO; 0.008–8), ciclopirox olamine (CIC; 0.008–8) and efinaconazole (EFI; 0.008–8).

The isolates were incubated at 25°C on Sabouraud dextrose agar (Trios, Prague, Czech Republic). Inoculum suspensions were prepared from colonies that were 1–3 weeks old to achieve sufficient sporulation. The suspensions were prepared in sterile distilled water supplemented with 0.1% Tween 20 and filtered using sterile nylon filters with a pore size of 11 um (Merck, Prague, Czech Republic). To adjust the density of the suspension to 0.5 McFarland (concentration of $2–5 \times 10^6$ conidia mL^{-1}), distilled water was added and the resulting density was checked using the densitometer DEN-1B (BIOSAN, Riga, Latvia). The suspension was then diluted 1:10 with sterile distilled water to obtain a final working inoculum of $2–5 \times 10^5$ cfu mL^{-1}. Inoculum suspension was supplemented with chloramphenicol (Sigma Aldrich, St. Luis, USA; final concentration 50 mg L^{-1}) and cycloheximide (Sigma Aldrich, St. Louis, USA; final concentration 300 mg L^{-1}). The microplates were incubated at 25°C in ambient air for 5–7 days. The endpoints (MICs) were read spectrophotometrically as 50% inhibition. *Aspergillus flavus* (ATCC 204304) was used as a QC strain.

3 | RESULTS

3.1 | Examined clinical isolates

In this study, a total of 514 strains of *T. rubrum* and 240 strains of *T. mentagrophytes* were collected from four institutions and examined for terbinafine resistance. *Trichophyton rubrum* isolates were collected in 2020 ($n = 171$) and 2021 ($n = 343$), while *T. mentagrophytes* isolates were collected from 2018 to 2021 (2018, $n = 32$; 2019, $n = 84$; 2020, $n = 54$; 2021, $n = 70$). The most common type of dermatophytosis among patients with *T. rubrum* infection was tinea unguium, followed by tinea pedis and tinea corporis. The median age of the patients was 48 years (Table 1). Isolates of *T. mentagrophytes* were predominantly collected from patients with tinea unguium, followed by tinea corporis and tinea pedis. The median age of the patients was 47 years (Table 1). Based on molecular identification using ITS marker, seven strains were classified as *T. mentagrophytes* var. *indotinae* (ITS genotype VIII), 162 as *T. mentagrophytes* var. *interdigitale* (including mixed genotype II*) and 71 as *T. mentagrophytes* var. *mentagrophytes* (Figure S1).

3.2 | Screening for terbinafine resistance and antifungal susceptibility testing

Antifungal resistance to terbinafine was tested in all strains of *T. rubrum* and *T. mentagrophytes* using a screening method. Among the *T. rubrum* strains, no resistance was found. However, six resistant strains were revealed among the *T. mentagrophytes* strains, resulting in an overall resistance rate of 2.5% between 2018 and 2021. These

TABLE 1 Clinical-epidemiological data associated with patients infected by *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

Species	Descriptive categories	All strains	Clinical manifestation: Tinea unguium	Tinea pedis	Tinea corporis
T. rubrum	Number of strains	514	316	129	69
	Males: *n* (%)	341 (66.3%)	204 (64.6%)	82 (63.6%)	55 (79.7%)
	Females: *n* (%)	173 (33.7%)	112 (35.4%)	47 (36.4%)	14 (20.3%)
	Age range-all (median)	2–96 (48)	2–96 (47)	7–86 (48)	18–83 (52)
	Age range males (median)	2–86 (50)	2–86 (50)	17–80 (47.5)	18–83 (52)
	Age range females (median)	4–96 (45)	4–96 (44)	7–86 (48)	18–77 (46)
T. mentagrophytes	Number of strains	240	96	66	78
	Males: *n* (%)	115 (47.9%)	53 (55.2%)	34 (51.5%)	28 (35.9%)
	Females: *n* (%)	125 (52.1%)	43 (44.8%)	32 (48.5%)	50 (64.1%)
	Age range-all (median)	1–84 (47)	21–84 (59)	5–81 (46)	1–76 (27)
	Age range males (median)	3–84 (50)	21–84 (57)	8–81 (43.5)	3–70 (27)
	Age range females (median)	1–80 (46)	21–80 (63)	5–75 (46)	1–76 (25.5)

resistant strains were collected in 2019 ($n=1$) and 2020 ($n=5$), with no resistant strains detected in 2018 and 2021. The resistance level in 2018 was 0%, 1.2% in 2019 (1/84), 9.3% in 2020 (5/54) and 0% in 2021 (0/70). In addition to these six resistant strains, all belonging to *T. mentagrophytes* var. *indotineae*, one additional strain of *T. mentagrophytes* var. *indotineae* (CCF 7006) was revealed (which tested negative in the screening but was detected using phylogenetic analysis based on the ITS region - Figure S1).

All *T. mentagrophytes* var. *indotineae* strains were tested using the EUCAST method to determine the MIC for several antifungal drugs (Table 2). For all resistant isolates, the MIC for terbinafine was >4 mg L^{-1}, while in isolate CCF 7006, the MIC was 0.008 mg L^{-1}. The MICs of azole antifungals (itraconazole, ketoconazole, amorolfine and efinaconazole), ciclopirox olamine and clotrimazole were low in all tested isolates, while for fluconazole, the MICs ranged between 8 and >64 (Table 2).

3.3 | SQLE sequencing and analysis of mutations

Six out of seven *T. mentagrophytes* var. *indotineae* isolates had a missense mutation F397L. No mutations in the SQLE gene were found in the terbinafine-susceptible strain CCF 7006. The information regarding patients with resistant strains is summarised in Table 2. The SQLE and ITS sequences of *T. mentagrophytes* var. *indotineae* strains identified in this study were deposited into the GenBank database. The corresponding accession numbers can be found in Table 2.

4 | DISCUSSION

Terbinafine-resistant *T. rubrum* and *T. mentagrophytes* are increasingly reported worldwide.[7] However, the prevalence of terbinafine resistance in a large and unbiased dataset of strains (which includes all cases, not just the suspected resistant ones) has only been reported from several countries, including India,[9] Switzerland,[25,26] Greece,[8] France,[27] and the USA.[28] There is still lack of data from many countries, including the Czech Republic. Although the occurrence of a single *T. mentagrophytes* strain with the ITS genotype VIII (*T. mentagrophytes* var. *indotineae*) has been reported from the Czech Republic already in 2016, this isolate showed low MICs to terbinafine.[36] Further studies dealing with dermatophyte antifungal susceptibilities in the Czech Republic were restricted to *Trichophyton benhamiae* complex and *T. quinckeanum* and no terbinafine resistance was detected.[42,43]

In this study, we screened 514 *T. rubrum* isolates collected between 2020 and 2021, as well as 240 *T. mentagrophytes* isolates obtained between 2018 and 2021 for terbinafine resistance. Figure 1 provides a comprehensive summary of the methodologies used to detect resistance, as well as the underlying molecular mechanisms. We found only six resistant *T. mentagrophytes* isolates and no resistant *T. rubrum* isolates. Molecular identification using ITS rDNA revealed that all of these resistant isolates, along with one susceptible isolate, belonged to the *T. mentagrophytes* var. *indotineae* (Figure S1).[36,44] All six resistant *T. mentagrophytes* var. *indotineae* strains showed missense mutation F397L, while the remaining susceptible strain showed no mutation in the SQLE gene (Table 2). The mutation F397L belongs to the most common mutations reported worldwide and is responsible for the high level of resistance to terbinafine.[8,9,26,28,45] All *T. mentagrophytes* var. *indotineae* strains identified in this study showed low minimum inhibitory concentrations (MICs) to antifungals amorolfine, ciclopirox olamine and azole antifungals except for fluconazole (Table 2), to which most dermatophytes are naturally resistant.[46] Dermatophytes are usually susceptible to azole antifungals, which makes azoles a suitable alternative for treating of dermatomycoses if terbinafine treatment fails.[47]

According to our study, the prevalence of terbinafine resistance in the Czech Republic is 0% in *T. rubrum* and 2.5% in *T.*

TABLE 2 Clinical information, antifungal susceptibility and genetic data associated with patients infected by *Trichophyton mentagrophytes* var. *indotinae*.

Strain number	Age/sex	Clinical manifestation	Place of residence	Isolation date	Mutation (SQLE)	GenBank accession numbers		MICs (mg L^{-1}) determined using EUCAST E.def 11.0							
						ITS	SQLE	TRB	FLU	ITR	KET	CLO	AMO	CIC	EFI
CCF 6687	44 ♂	Tinea pedis	Prague	IV/2019	F397L	OR863411	OR862934	>4	16	0.008	0.064	0.5	0.064	1	0.016
CCF 6639	21 ♂	Tinea unguium	Hostivice	I/2020	F397L	OR863412	OR862935	>4	16	0.016	0.125	1	0.125	1	0.125
CCF 6685	29 ♂	Tinea corporis	Prague	I/2020	F397L	OR863413	OR862936	>4	>64	≤0.008	0.125	1	0.064	1	0.032
CCF 6688	23 ♀	Tinea corporis (cruris)	České Budějovice	III/2020	F397L	OR863414	OR862937	>4	8	≤0.008	0.032	0.125	0.064	0.5	0.016
CCF 6640	36 ♀	Tinea unguium	Prague	IV/2020	F397L	OR863415	OR862938	>4	32	0.032	0.25	1	0.064	1	0.064
CCF 6686	59 ♀	Tinea unguium	Prague	IV/2020	F397L	OR863416	OR862939	>4	16	≤0.008	0.125	0.5	0.064	1	0.016
CCF 7006	27 ♂	Tinea corporis (faciei)	Plzeň	XI/2021	Absent	OR863417	OR862940	0.008	1	≤0.008	≤0.008	0.064	0.064	0.5	≤0.008

Abbreviations: ♂, male; ♀, female; AMO, amorolfine; CIC, ciclopirox olamine; CLO, clotrimazole; EFI, efinacozole; FLU, fluconazole; ITR, itraconazole; KET, ketoconazole; MIC, minimal inhibition concentration; SQLE, squalene epoxidase; TRB, terbinafine.

mentagrophytes. These rates have increased from 0% in 2018 to 1.2% in 2019 and further to 9.3% in 2020. The absence of resistance in *T. rubrum* is in agreement with study from Greece and contrasts with studies from Denmark, France, Switzerland and the USA where resistance rates ranging from 0.23% to 3.6% have been found.[8,14,25,27,28] In *T. mentagrophytes*, the level of resistance detected (2.5%) is higher than the levels detected in Switzerland (0.24%–0.57%) and France (0.65%),[25,26] but lower than the levels in the USA (4.3%), Greece (25%) and India (up to 76%).[8,9,11,28] The predominant clinical manifestation of infections with terbinafine-resistant *T. mentagrophytes* in Czechia was tinea unguium (3/6), as well as in patients from Switzerland.[25] This is in contrast to the predominant clinical manifestation reported for *T. mentagrophytes* var. *indotineae*, which is tinea corporis, tinea cruris and tinea faciei.[11]

The origin of resistant strains detected in our study can be only partly attributed to travel and migration. One resistant strain was found in a patient of Indian nationality, and one isolate originated from a patient whose partner was recently living in Vietnam. Remaining four resistant isolates were found in patients with Czech nationality or long-term residence where travel to areas with a high level of resistance was not reported in case history. Interestingly, five of the six resistant *T. mentagrophytes* strains were isolated in the short window of a few months (January 2020 to April 2020) preceding the strict lockdowns in the Czech Republic during COVID-19 pandemic. We can hypothesize that the reduction in contacts during lockdowns and the inability to travel may have had an impact on preventing the initial community spread of these strains, as no resistant isolates were detected during the period May 2020 to December 2021.

Although similar resistance screening did not continue in 2022, we continued to identify *T. mentagrophytes* strains prospectively collected from several clinical institutes using ITS region sequencing. Among 92 strains received in 2022, we detected only two strains of *T. mentagrophytes* var. *indotineae*. Antifungal susceptibility testing and mutation analysis showed that only one of these strains was resistant to terbinafine (mutations F397L and A448T; isolate CCF 6704; ITS sequence GenBank: OR863418; SQLE sequence GenBank: OR862941; isolated from patient with Indian nationality) while the second strain was susceptible to terbinafine (mutation A448T; isolate CCF 6705; ITS sequence GenBank: OR863419; SQLE sequence GenBank: OR862942; patient with Czech nationality). These data show that the only resistant isolate detected so far after the pandemic can probably be attributed to migration, and that these isolates are different in their combination of mutations from pre-pandemic isolates.

Despite the current low levels of resistance, it is crucial to continue monitoring the situation, conducting routine screening of clinical isolates, and remaining vigilant due to the increasing trend of resistance in neighbouring countries and the potential for increased population migration. At present, the established guidelines for the treatment of dermatomycosis in the Czech Republic, specifically the use of terbinafine, do not require immediate revision due to the low level of resistance. However, ongoing monitoring and surveillance are necessary to address any potential future increase in resistant isolates of the genus *Trichophyton*.

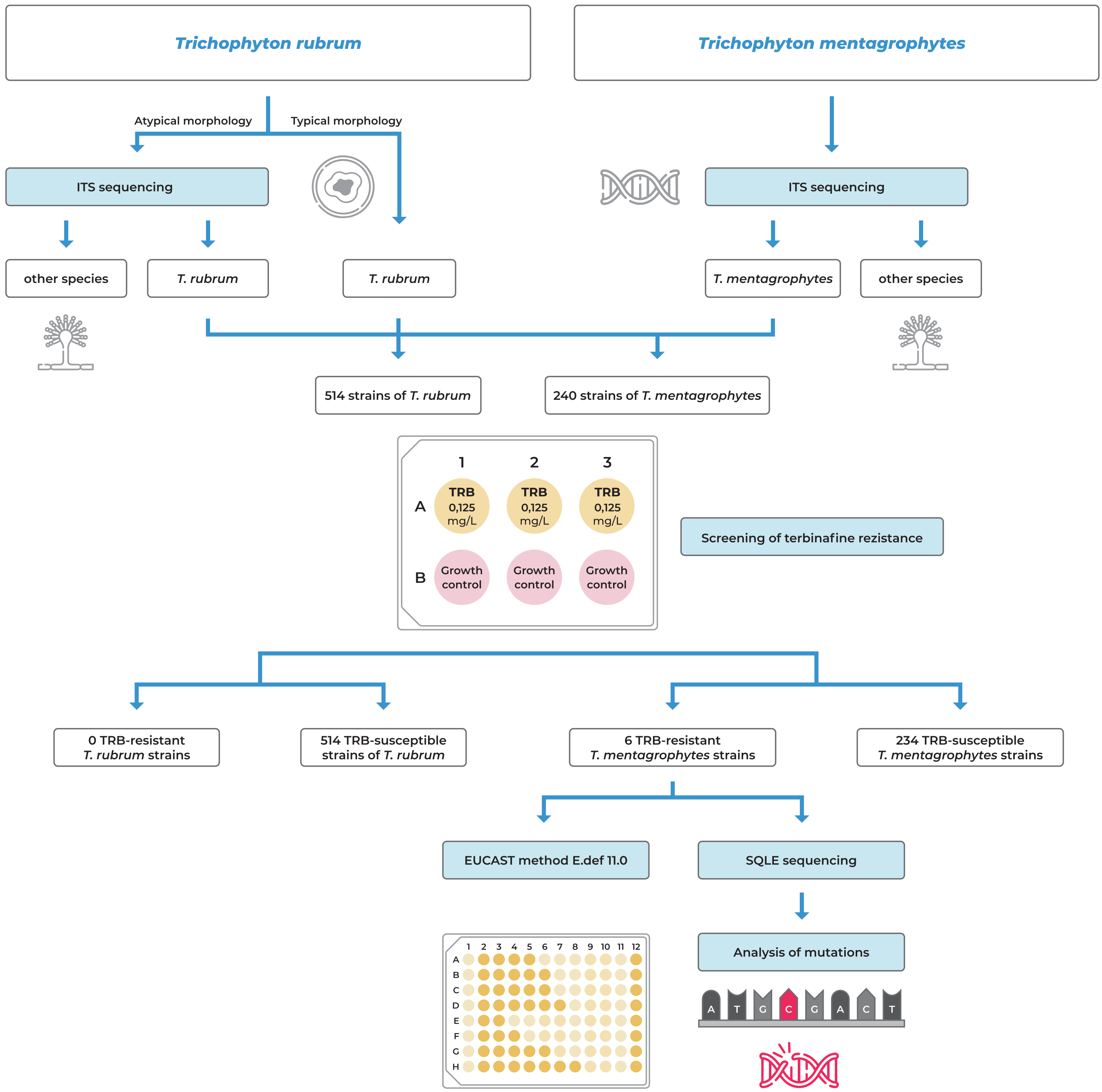

FIGURE 1 Methodological procedure for the detection of terbinafine resistance in *Trichophyton rubrum* and *T. mentagrophytes* in the present study.

ACKNOWLEDGEMENTS

Daniela Kolarczyková was supported by the project of Charles University Grant Agency (GAUK 379821). The contribution of Vit Hubka and Miroslav Kolařík were supported by the Czech Ministry of Health (grant NU21-05-00681), project Strategie AV21'VP33 Houby kolem nás i v nás' of the Czech Academy of Sciences and Czech Academy of Sciences Long-term Research Development Project (RVO: 61388971). We are grateful to Jan Karhan for help with graphical adjustments of Figures. We thank Lenka Zídková and Kateřina Glässnerová for their invaluable assistance in the laboratory. The research reported in this publication was part of the long-term goals of the ISHAM working group Onygenales. Open access publishing facilitated by Univerzita Karlova, as part of the Wiley - CzechELib agreement.

CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available. The DNA sequences of ITS rDNA region and squalene epoxidase genes of *T. mentagrophytes* var. *indotineae* were deposited to

GenBank database (Table 2). The strains are available in the CCF collection of fungi upon request (Prague, Czech Republic).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kolarczyková D, Lysková P, Švarcová M, et al. Terbinafine resistance in *Trichophyton mentagrophytes* and *Trichophyton rubrum* in the Czech Republic: A prospective multicentric study. *Mycoses*. 2024;67:e13708. doi:10.1111/myc.13708