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Carolina Barbosa Vidal Ferreira Boysen

Emergence of Terbinafine-Resistant *Trichophyton indotineae* in Europe

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Microbiologia Clínica

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Emergence of Terbinafine-Resistant *Trichophyton indotineae* in Europe

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UC Dissertação - DECLARAÇÃO DE TRANSPARÊNCIA RELATIVAMENTE À UTILIZAÇÃO DE FERRAMENTAS DE CHATBOT GENERATIVO BASEADAS EM LARGE LANGUAGE MODELS

Eu, **Carolina Barbosa Vidal Ferreira Boysen,** abaixo assinado, nº mecanográfico **201910430**, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro que:

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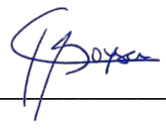

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Ao Gunther, *sine qua non*

A Systematic Review on the Emergence of Terbinafine-Resistant *Trichophyton indotineae* in Europe: Time to Act?

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Data availability
Data is available in the articles referenced in the study and listed in the References.

Author contributions
Conceptualization, C.B.F. and C.L.; methodology, C.B.F. and C.L.; Investigation, C.B.F. and C.L.; Writing - original draft preparation, C.B.F; Writing - review and editing, C.B.F. and C.L.; Visualization, C.B.F. and C.L.; Supervision, C.L.. Both authors have agreed to the published version of the manuscript.

Abstract

There is a recent growing global concern regarding the rise in antifungal resistance of dermatophytosis. The emergence of terbinafine-resistant *Trichophyton indotineae* (*T. indotineae*), previously known as *Trichophyton mentagrophytes* genotype VIII, is particularly alarming given that terbinafine is considered the treatment of choice for dermatophyte infections and the limited number of available antimycotics. This strain surfaced in the Indian subcontinent in the mid 2010s and more recently there have been reports of its arrival in Europe. The objective of this review is to document the presence of terbinafine-resistant *T. indotineae* in Europe with the aim to enlighten antifungal stewardship and ultimately prevent the spread of resistance. We extracted information from 16 studies published between 2019 and 2023 that described a total of 63 cases of antifungal-resistant dermatophytosis caused by *T. indotineae* in Europe. Antifungal susceptibility testing (AFST) revealed a wide range of terbinafine minimum inhibitory concentrations (MICs), varying from 0.014 to ≥ 16 µg/mL. However these values are hard to interpret due to variations among used methodologies as well as the lack of clinical breakpoints. In all cases included in this study, elevated terbinafine MICs was associated with mutations in the squalene epoxidase (SQLE) gene, including single mutations F397L and L393S, as well as double mutations such as F397L/A448T. Itraconazole was the most frequently used alternative to terbinafine in this study and complete remission of *tinea* lesions after treatment switch was shown. Nevertheless, relapse rates are concerningly high. To prevent the further spread of resistance across Europe and worldwide, we propose the implementation of surveillance programs for a fast identification of *T. indotineae* (and SQLE mutations) through sequencing and standardization of procedures for AFST to facilitate the establishment of clinical breakpoints. This may enable guidance on the appropriate use of antifungals and prevent the therapeutic challenges associated with resistance.

Introduction

Dermatophytosis, otherwise known as tinea, is the most common fungal infection of keratinised tissues causing superficial infections of the skin, hair and nails. These diseases have a significant negative impact on quality of life (QoL) due to the symptoms associated (intractable itching, burning sensation, sleep disturbance, stigmatization and depression).[1] In recent years, an outbreak of antifungal-resistant and recalcitrant dermatophytosis in South Asia led to the identification of a novel Trichophyton species, *Trichophyton indotineae* (*T. indotineae*). The most common clinical presentation is multiple inflammatory erythematous skin lesions on the trunk (*tinea corporis*), inguinal and gluteal regions (*tinea cruris et glutealis*). Initially named *Trichophyton mentagrophytes*, later reclassified as Trichophyton mentagrophytes/Trichophyton interdigitale complex or just *Trichophyton interdigitale* and finally *Trichophyton mentagrophytes* genotype VIII, these terms were used interchangeably making gathering and reviewing of the evidence rather challenging. Classification errors stemmed from the fact that these strains are indistinguishable morphologically and it is necessary to sequence the internal transcribed spacer (ITS) region in order to differentiate them.[2,3]

Terbinafine has been the cornerstone for the treatment of dermatophytosis. Terbinafine is a fungicidal drug that inhibits the ergosterol biosynthesis by targeting the squalene epoxidase enzyme (SQLE). Inhibition of this enzyme causes the accumulation of squalene inside the cell and the depletion of ergosterol which ultimately leads to cell death. Compared to azoles, it has an excellent safety profile and minimal risk of drug interactions making it the gold standard for dermatophytosis treatment.[4] Reports describing terbinafine-resistant strains were almost absent until 2017.[5-7] Since then, resistant dermatophytosis has emerged as a major health problem due to the alarming epidemic of terbinafine-resistant and recalcitrant dermatophyte infections spreading in India.[8-10] The inappropriate use of over-the-counter creams containing corticosteroid-antifungal combinations has been suggested as one likely cause for the emergence of resistance.[11]

On a molecular level, terbinafine resistance has been associated with various mutations in the gene encoding SQLE. Point mutations leading to single amino acid substitutions, such as F397L or L393F, are the most commonly reported substitutions.[8] However substitutions at other positions within the encoded protein have also been described (Q409, F415, H440).

Migration and travel have enabled the spread of terbinafine-resistant strains to Europe with sporadic reports of resistance from France, Italy, Greece, Germany, Spain, Switzerland and Denmark.[12-20] There has also been a pilot study confirming the existence of clinical and mycological resistance to antifungals in Europe.[21] However, susceptibility testing is not performed routinely due to its technical complexity and difficult implementation, suggesting a likely underestimation of antifungal resistance.[22] The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently released an updated method (E.Def 11.0) with validated tentative epidemiological cut-off values (ECOFFs) against *T. Indotineae.*[23] Nevertheless, studies on the current epidemiology of terbinafine-resistant strain *T. indotineae* in Europe are still lacking. Moreover, the absence of uniformity in dermatophyte nomenclature could mean that previously reported resistant *T. mentagrophytes* or *T. interdigitale* were in fact *T. indotineae* demanding a new look and interpretation of previously published data.[24-26] This systematic review aims at collating and interpreting the available evidence on the presence of terbinafine-resistant *T. indotineae* in Europe.

Methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[27] outlined in the PRISMA 2020 checklist. A systematic literature search was conducted in January 2024 using Pubmed, Scopus and Web of Science databases using the terms “(Trichophyton mentagrophytes genotype VIII OR Trichophyton indotineae OR Trichophyton interdigitale) AND (resistance OR treatment failure)”. The results were uploaded to Covidence systematic review manager (https://www.covidence.org/) for title and abstract screening. Reference

screening was conducted by two independent reviewers (CBF and CL) and any conflicts were resolved through discussion. References from included articles were searched for further relevant publications.

The inclusion criteria were studies reporting *T. indotineae* cases in Europe with description of clinical presentation, dermatophyte species confirmed by ITS sequencing and demonstrated clinical and/or mycological antifungal resistance. Reviews and animal studies were excluded. No publication language filters were applied.

Data extraction was performed following a protocol previously outlined by the authors which included first author, publication year, study design, study objectives, total number of *T. indotineae* cases and number of antifungal-resistant *T. indotineae* cases, European country where study was performed, country of origin or history of travel to Asia or Middle East, other dermatophyte species identified, age and gender of patients, medical history, clinical presentation, treatment (topical and systemic), dermatophyte species identification method, antifungal susceptibility testing method, susceptibility to antifungals (terbinafine, itraconazole, voriconazole and/or amorolfine), SQLE gene mutations and study conclusions.

Quality assessment to inform synthesis and interpretation of results was carried out by 2 independent reviewers (CBF and CL) and any disagreements were solved through discussion. National Institute of Health (NIH) Study Quality Assessment tools were used for observational cohort, cross-sectional and case series studies. Joanna Briggs Institute (JBI) critical appraisal tools were used for case reports and the results were considered “Good” when overall appraisal of the checklist resulted in “Include”, “Fair” when “Seek further info” and “Poor” in case of “Exclude”.

Definitions

Resistance is the term used for describing treatment failure. It encompasses lack of clinical response (clinical resistance) and/or laboratory resistance (*in*

vitro resistance). Recalcitrance refers to the chronic evolution of fungal infection with relapses and recurrences.

ECOFFs are defined as the highest MIC observed in isolates that are not considered to have resistance and hence representative of the wild type. However, for the sake of simplicity, we hereby considered strains exhibiting MIC values above the corresponding tentative ECOFF as resistant.

Clinical breakpoints are reference concentration values that can help predict likelihood of treatment response based on the MICs.

Results

Literature search

A total of 378 publications were identified from Pubmed (102), Scopus (147) and Web of Science (129). Three additional references were added by citation searching. References were uploaded to Covidence systematic review manager and 169 duplicates were removed. We performed title and abstract screening of 212 articles and 187 were excluded. Full text review was performed on 24 and 16 met the inclusion criteria (Fig. 1).

Study characteristics

The included studies were published between 2019 and 2023 and report cases identified in Europe of difficult-to-treat tinea caused by *T. indotineae*. There are studies reporting terbinafine resistance in other european countries (Belgium, Denmark, Russia, Poland) though these did not fulfil the inclusion criteria for this review[12,24-26,28,29] (Fig. 2).

Among the 16 studies, one is a prospective cohort, 7 are cross sectional, one is a case series and 7 are case reports (Table 1). The prospective cohort by Bortoluzzi and colleagues[13] describes an intervention in which patients from Italy with confirmed dermatophytosis by *Trichophyton spp.* were treated with topical and systemic terbinafine. After 12 weeks, in the absence or

presence of incomplete response, dermatophyte species identification and antifungal susceptibility testing was performed.

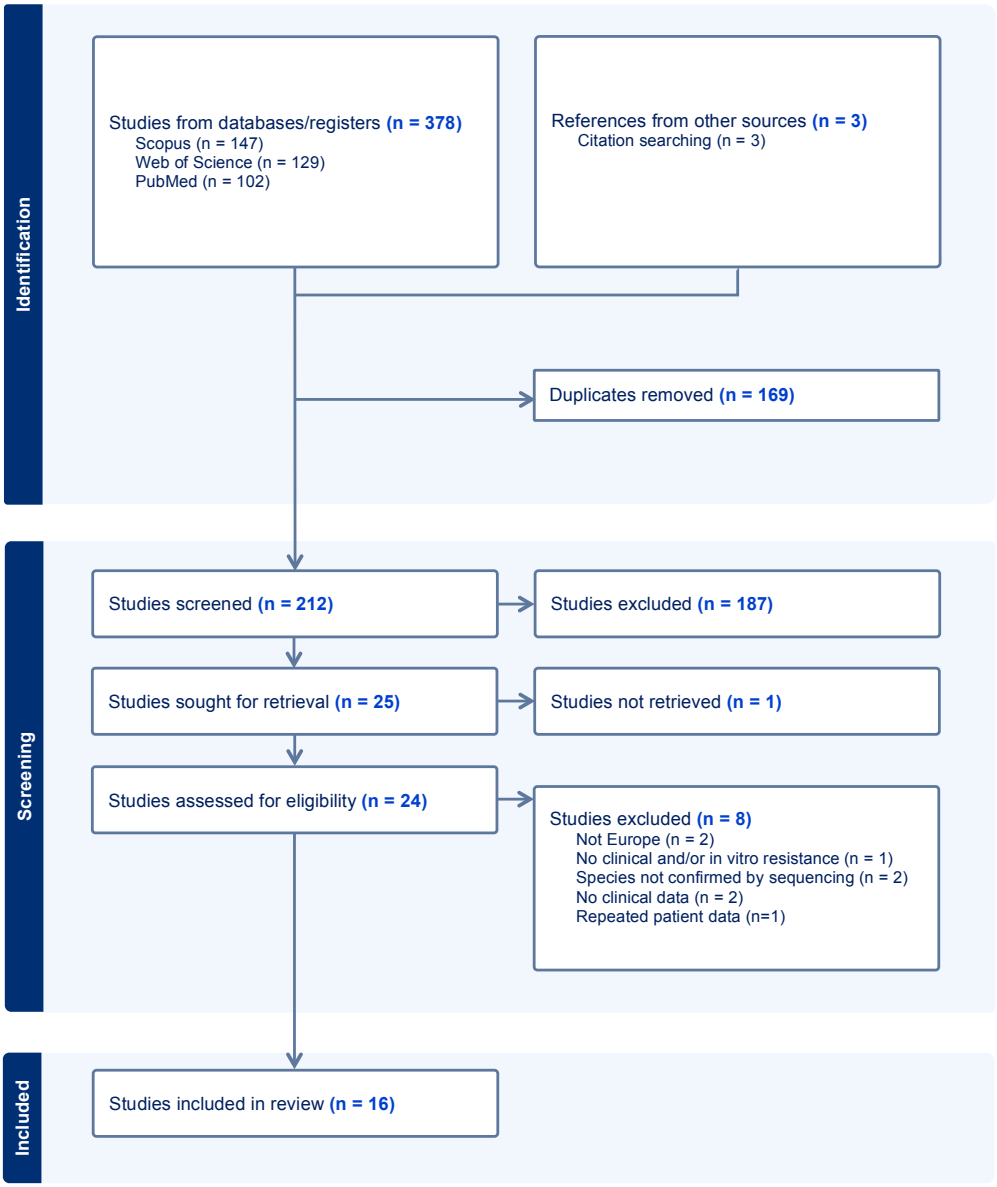

Figure 1. Prisma flowchart.

The cross-sectional studies aimed to determine the epidemiology of terbinafine-resistant *T. indotineae* in France,[15,30] Greece,[20] Switzerland[31,32] and Europe-wide.[21] The last study conducted by Saunte *et al.*[21] explore the occurrence of dermatophyte antifungal resistance in Europe through distribution of a standardised questionnaire to dermatologists in 23 different European countries.

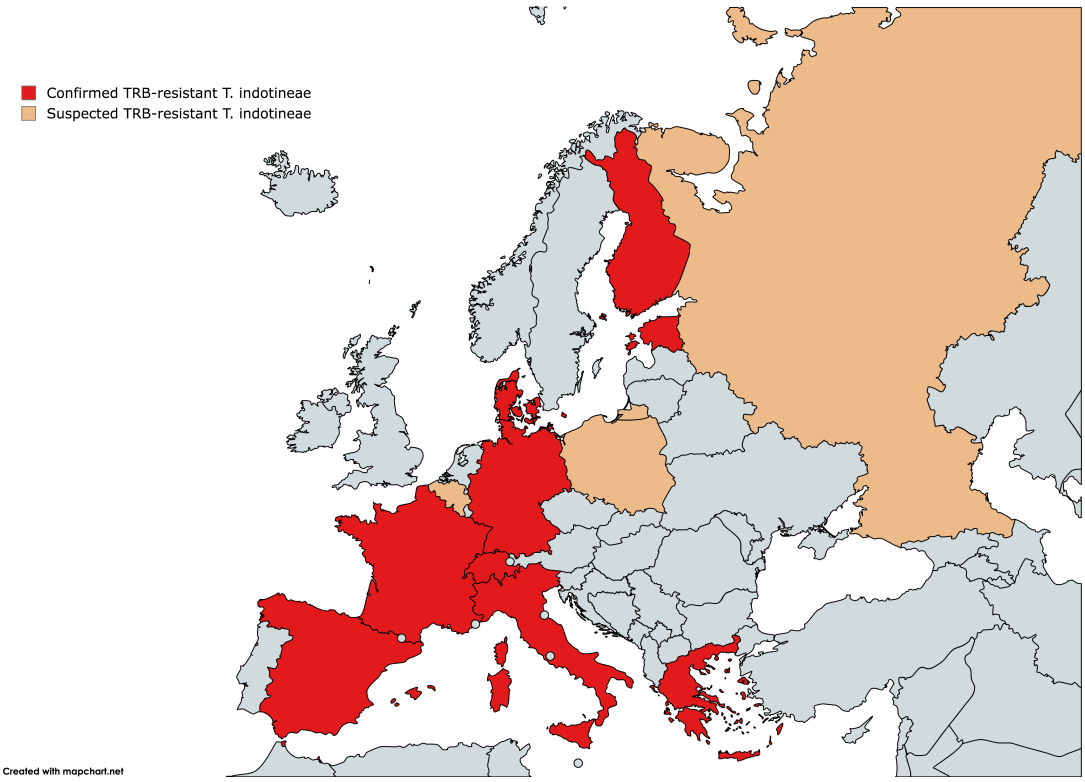

Figure 2. European countries with reported cases of dermatophytosis caused by terbinafine-resistant *T. indotineae*. Confirmed cases of *T. indotineae* are represented in red while suspected cases are represented in orange. Species identity confirmation was achieved by sequencing of the ITS region.

Jabet *et al.*[18] present a series of cases of extensive tinea in France caused by *T. indotineae* of which 57% (4/7) showed resistance to terbinafine.

There are 7 case reports describing extensive tinea cases caused by terbinafine-resistant *T. indotineae* in Switzerland,[17,19] Spain,[33,34] Germany[14,35] and France[16] (Fig. 2). Hsieh *et al*[17] in 2019 reported the case of a couple in Switzerland with disseminated terbinafine-resistant tinea associated with *T. mentagrophytes*. Given the terbinafine resistance and that this publication was previous to the reclassification of *T. indotineae* in 2020, we checked the available sequences and were able to identify these cases of resistance as caused by *T. indotineae*.

Table 1. Characteristics of studies reporting cases in Europe of difficult-to-treat dermatophytosis due to *T. indotineae*

Study	Study design	Objectives	Country	Country of origin	*T. ind*[*]	Clinical presentation	Treatment (duration in weeks)		Conclusions	Quality score[%]
							Topical	Systemic		
Dellière *et al.*, 2022[15]	Cross-sectional	To investigate the origin of clinically resistant *tinea corporis* that do not respond to TRB by looking at mutations in SQLE gene and determine the MIC of antifungal drugs (TRB, ITZ, VOR, Posaconazole, Isavuconazole)	FRA	BAN (n=3), IND (n=3), SRI (n=1)	7/350 (2282)	*Tinea corporis* (n=6), *Tinea cruris* (n=5), *Tinea faciei* (n=1)	TRB (n=1), BFN (n=2), Ciclopirox (n=1), steroids (n=1)	TRB (n=6), FCZ (n=1), ITZ (n=4), GSF (n=1)	Evidence of *T. indotineae* spread through travel and immigration. Surveillance should focus on identifying *T. indotineae* rather than SQLE sequences or MIC testing. Optimal treatment when TRB resistance is observed remains to be established	Fair

Moreno-Sabater *et al.*, 2022[30]	Cross-sectional	To determine the relative frequency of terbinafine-resistant clinical isolates and of *T. indotineae* species in Paris, France; To gain insights into the current epidemiology of *T. intergitale, T. mentagrophytes and T. indotineae* and determine their susceptibility to antifungal agents (TRB, ITZ, AMO and VOR)	FRA	FRA[#]	1/ND (580)	*Tinea corporis*	NA	ITZ (12w)	TRB resistance in France and dermatophyte epidemiology is changing with the introduction *T. indotineae*. The implementation of systems to survey the evolution of introduced species and identify TRB resistance is proposed. Dermatologists should be alerted to the emergence of TRB resistance and the local transmission of *T. indotineae* in France	Good
Jabet *et al.*, 2022[18]	Case series	To present a series of extensive dermatophytosis cases caused by TRB-resistant *T. indotineae* and document spread of *T. indotineae* using sequence-based screening	FRA	BAN (n=4)	4/7 (10)	*Tinea cruris* or *Tinea corporis*	ECZ (n=1), BFN (n=1)	TRB (n=4), GSF (n=2)	Points the need to implement systems to promptly identify TRB-resistant *T. indotineae* to prevent spread throughout Europe	Good

Gueneau *et al.*, 2022 [16]	Case report	To report a case of extensive dermatophytosis caused by TRB-resistant *T. indotineae*, successfully treated with topical VOR	FRA	BAN	1	*Tinea corporis et glutealis*	Steroids, TRB (8w), ECZ (9w), TRB (13w), BFN (9w), VOR (24w), VOR (8w)	TRB (8w), GSF (9w), TRB (13w), TRB (9w)	VOR topical cream is an effective and well-tolerated option for the management of TRB-resistant dermatophytic skin infections	Good
Nenoff *et al.*, 2020[38]	Cross-sectional	To identify the dermatophyte genotypes associated with treatment failure of chronic dermatophytosis with topical and oral TRB	GER	BAN (n=1), IND (n=2), GER (n=1), GER[#] (n=2), BRN (n=1), LBA (n=1), Not GER (n=4), NR (n=1)	13/29 (NA)	*Tinea corporis* (n=10), *Tinea cruris* (n=1), *Tinea faciei* (n=2), *Tinea pedis* (n=1)	TRB, ciclopirox olamine + miconazole , sertaconazole or luliconazole	TRB	Increased transmission of TRB-resistant *T. indotineae* from the Indian continent to Germany has been observed	Fair
Gawaz *et al.*, 2021[35]	Case report	To report a case of TRB-resistant *tinea corporis* on a traveller returning from Asia	GER	GER[#]	1	*Tinea corporis et cruris*	Steroids, antifungals	TRB (8w), ITZ (16w)	In people returning from Asia with TRB-resistant tinea, *T. indotineae* should be considered. In case of TRB treatment failure, systemic therapy should be switched to an azole and accompanied by a topical antifungal	Good

Burmester *et al.*, 2019[14]	Case report	To identify TRB-resistant indian T. mentagrophytes ITS genotype VIII strain and SQLE gene mutations by sequencing of DNA extracted from infected scales of a male with extensive *tinea corporis*	GER	IND	1	*Tinea corporis, Tinea glutealis et cruris*	Ciclopirox	TRB (2w), ITZ (NA)	Determining the genotype of dermatophyte species will not be sufficient and analysis of resistance genes will be necessary to guide adequate therapy	Poor
Siopi *et al.*, 2022[20]	Cross-sectional	To study the molecular epidemiology and *in vitro* antifungal susceptibility patterns of Greek Tricophyton isolates over 10 years with EUCAST E.DEF 11.0 reference method	GRE	IRI (n=1), SYR (n=1), GRE (n=5)	7/24 (112)	*Tinea corporis* (n=3), *Tinea cruris* (n=7)	Azole ointments (n=5), TRB (n=1)	TRB (n=3), FCZ (n=1), ITZ (n=1)	Highlights the need for surveillance studies to establish baseline *in vitro* susceptibility data to allow monitoring of antifungal resistance trends in dermatophytes and defining ECOFFs with the ultimate goals of optimizing antifungal therapy and minimizing administration of inappropriate drugs at different doses and frequency	Good

Bortoluzzi *et al.*, 2023[13]	Cohort	To describe first isolates of Trichophyton spp. resistant to TRB in Italy and to study the resistance mechanism	ITA	ITA (n=1); PAK (n=3); EGY (n=1)	4/4 (5)	*Tinea corporis et cruris* (n=4)	TRB (5w)	TRB (4w), ITZ (2w)	Reports first cases of TRB-resistant Trichophyton in Italy and suggests the implementation of an active surveillance program for resistance such as with Candida and Cryptococcus resistance to azoles. It also demonstrates the arrival of *T. indotineae* in Italy	Good
Villa-González *et al.*, 2023[34]	Case report	To report the first described case in Spain of extensive *tinea corporis* caused by TRB-resistant *T. indotineae* successfully treated with ITZ	ESP	BAN	1	*Tinea corporis*	NA	TRB (8w), ITZ (12w), ITZ (12w)	A case of TRB-resistant extensive *tinea corporis* caused by *T. indotineae* is presented. Complete resolution and no relapse of lesions were achieved after 6 months of treatment with ITZ	Fair

Hernández *et al.,* 2023[33]	Case report	To report the second described case in Spain of extensive tinea corporis caused by TRB-resistant *T. indotineae* that could not be treated with oral ITZ due to pregnancy	ESP	MAR	1	*Tinea corporis*	NA	TRB (4w), ITZ (6w)	Highlights the diagnostic challenges of this emergent new species, which is easily transmitted and resistant to TRB in a high percentage of cases. Addresses therapeutic challenges such as in the case of pregnant patients.	Poor
Blanchard *et al.,* 2023[31]	Cross-sectional	To determine the proportion of resistant fungal skin infections, analyse the molecular mechanisms of TRB resistance and validate a method for its rapid and reliable identification	SUI	NR	3/8 (5634)	*Tinea corporis* (n=2), *Tinea cruris* (n=1)	NA	TRB (n=2)	A MIC of 0.015 mg/L is proposed as a minimum breakpoint for predicting clinically relevant TRB treatment. Two methods are proposed for a rapid and reliable detection of TRB resistance that involve using growth medium containing TRB and SQLE gene sequencing	Good

Klinger *et al.*, 2021[32]	Cross-sectional	To determine the epidemiology of *T. mentagrophytes* and *T. interdigitale* genotypes in Zurich, Switzerland	SUI	IND (n=3), BAN (n=1), EU (n=3), NA (n=4)	4/11 (162)	*Tinea corporis, Tinea glutealis et cruris*	TRB or cotrimazole or KCZ or AMO or miconazole or ECZ or isoconazole	TRB or ITZ or FCZ	Underlines the importance of identifying the Tricophyton strain through ITS genotyping. Establishes differences in mode of transmission, affected patient group, type of dermatophytosis, degree of inflammation and patient management	Good
Russo *et al.*, 2023[19]	Case report	To report two cases of *T. indotineae tinea corporis* in Switzerland, one with *in vitro* resistance to TRB and a second with *in vitro* susceptibility but a clinical resistance	SUI	AFG (n=1), IND (n=1)	2	*Tinea corporis* (n=2), *Tinea cruris* (n=1)	KCN (n=2)	TRB (8w) (n=1)	Systematic clinical monitoring and AFST for dermatophytic infections that do not respond to treatment Early detection of TRB resistance should be required in the management of *T. indotineae* infections as a way to mitigate the emergence of new resistances	Good

Hsieh *et al.*, 2019[§,17]	Case report	To report two cases of *tinea corporis* resistant to TRB caused by a *T. mentagrophytes* strain exhibiting a firstly described point mutation in the SQLE gene	SUI	SUI[#]	2	*Tinea corporis et cruris* (n=2)	TRB, KCN, EBZ	TRB, ITZ	Systematic approach to dermatophytic infections that are unresponsive to conventional therapy including clinical follow-up and AFST. Access to over-the-counter antifungal treatments should be cut down to prevent spreading of TRB resistant strains	Good
Saunte *et al.*, 2021[21]	Cross-sectional	To explore the occurrence of clinical and mycological antifungal drug resistance in dermatophytosis in Europe	EST, FIN, GRE, SUI	EU[#]	11/ND (126)	*Tinea capitis, Tinea corporis, Tinea cruris*	NA	TRB or ITZ or FCZ or GSF or	Confirms the existence of clinical and/or mycological antifungal resistance in Europe. Highlights the need for standardized AFST and breakpoints for predicting clinically relevant resistance	Fair

[*] *T. ind*: Number of confirmed cases of antifungal-resistant *T. indotineae*/Number of *T. indotineae* cases (total Number of cases of dermatophytosis)
[#] Visited Asia/Middle East or close contact with someone who did; [§] Sequences compatible with *T. indotineae*
[%] NIH study quality assessment tools and JBI critical appraisal tools were used to assess study quality and a Quality Score was attributed
ITS: Internal transcribed spacer region; **MIC**: Minimal inhibitory concentration; **ECOFF**: epidemiological cut-off values; **AFST**: Antifungal susceptibility testing; **SQLE**: Squalene epoxidase enzyme; AFG: Afghanistan, BAN: Bangladesh, BRN: Bahrain, EGY: Egypt, **ESP**: Spain, EST: Estonia, **EU**: Europe, FIN: Finland, **FRA**: France, **GER**: Germany, **GRE**: Greece, IND: India, IRI: Iran, **ITA**: Italy, LBA: Libya, MAR: Morocco, PAK: Pakistan, SRI: Sri Lanka, **SUI**: Switzerland, SYR: Syria, **NA**: Not applicable or Not available, **NR**: Not reported. **TRB**: Terbinafine; **ITZ**: Itraconazole; **VOR**: Voriconazole; **AMO**: Amorolfine; **BFN**: Bifonazole; **FCZ**: Fluconazole; **GSF**: Griseofulvine; **ECZ**: Econazole.
Studies are grouped according to country and study design and are presented in reverse chronological order.

Patient characteristics (Table 1)

There were a total of 63 cases of resistant *T. indotineae* reported in Europe across the 16 studies included in this review. The mean age of patients was 34.3 years old (n = 51, range 0.5 to 90) and there were 15 females and 21 males (gender of 27 patients was not specified) (Supplementary Table S1). In 76% of the cases (37/49) where geographical information was available, origin or previous travel to Asia or Middle East or close contact with someone who visited Asia or Middle East was reported. Ten and eleven of these cases originated from or visited India (20%, 10/49) and Bangladesh (22%, 11/49), respectively.

Only 9 patients were documented as having co-morbidities (Supplementary Table S1): diabetes mellitus (n = 3), psoriasis (n = 2), asthma (n = 1), Crohn's disease (n = 1), myasthenia gravis/thymoma (n = 1), chronic hepatitis B (n = 1). Two women were reported as being pregnant (n = 1) or breastfeeding (n = 1).

Tinea corporis was the most common clinical presentation having been reported in all 16 studies. *Tinea glutealis* and *tinea cruris* were also very common forms of presentation of the disease and these were mentioned in every study but 3.[30,33,34]

Dermatophyte species identification

Dermatophyte species were identified through phenotypic methods (direct mycological examination, microscopic morphology and presence of hyphal structures) and confirmed by ITS sequencing in every study except one that used molecular methods only[14] (Supplementary Table S1). Some studies also factor into species identification the clinical localization and manifestation of dermatophytosis.[36] For example, *T. interdigitale* is more frequently isolated from non-inflammatory onychomycosis, whereas T. mentagrophytes strains and its clonal offshoot *T. indotineae* (*T. mentagrophytes* genotype VIII) are associated with inflammatory *tinea corporis* and *tinea cruris.*[20,32,36]

When cultured on Sabouraud dextrose agar, *T. indotineae* colonies are flat, white in colour and have a velvety surface, with a slight central elevation and a brown tint on the reverse side. In 3 studies[13,17,19], presumptive identification was attempted using matrix-assisted laser desorption/ionisation

time of flight mass spectrometry (MALDI-TOF-MS).[37] Molecular identification performed by amplification and sequencing of the ITS region using polymerase chain reaction (PCR) was performed in all included studies.[13-21,30,31,33-35,38,39]

Other dermatophyte species have been reported in these studies such as *T. rubrum*, *T. interdigitale* genotypes I-II, *T. mentagrophytes* genotypes II-VII and, less frequently, *T. tonsurans*, *T. verrucosum*, *T. violaceum*, *Microsporum canis* (*M. canis*), *M. audouinii* and *Nannizzia gypsea* (Supplementary Table S1).

Antifungal susceptibility testing (Table 2)

All studies except 2[14,33] performed antifungal susceptibility testing (AFST) to demonstrate mycological resistance in 61 of the total 63 cases (Table 2). Twenty-six resistant *T. indotineae* isolates were identified using the method developed by the European Committee for Antimicrobial Susceptibility testing (EUCAST): 3 studies used a modified version of the EUCAST E.Def 9.3.1 to identify a total of 13 resistant isolates of *T. Indotineae,*[15,18,19] 3 studies used the most recent method, the EUCAST E.Def 11.0,[13,20,30] identifying 12 resistant isolates of *T. indotineae* and one study did not specify which version of EUCAST was used to identify the remaining resistant isolate[34]. Eighteen other isolates were defined as resistant across 3 different studies using the Clinical and Laboratory Standards Institute (CLSI) method.[17,31,38] Seven studies combined AFST methods with terbinafine containing agar method (TCAM) defining 25 isolates as terbinafine-resistant. One study used a specific broth microdilution method[40] and another study[21] mentioned the use of other methods such as disk diffusion and E-test.

The most frequently tested antifungals were terbinafine, itraconazole, fluconazole, voriconazole, posaconazole, griseofulvin and amorolfine. Based on the available tentative ECOFF values, MIC values for terbinafine (TRB), itraconazole (ITZ), voriconazole (VOR) and amorolfine (AMO) were extracted and analyzed, when available (Table 2). In the absence of clinical breakpoints, selection of antifungals was based on the availability of tentative ECOFFs proposed for *T. indotineae*. These were established based on results from a multicenter study[41] with the values of 0.125 µg/mL for terbinafine,

0.25 µg/mL for itraconazole, 1.0 µg/mL for voriconazole and 0.5 µg/mL for amorolfine. Terbinafine and itraconazole MICs varied from 0.014 to ≥ 16 µg/mL while voriconazole MICs ranged from 0.06 to 2 µg/mL.

A total of 94% of tested isolates were reported as being terbinafine-resistant (47/50), 3 were considered sensitive although clinically resistant (n = 1) or resistant to azoles (n = 2). Antifungal-susceptibility information was not available for 11 isolates and the remaining 2 were not tested for terbinafine-susceptibility. Only 36 isolates were tested for itraconazole-susceptibility and 3 showed resistance (8%, n = 3/36) while 33 were reported as sensitive. For voriconazole, only one isolate was considered resistant from the total 23 tested. Finally, no isolate was found resistant to amorolfine.

SQLE mutational analysis (Table 2 and Figure 3)

Of the 16 included studies, mutational analysis of the SQLE gene was performed in all included studies but 3[21,33,34] corresponding to a total of 50 isolates of *T. indotineae*. All tested samples had a mutation of the SQLE gene (Table 2). Single amino-acid substitutions were identified in 46 isolates (92%) while the remaining 4 (8%) had double substitutions. The most prevalent mutation was F397L (substitution of phenylalanine by leucine at position 397) at a rate of 62% (31/50). This was followed by the substitution of leucine at position L393 (L393S or L393F) detected in 20% (10/50) of isolates, A448T (substitution of alanine by threonine at position 448) detected in 15% (7/50) of isolates, Q408L (substitution of glutamine by leucine at position 408) detected in 4% of the isolates (2/50) and F415C (substitution of phenylalanine by cysteine at position 415) detected in 2% of the isolates (1/50). The double mutations found were F397L/A448T (n = 4).

Our study found that the most common mutations present in *T. indotineae* strains isolated in Europe were at positions L397 and L393 comprising 82% of all mutations detected and presenting high terbinafine MIC values (Fig. 3, in red and orange). Intermediate-high MIC values were found in strains which displayed mutations at position F415 or L393 at a lower frequency. Finally, strains exhibiting amino acid substitutions at A448 had the lowest terbinafine-MIC values that did not surpass the ECOFF value and were therefore

Table 2. Clinically and/or *in vitro* antifungal-resistant *T. indotineae* reported in Europe

Study	Country	*T.ind* * (n = 63)	AFST method	TRB		ITZ		VOR		AMO		SQLE mutation (No of isolates)
				MIC µg/mL, (No of isolates)								
				S ≤ 0.125	R > 0.125	S ≤ 0.25	R > 0.25	S ≤ 1.0	R > 1.0	S ≤ 0.5	R > 0.5	
[15]	FRA	7	Modified EUCAST E.Def 9.3.1[18]	0.014 (1), 0.03 (1)	1 (1), 4 (4)	0.014 (2), 0.06 (4)	16 (1)	0.125 (1), 0.25 (5)	2 (1)	ND	ND	L393S (1), F397L (4), A448T (2)
[30]	FRA	1	TCAM + EUCAST E.Def 11.0[19]		2	1		0.06 (1)		0.125 (1)		L393S
[18]	FRA	4	TCAM + modified EUCAST E.Def 9.3.1[18]		2 (2), > 8 (1), 2 (1)	(3), ND (1)		(3), ND (1)		(3), ND (1)		L393S (2), F397L (1), F397L/A448T (1)
[16]	FRA	1	TCAM		≥ 0.2	ND	ND	ND	ND	ND	ND	L393S

[38]	GER	13	TCAM + CLSI		0.2 (4), 8 (4), 16 (2), 16 (1), 8 (1), 16 (1)	(4), ND (8)	1	(5), ND (8)		ND	ND	F397L (10), L393F (1), F397L/A448T (2)
[35]	GER	1	TCAM		≥ 16	ND	ND	ND	ND	ND	ND	F397L
[14]	GER	1	NA		ND	ND	ND	ND	ND	ND	ND	F397L
[20]	GRE	7	EUCAST E. Def 11.0[19]		0.25 (2), 2 (1), 2 (1), 4 (2), 8 (1)	(7)		(7)		(7)		L393S (3), F397L (4)
[13]	ITA	4	EUCAST E.Def 11.0[19]		0.25 (1), 0.5 (1), 1 (1), 4 (1)	(4)		ND	ND	ND	ND	L393S (1), F415C (1), F397L (2)
[34]	ESP	1	EUCAST		4		> 8 (1)	ND	ND	ND	ND	ND
[33]	ESP	1	NA		ND	ND	ND	ND	ND	ND	ND	ND
[31]	SUI	3	TCAM + CLSI		2 (1), 4 (1), ND (1)	ND		ND	ND	ND	ND	F397L (3)
[32]	SUI	4	BM[(3)]		≥ 4 (4)	4		ND	ND	ND	ND	F397L (4)

[19]	SUI	2	Modified EUCAST E. Def 9.3.1[18]	0.06 (1)	≥ 16 (1)	0.06 (1), 0.25 (1)		ND	ND	ND	ND	A448T (1), F397L/A448T (1)
[17]	SUI	2	TCAM + CLSI		≥ 1 (2)	0.015 (2)		ND	ND	ND	ND	Q408L (2)
[21]	EU[§]	11	Disk diffusion, E-test, CLSI, EUCAST, BM[(1)]		NA	NA	NA	NA	NA	NA	NA	NA

[§] EU: European countries Estonia, Finland, Greece and Switzerland
[*] *T.ind*: No of confirmed cases of antifungal-resistant *T. indotineae*
[(1)] BM: Broth microdilution [40]

FRA: France, **GER**: Germany, **GRE**: Greece, **ITA**: Italy, **ESP**: Spain; **SUI**: Switzerland; **NA**: Not applicable or Not available; **NR**: Not reported. **MIC**: Minimal inhibitory concentration; **TRB**: Terbinafine; **ITZ**: Itraconazole; **VOR**: Voriconazole; **AMO**: Amorolfine; **TCAM**: Terbinafine containing agar method; **SQLE**: Squalene epoxidase enzyme-codifying gene; Mutations: A448T (blue), F397L (red), L393S (orange), L393F (purple).

considered not resistant (Fig. 3, in blue). However, some isolates included in this study carrying the A448 amino acid substitution exhibited high MICs for azoles.[15,38]

L393S substitution originated in Bangladesh and was predominant in France due to a wave of migrants applying for asylum in France since 2016.[42] Meanwhile, L393F was predominantly found in isolates from India and Germany, likely related to movement of people between the countries.

Finally, there is a report of clinical and *in vitro* resistance to terbinafine in a couple from Switzerland containing a previously unknown point mutation leading to substitution of a highly conserved residue in a hot spot region in the SQLE gene (Q408L).[17] This same mutation was later reported in India,[8] where the couple had been visiting before developing tinea.

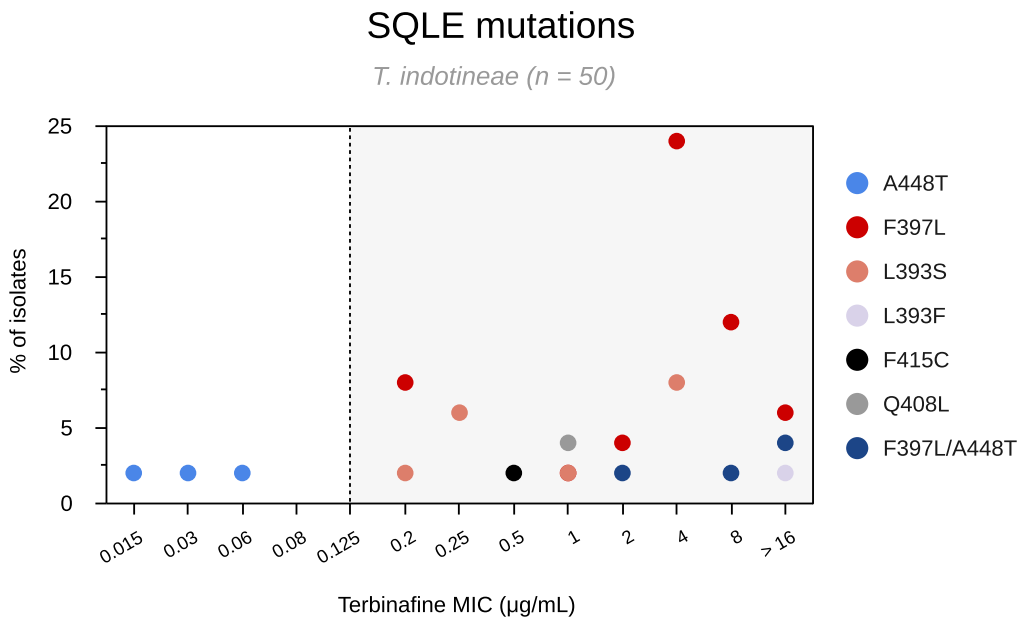

Figure 3. Terbinafine minimal inhibitory concentration (MIC) of *T. indotineae* isolates with amino acid substitutions in squalene epoxidase (SQLE) enzyme. Percentage of *T. indotineae* isolates (n = 50) exhibiting mutations in SQLE. ECOFF is represented by a dashed line. Grey area corresponds to MIC values considered resistant.

Treatment strategy (Table 1)

Terbinafine use was reported in 15 of the 16 studies included in this review. The remaining study[30] used oral itraconazole to treat *tinea corporis* as a prior screening of terbinafine resistance was performed on clinical isolates. Itraconazole is the most commonly used second-line antifungal used systemically and the treatment of choice for dermatophytosis caused by terbinafine-resistant *T. indotineae* with its use being referred to in 11 studies.[13-15,17,20,21,30,32-35] Among the selected studies, oral treatment with griseofulvin and fluconazole was also referenced.[15,16,18,20,21,32]

The use of topical antifungal agents, either alone or in combination with oral antifungals, has been reported in 11 studies. These included antifungals such as terbinafine, azole ointments (bifonazole, clotrimazole, econazole, bifonazole, miconazole, sertaconazole, voriconazole, ketoconazole, isoconazole, luliconazole) and ciclopirox. Some studies also mentioned the use of topical corticosteroids.[15,16,35]

Discussion

This systematic review examines the existing evidence on the presence of terbinafine-resistant *T. indotineae* in Europe, the molecular basis of treatment failure and *in vitro* resistance to terbinafine and its implications on the clinical management of dermatophytosis. We found 63 cases of terbinafine resistance in Europe reported from 2019 to 2023, all of which had mutations on the SQLE gene. Itraconazole is the antifungal most commonly used when treatment failure with terbinafine although relapse rates are concerningly high.

In our study, 78% of the strains were imported from Asia or the Middle East, 33% of patients were Europeans who recently visited endemic areas while 17% were Europeans with no connection to Asia or Middle East, demonstrating human-to-human transmission. Similarly, a study by Jabet and colleagues[18] screened the available sequences of *T. indotineae* on GenBank to study the global epidemiological characteristics of *T. indotineae*. It showed that 98.8% were of human origin indicating predominance of human-to-human transmission and that *T. indotineae* was also detected in animals, suggesting their potential role as reservoirs and the possibility of zoonotic transmission.

Comparable to our findings, 76% of documented sequences originated from India, 12.8% from the Middle East, 9.6% from Europe and the remaining 1.1% from other countries.[18] Nevertheless, dermatophytosis is not a notifiable disease and therefore, epidemiological numbers on *T. indotineae* are likely skewed.

The prevalence of *T. indotineae* might be underestimated given the inability of classical methods that rely on morphological identification of fungal cultures to distinguish taxonomically close species. In this study, MALDI-TOF was unable to accurately identify Trichophyton isolates at species level due to the phenotypic similarities between *T. indotineae,* T. mentagrophytes and *T. interdigitale*. New databases and improvements of the method are underway.[3,43] Currently correct assignment is only possible through sequencing of ITS region of ribosomal DNA which was the most common method of species identification among the included articles.[3,44]

In this study, terbinafine-resistant *T. indotineae* clinical lesions were mostly manifested as *tinea corporis* and/or *tinea cruris et glutealis*. Nevertheless, there have been cases of *T. indotineae* associated with onychomycosis and terbinafine-resistant onychomycosis has been shown in association with other strains (*T. interdigitale* and *T. rubrum*).[30] Antifungal resistance in onychomycosis might be overlooked as these patients are typically excluded from studies because management of fungal nail disease is very complex and relies on several factors that contribute to treatment failure and recalcitrance.

As demonstrated by this study, there is a diversity of methods used, alone or in combination, to determine susceptibility to terbinafine and other antifungals, making comparison and interpretation of values challenging. Methods used in Europe were: EUCAST (modified E.Def 9.3.1 and E.Def 11.0), TCAM, CLSI, unspecified broth microdilution, disk diffusion or E-test. At the moment, the EUCAST method is the only validated standardised protocol to determine *in vitro* susceptibility of dermatophytes, being the most commonly used method in the selected studies (identified 26 cases of

resistance) immediately followed by TCAM (25 cases). Some authors propose culturing isolates in terbinafine containing medium (TCAM) to allow a fast and inexpensive screening of terbinafine resistance before quantitative antifungal susceptibility testing.[30,31] There appears to be a good correlation between results obtained with TCAM and EUCAST and between *in vivo* and *in vitro* resistance in the presented studies which validates both the methods of susceptibility testing and the proposed ECOFFs. In most studies, the EUCAST cut-offs were able to reliably identify resistance as the strains with MIC values considered resistant were indeed non-wild-type for SQLE. CLSI was performed in fewer studies (18 cases) and the main difference to EUCAST is that it uses a visual endpoint (corresponding to 80% decrease in growth) vs a spectrophotometric endpoint (corresponding to 50% decrease in growth), respectively.

There were a few reports in which MIC values did not correlate with clinical response to antifungal drugs. Namely, a case from Spain[34] reporting an *in vitro* itraconazole and terbinafine non-wild-type isolate which had a favourable clinical response to itraconazole. Yet, SQLE mutational status of this strain and long-term follow up is unknown. Another study, from Switzerland,[19] reports one imported case of extensive *T. indotineae tinea corporis* with *in vitro* susceptibility to terbinafine but with clinical resistance. Other than resistance, there are other causes that may be responsible for a lack of response to antifungal therapy such as patient compliance, reinfection, low drug absorption or increased metabolism. When good patient compliance is ensured, treatment failure might be explained through close contact with infected people or contaminated textiles or with a reservoir such as a pet or by poor hygiene rather than true terbinafine resistance.

Every *T. indotineae* isolate described in this study presented SQLE mutations suggesting that antifungal resistance was most likely acquired. Given the wide use of topical antifungals in combination with potent corticosteroids, resistance has potentially arisen as a result of indiscriminate exposure to drugs targeting SQLE enzyme. In this review some patients with treatment failure had been indeed pretreated with oral or topical antifungals

but data on previous exposure was not available for most of the entries making it impossible to search for causality. The use of topical corticosteroids is strongly discouraged, even in combination with antifungals.[45]

In addition to SQLE mutations, other mechanisms of resistance such as efflux pumps[46] and biofilm formation[47] have been described but these were not observed in the European cases presented in this study.

Our study found SQLE mutations in *T. indotineae* strains isolated in Europe at positions F397, L393, F415 and Q408. Yamada and colleagues demonstrated that amino-acid substitutions at F397, L393 and F415 of the SQLE enzyme conferred resistance to terbinafine *in vitro*, when introduced into susceptible strains.[29]

We observed that the highest MIC increases were associated with the most common amino acid substitutions F397L (alone or in combination F397L/A448T), followed by intermediate-high MIC values linked to less frequent mutations at position F415 or L393 and the lowest terbinafine-MIC values corresponded to strains exhibiting amino acid substitutions at A448. In agreement with our observations, several studies have shown that mutations leading to amino acid substitutions F397L or F397L/A448T were linked to the highest MIC increases while the other variants were also associated with treatment failure even if MICs were not as elevated.[9,10,29] One study from India attempted to correlate antifungal susceptibility with mutations in the SQLE gene with similar findings to our study highlighting the concern of a similar epidemic of difficult-to-treat and recalcitrant tinea developing in Europe.[46]

Strains exhibiting amino acid substitutions at A448 had the lowest terbinafine-MIC that did not surpass the ECOFF value and were therefore considered not resistant. This mutation alone may not contribute to terbinafine resistance, as previously observed.[8,15,38,48] It has been speculated that it may contribute to increased MIC values for triazoles as shown in this study[15,38] and in another European study not included in this review.[49] SQLE mutations

seem to have an effect on terbinafine sensitivity hence combining SQLE mutational analysis with MIC might improve the overall utility of susceptibility testing.

Optimal treatment of dermatophytosis after treatment failure with terbinafine remains to be established. Itraconazole is the most commonly used second-line antifungal and has been recommended in case of terbinafine resistance.[50] Other systemic azoles, such as fluconazole, and griseofulvin have also been tested with inferior clinical efficacy.[51] Our study supports these findings with reports of complete tinea remission after switch to itraconazole in 8 publications.[13-15,17,30,33-35] In a pilot study which surveyed dermatologists in 23 European countries,[21] questionnaire responders said using itraconazole as the most frequent alternative treatment when terbinafine resistance was suspected or confirmed. In addition, many survey participants suggested the use of topical antifungals from a different drug class in combination with the oral treatment.

Despite initial efficacy of itraconazole in lesion clearance, relapse rates after treatment are hard to ignore. Indeed, in a randomised clinical trial testing different doses of itraconazole for the treatment of *tinea corporis*, a relevant number of patients relapsed.[52] Although long-term clinical follow-up was mostly not available, Delliere *et al.*[15] reports that more than half of the patients had relapse of skin lesions months after the end of itraconazole treatment. Prolonging therapy with systemic azoles poses a risk of selecting resistant isolates and exposes the patient to possible side effects such as kidney and liver injury. In fact, one study[17] reports that second-line treatment with itraconazole had to be interrupted due to elevated liver enzymes.

Topical agents such as Voriconazole might be preferred for relapsing dermatophytosis requiring extended treatment due to a more favourable safety profile. This recently developed topical antifungal, seems to be a valuable alternative option to bypass systemic treatments with one study [16] showing clinical cure after 2 months, good tolerance and no local or systemic side effects.

This systematic review presents some limitations, many of which are intrinsic to the included studies. Overall findings are heavily based on case reports, which often lack generalizability due to their circumstantial nature. There is also the risk of publication bias, as case reports documenting terbinafine resistance of dermatophytosis caused by *T. indotineae* are more likely to be published than cases of sensitive strains. While this may promote overestimation of antifungal resistance, the fact that we only included reports of *T. indotineae* with confirmed identity by ITS sequencing should do the opposite as many *T. indotineae* strains are wrongly labelled as *T. mentagrophytes* or *T. interdigitale*. Beside study design, other limitations of the included studies are small sample sizes, inadequate follow-up, variability of oral and systemic treatment combinations and inconsistencies in laboratory methodologies for AFST. Furthermore, there is a lack of consensus regarding the definition of *in vitro* and clinical resistance in the absence of clinical breakpoints which in turn makes AFST becomes partly redundant and perhaps the reason why these are often not performed. We used ECOFFS which allow differentiation between wildtype microorganisms and those which have likely acquired resistance. This seems to have been a reliable method of determining resistance as there was an overall good correlation between *in vitro* and clinical response, with all strains exhibiting resistance carrying SQLE mutations. Nevertheless, the main focus of this work was to provide evidence of terbinafine-resistant *T. indotineae* spread to Europe and highlight that measures must be taken to halt its dissemination, which was achieved regardless of these limitations.

Currently, *T. indotineae* is the predominant dermatophyte in India[53] and we are likely facing a similar epidemiological shift in Europe. In addition to natural population flows, an unprecedented number of migrants seeking asylum in Europe[18,20,38] has contributed to the increasing number of imported *T. indotineae* cases detected in different European countries and shows that European dermatophyte epidemiology is shifting. Antifungal stewardship should promote surveillance programs for a fast detection of terbinafine-resistant *T. indotineae* and the implementation of standardised AFST. There is currently an unmet need for standardised clinical trials to establish clinical

breakpoints, treatment guidelines and adequate combinations of topical and oral antifungals. This might encourage a more responsible use of antimicrobials while safeguarding their therapeutic effectiveness, specially considering the limited number of antifungals available to treat dermatophytosis, and prevent further spread of this pathogen across Europe.

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Supplementary Table S1. Characteristics of studies reporting cases in Europe of difficult-to-treat dermatophytosis due to antifungal-resistant *T. indotineae*

Study	Country	ID method[#]		Other dermatophytes	Age (years), gender	Medical history
		PM	MM			
Dellière *et al.,* 2022[15]	FRA	✔	✔	ND	44 (20-57), F (n=4), M (n=3)	DM (n=3), Psoriasis (n=1), Chronic hepatitis B (n=1), Crohn's disease (n=1)
Moreno-Sabater *et al*., 2022[30]	FRA	✔	✔	*T. interdigitale* (n=1), *T. rubrum* (n=1)	NA	NR
Jabet *et al*., 2022[18]	FRA	✔	✔	ND	30 (16-53)	NR
Gueneau *et al.*, 2022[16]	FRA	✔	✔	ND	28, M	Asthma; No signs of immunodeficiency, negative HIV and HTLV serologies, no DM, no acquired haematological malignancy, normal TAP CT scan.
Nenoff *et al*., 2020[38]	GER	✔	✔	ND	32 (0.5-58), F (n=5), M (n=8)	NR

Gawaz *et al*., 2021[35]	GER	✔	✔	ND	21, M	NR
Burmester *et al.,* 2019[14]	GER	✖	✔	ND	26, M	NR
Siopi *et al.,* 2022[20]	GRE	✔	✔	*T. rubrum* (n = 70), *T. mentagrophytes* (n=24), *T. interdigitale* (n = 12), *T. tonsurans* (n = 6)	42 (0.8-90), F (n=2), M (n=5)	NR
Bortoluzzi *et al.,* 2023[13]		✔	✔	*T. rubrum* (n=1)	44 (32-59), F (n=1), M (n=4)	Myastenia gravis secondary to thymoma (n=1; Italian)
Villa-González *et al.,* 2023[34]		✔	✔	ND	17, F	NR
Hernández *et al.,* 2023[33]		✔	✔	ND	33, F	Pregnant at 33 weeks of gestation
Blanchard *et al.,* 2023[31]		✔	✔	*T. rubrum* (n=39)	45.7 (9-82)	NR
Klinger *et al.,* 2021[32]		✔	✔	*T. interdigitale* genotypes I-II, *T. mentagrophytes* genotypes II-VIII	32 (31-41), F (n=7), M (n=4)	
Russo *et al.,* 2023[19]		✔	✔	ND	29 (26-32), F (n=1), M (n=1)	Breastfeeding (n=1)

Hsieh *et al.,* 2019[17]	✔	✔	ND	56 (51-60), F (n=1), M (n=1)	Psoriasis (n=1)
Saunte *et al.*, 2021[21]	✔	✔	*T. interdigitale,* *T. rubrum,* *T. tonsurans,* *T. verrucosum,* *T. violaceum,* *M. canis,* *M. audouinii,* *N. gypsea*	NA	NA

[#] Species identification method: Phenotypic methods (PM) by direct mycological examination, microscopic morphology and presence of hyphal structures and/or molecular methods (MM) by ITS region sequencing.
NA: Not applicable or Not available, **ND**: Not determined, **NR**: Not reported

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	2
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	4-5
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	4-6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	4-5
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	5
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	5
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	5
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	5
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	NA
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	NA
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	NA
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	NA
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	NA
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	NA
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	NA
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	6
Study characteristics	17	Cite each included study and present its characteristics.	Table 1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Table 1
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 1
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	NA
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	NA
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	11-15
	23b	Discuss any limitations of the evidence included in the review.	14-15
	23c	Discuss any limitations of the review processes used.	14-15
	23d	Discuss implications of the results for practice, policy, and future research.	11-15
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	NR
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	NA
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	1
Competing interests	26	Declare any competing interests of review authors.	1
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	1

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

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Time frame: past 36 months			
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3	Royalties or licenses	None	
4	Consulting fees	None	

5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	None	
6	Payment for expert testimony	None	
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8	Patents planned, issued or pending	None	
9	Participation on a Data Safety Monitoring Board or Advisory Board	None	
10	Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid	None	
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8. Any conflict-of-interest disclosures (For each co-author, please refer to sections 3 and 6)
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1. de Berker DAR, Baran R, Dawber RPR. The nail in dermatological diseases. In: *Baran and Dawber's Diseases of the Nails and their Management* (Baran R, Dawber RPR, de Berker DAR, Haneke E, Tosti A, eds), 3rd edn. Oxford: Blackwell Science Ltd, 2001; 172–92

2. Wollina U, Hansel G. The use of topical calcineurin inhibitors in lupus erythematosus: an overview. *J Eur Acad Dermatol Venereol* 2008; **22:1**–6.

3. Graham-Brown R, Burns T. *Lecture Notes: Dermatology*. Oxford: Wiley-Blackwell, 2006.

4. British Lymphology Society. *Consensus Document on the Management of Cellulitis in Lymphoedema*. 2007. Available at: **https://www.thebls.com/public/uploads/documents/document-75091530863967.pdf** (last accessed 28 November 2007).

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