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CASE REPORT

A case of double-mutant resistant tinea indotineae

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Abstract

In recent years, there has been a concerning increase in a challenging-to-treat dermatophyte lineage of the *Trichophyton mentagrophytes* complex, known as *Trichophyton indotineae*. We report the first case of resistant *T. indotineae* bearing a double mutation in the squalene epoxidase gene (SQLE), isolated in Italy from a 26 -year-old Nepali woman affected by a persistent tinea corporis. Topical terbinafine yielded minimal to no improvement while itraconazole provided partial relief but failed to eradicate the infection. Molecular identification through Sanger sequencing of the internal transcribed spacer region confirmed *T. indotineae* and sequencing of the SQLE gene revealed the presence of a double mutation (Phe397Leu/Ala448Thr) associated with antifungal resistance. Broth microdilution susceptibility testing demonstrated resistance to fluconazole, but susceptibility to itraconazole. Ultimately complete cure was achieved with prolonged high-dose itraconazole therapy.

KEYWORDS
azoles, drug-resistance, tinea corporis, *Trichophyton indotineae*

INTRODUCTION

Trichopyton rubrum has traditionally been the main cause of dermatophytosis. However, there is a shifting trend, particularly notable in countries like India, where *Trichophyton mentagrophytes* is increasingly replacing *T. rubrum* in terms of prevalence.[1]

In recent years, a resistant challenging-to-treat dermatophyte lineage of the *T. mentagrophytes* complex known as *Trichophyton indotineae*, has emerged.[2] Point mutations in the squalene epoxidase (SQLE) gene, most commonly Phe397Leu and Leu393Phe, are responsible for resistance to terbinafine.[3,4] Other point mutations, such as the substitution Ala448Thr, have proved to be

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associated with an increased minimal inhibitory concentration (MIC) of itraconazole and voriconazole, thus leading to potential in-vivo azole resistance.[5] The presence of a double mutation (Phe397Leu/Ala448Thr) has been described to be linked to resistance to both terbinafine and azoles.[6,7]

Cases of drug-resistant tinea have been detected across various European countries.[8–10] Moreover, the frequency of this infection could be underestimated, as it is frequently misidentified in laboratory settings as *T. mentagrophytes*.[11] We report on the first finding of resistant *T. indotineae* harboring a double hit mutation (Phe397Leu/Ala448Thr) in the SQLE gene in Italy.

CASE REPORT

A 26-year-old Nepali female patient presented to our clinic for a persistent tinea corporis. Seven months before her arrival in Italy, the patient developed a pruritic patch on her right leg; treatment with an unspecified systemic antifungal drug led to clearance of the lesion. However, lesion recurred after discontinuation of the treatment. While in Italy, the patient started to develop pruritic patches across her body. Topical terbinafine yielded minimal to no improvement, thus oral itraconazole (100 mg daily for a week, for 3 months) was prescribed, resulting in partial relief. Nevertheless, lesions and symptoms reappeared after end of treatment. Upon examination, we found characteristic findings consistent with extensive tinea corporis, including highly pruritic large, annular, scaly, and erythematous concentric patches and plaques distributed across the arms, trunk, axillae, groins, and legs (Figure 1). Blood tests revealed normal full blood cell counts, glucose, renal and liver function, as well as electrolyte levels.

A sample obtained from cutaneous scrapings of the lesions was cultured on Sabouraud Gentamicin Chloramphenicol 2 Agar (bioMérieux) at 30°C that revealed white, powdery, flat colonies with reddish-yellow bottom color after 2 weeks (Figure 2). The strain was identified as *T. mentagrophytes* at microscopy examination, then confirmed by Maldi-ToF assay (bioMérieux).

Molecular identification was carried out by Sanger sequencing of internal transcribed spacer (ITS) region of ribosomal DNA. End-point PCR was run using V9G (5′-TTACGTCCCTGCCCTTTGTA-3′) and LS266 (5′-GCATTCCCAAACAACTCGACTC-3′) primers as previously described.[12] The amplified products were sequenced with the same primers and the BigDye Terminator Kit v.3.1 (Applied Biosystems) in a SeqStudio Genetic Analyzer (Applied Biosystems). Consensus sequence was obtained by Sequencer software (Gene Codes Corporation) and aligned on NCBI BLAST. showing a 100% identity with GenBank sequence ON182016 for *T. indotineae*.

A partial coding region of SQLE gene was amplified with the TrSQLE-F1 (5′-ATGGTTGTAGAGGCTCCTCC C-3′) and TrSQLE-R1 (5′-CTAGCTTTGAAGTTCGGCA AA-3′) primers pair as previously described.[12] Nucleotide substitutions 1189 TTC to CTC and 1347 ATG to GTC were found leading to Phe397Leu and Ala448Thr aminoacids substitution in the protein.

Antifungal susceptibility testing was performed using the broth microdilution method according to the CLSI M38-A3 standard (Clinical and Laboratory Standards Institute). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. 3rd ed. Results are shown in Table 1.

Given the patient's previous improvement with itraconazole and the result of the antifungal susceptibility test, we prescribed a daily dosage of 200 mg itraconazole and scheduled regular follow-up visits. After 3 weeks, the patient reported a significant reduction in pruritus, with a resolution of nearly all patches and plaques leaving

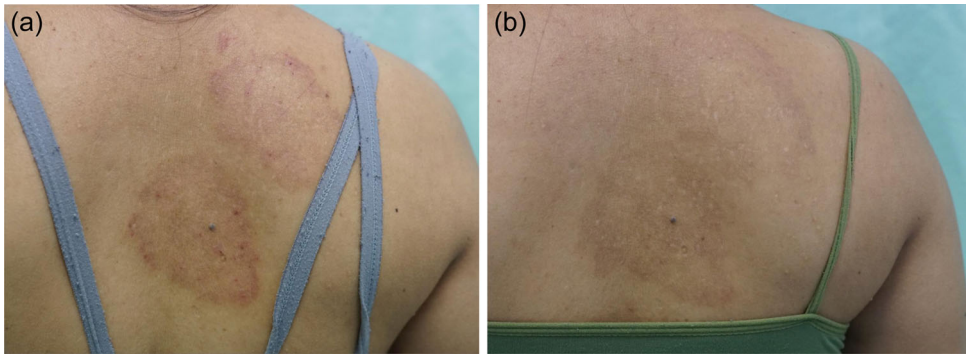

FIGURE 1 Two large, annular, scaly, and erythematous concentric plaques on the dorsum before treatment (a) and after treatment (b) with itraconazole 200 mg daily for 6 weeks.

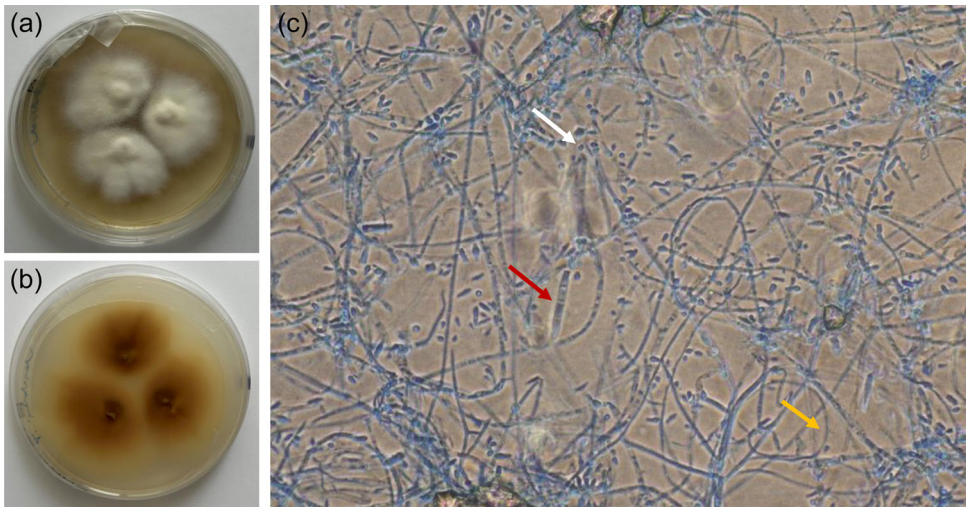

FIGURE 2 Macro and microscopic aspect of isolated *Trichophyton indotineae* strain cultured on Sabouraud Gentamicin Chloramphenicol 2 Agar (bioMérieux) at 30°C after 2 weeks: (a) colonies obverse; (b) colonies reverse; (c) microscopic elements stained by lactophenol blue: macroconidia (red arrow), microconidia (white arrow), spiral hyphae (yellow arrow).

TABLE 1 MIC (80% growth inhibition) values for antimicrobials tested by microdilution assay following CLSI M38-A3 standard method (ISBN 1-56238-830-4 [Print]; ISBN 1-56238-831-2 [Electronic]).

Antimicrobials	MIC (mg/L)
ISAVUCONAZOLE	2
POSACONAZOLE	0.25
VORICONAZOLE	0.5
ITRACONAZOLE	0.25
FLUCONAZOLE	32
AMFOTERICIN B	2

Note: Drugs concentration ranges tested were: Fluconazole 0.125 to 64 μg/mL, Itraconazole, Posaconazole and Voriconazole 0.03 to 32 μg/mL, Amphotericin B 0.125 to 8 μg/mL. Terbinafine was not available. The MIC was determined using the endpoint of 80% inhibition of growth compared to control except for Amphotericin B were inhibition of 100% was considered. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality controls.

post-inflammatory hyperpigmentation. A mild pruritic small erythematous and scaly patch on the patient's right arm persisted, thus treatment was not halted. After 6 weeks of treatment complete recovery was achieved (Figure 1).

DISCUSSION

Topical therapy with allylamines like terbinafine is commonly employed for the majority of dermatophyte infections.[13] Oral therapy may be necessary in cases of widespread infections or when topical treatment fails to yield satisfactory results. Oral administration of terbinafine or itraconazole is the preferred initial therapeutic approach, with the expectation of resolution within a time frame of approximately 2 to 3 weeks. Oral Griseofulvin, has been recently proved as less effective drug in chronic and relapsing dermatophytosis.[14]

However, the global emergence of a novel species exhibiting multidrug resistance presents a significant challenge to the aforementioned treatment protocols. Identification of *T. mentagrophytes* through culture alone may not provide adequate information for effective treatment decision-making.

Before being referred to our care, our patient underwent unsuccessful treatments with topical terbinafine and oral itraconazole at standard dosages, the infection had most likely occurred in Nepal, but lack of effective therapy led to reappearance while her stay in Italy. The detection of *T. indotineae* through molecular techniques prompted us to prioritize itraconazole-based therapy, despite an initial therapeutic setback. This decision was further reinforced by the identification of the double hit mutation in the SQLE gene (Phe397Leu/Ala448Thr) which has been associated with resistance to terbinafine and fluconazole, but not to the most recent azoles.[12]

A high-dosage itraconazole-based therapy of 200 mg daily for 6 weeks was further affirmed by the clinical response to the infection but further research is necessary as other factors may also play a role in contributing to resistance against the latest generation azoles.[7] In our case, we noticed a connection between in-vitro itraconazole sensitivity (Figure 3) and the clinical effectiveness of treatment.

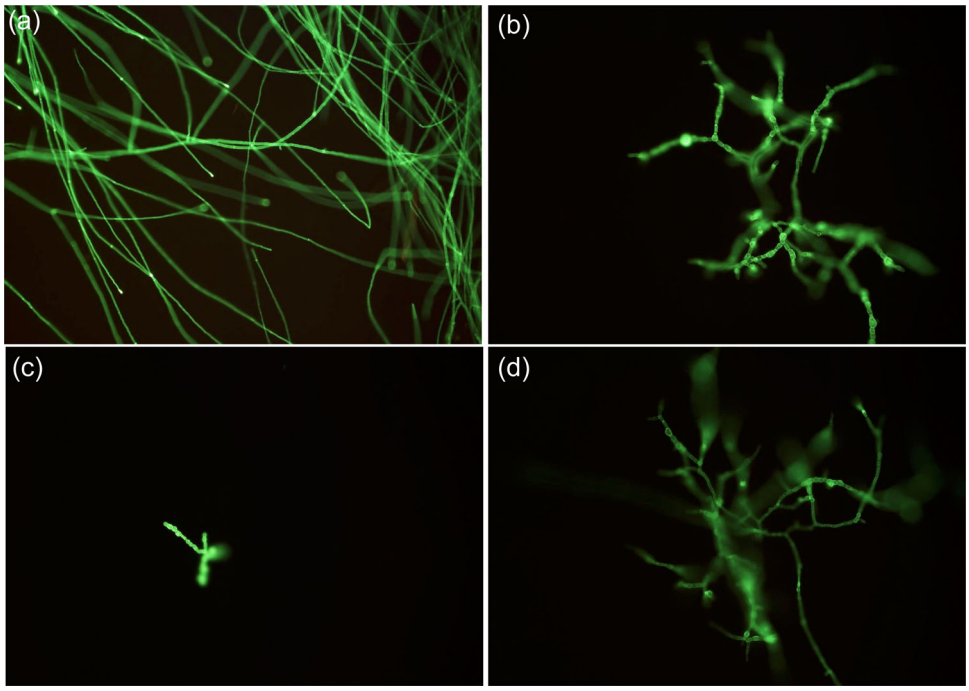

FIGURE 3 Hyphae of *Trichophyton indotineae* observed by fluorescence microscopy when growing in 1640 rpmI medium according to the CLSI M38-A3 standard Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi and different antifungal compound: (a) in pure medium; (b) with 0.12 μg/mL Itraconazole; (c) with 0.25 μg/mL Itraconazole (MIC); (d) with 2 μg/mL Amphotericin B (MIC); a round shape of hyphal cells due to osmotic effect can be observed in all spots with antifungal drugs compared to pure medium.

This might suggest that the values we gathered for all the azoles tested are reliable and could provide valuable guidance for treatment decisions. While broth microdilution remains the gold standard for antifungal susceptibility testing of dermatophytes, its technical complexity limits its widespread use.[15] Despite these challenges, the growing prevalence of dermatophyte resistance necessitates expanded susceptibility testing capabilities, especially in case of chronic or recurrent infections. The absence of established clinical breakpoints hinders optimal treatment strategies for potentially resistant strains. Therefore, defining epidemiological cut-off values is crucial to aid clinicians in managing these challenging cases.

AUTHOR CONTRIBUTIONS

A. Benini and R. Koncan conceived/organized the study and wrote the original draft. R. Koncan performed sequencing. G. Lo Clemente, V. Lepera, G. Palladino performed antifungal susceptibility assay and pictures of sample. G. Lo Clemente performed microbiological culture. N. Di Meo, F. Barbone, I. Zalaudek reviewed the manuscript. I. Zalaudek provided clinical sample.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The patient in this manuscript has given written informed consent to publication of deidentified, anonymized, aggregated data and case details (including photographs) for publication. Ethical Approval: not applicable.

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