Emerging Microbes & Infections
2025, VOL. 14, 2498571 (11 pages)
https://doi.org/10.1080/22221751.2025.2498571



# Genomic epidemiology and antifungal resistance of emerging *Trichophyton indotineae* in China

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**ABSTRACT**

The emergence and spread of antifungal-resistant *Trichophyton indotineae* pose an increasing public health concern worldwide. Multidrug-resistant *T. indotineae* infections have been reported in China in the past few years. To understand the genetic relationship and the origin of these Chinese isolates, as well as their relationship to the global collections, we sequenced the whole genomes of 31 isolates using the Illumina platforms. Genomic epidemiology was performed on a dataset of 181 *T. indotineae* isolates from China and 8 other countries, representing the largest genome-wide analysis. Single nucleotide polymorphism analysis revealed that *T. indotineae* can be divided into four distinct phylogenetic groups (I, II, III, IV), with regional clonal transmission clusters identified in eastern China; *T. indotineae* was introduced into China more than once given the genetic variability. The isolates from South Asia may be the source of Chinese isolates based on epidemiological information. There were differences in the prevalence and resistance profiles among four phylogenetic groups, with Group III being predominant and exhibiting a higher terbinafine resistance rate of 88.24% and azole resistance. Also, we characterized the role of gene mutation, copy number variation, and gene expression in antifungal drug resistance. Terbinafine resistance could be mainly associated with Phe397Leu substitution in *SQLE*, and azole resistance might be related to increased copy number of *CYP51B*, as well as elevated *MDR2* and *MDR3* expression. Given the clinical challenges posed by *T. indotineae*, this emerging dermatophyte should be recognized as a global threat, necessitating urgent collaborative surveillance and management strategies.

**ARTICLE HISTORY** Received 22 September 2024; Revised 27 March 2025; Accepted 17 April 2025

**KEYWORDS** *Trichophyton indotineae*; single nucleotide polymorphism; clonal dissemination; terbinafine resistance; azoles resistance; copy number variation

## Introduction

*Trichophyton indotineae* is an emerging pathogenic fungus of growing concern due to its drug resistance and therapeutic challenges posing to dermatophytosis [1, 2]. Initially classified as *T. mentagrophytes* ITS genotype VIII using rRNA phylogenies [3], it has recently been proposed as *T. mentagrophytes var. indotineae* based on RAD-seq analysis [4, 5]. Since its discovery, *T. indotineae* has been identified in at least 30 countries across Asia, Europe, North America, Oceania, and Africa, with a notable prevalence in the Indian subcontinent [6]. Numerous cases of generalized and refractory dermatophytosis caused by *T. indotineae* have been reported, affecting patients' health, and increasing the public economic burden [7]. *T. indotineae* could spread through contact with infected people or contaminated items, and it has rapidly replaced *T. rubrum* as the dominant pathogen of dermatophytosis in India [8]. Most infected patients have travel, trade, and migration history to South Asia [9]. However, *T. indotineae* has also been isolated in residents without known exposure history in China [10], the USA [11], and Germany [12], emphasizing the importance of ascertaining its origin.

Whole genome sequencing (WGS) approach is useful for investigating the epidemiology and evolution of microbial pathogens, and characterizing potential resistance mechanisms [13]. A genome-wide analysis of 20 *T. indotineae* isolates causing recalcitrant dermatophytosis in north India indicated

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/22221751.2025.2498571.

that the alarming outbreaks are caused by the spread of multidrug-resistant clones, with average single nucleotide polymorphism (SNP) differences of ≤ 42 bp between strains [14]. Meanwhile, 11 *T. indotineae* isolates identified in New York City from May 2022 to May 2023 clustered together in a phylogenetic k-mer analysis, with SNP differences ranging from 0 to 373 bp [15]. These two previous studies included limited number of national isolates and focused on *T. indotineae* terbinafine resistance. Recently, Rhode et al. [16] characterized the genomic epidemiology of 90 *T. indotineae* isolates from 5 countries, and the tree topology indicated all isolates formed a monophyletic group, supporting a single evolutionary origin. The lack of geographic clustering of isolates indicated the spread of *T. indotineae* was recent and likely originated from South Asia [16]. Apart from the mutations in squalene epoxidase (*SQLE*), the multidrug resistance mechanisms among global strains have not been characterized.

Recently, we reported 14 multidrug-resistant *T. indotineae* cases series in China, of which 13 were identified in patients without any history of travelling abroad between June 2022 and August 2023 [10]. Then, we collected 11 additional *T. indotineae* isolates from three different locations where possible. The identification of numerous locally acquired cases has raised questions about the source of isolates and serious concerns about the potential for epidemics of multidrug-resistant *T. indotineae* in localized regions of China. In addition, the evolutionary relationship between Chinese and global isolates is currently unknown. In the present study, firstly, we studied the emergence and transmission patterns of 25 *T. indotineae* isolates within China using a WGS approach. Secondly, we investigated the possible source of Chinese isolates using a phylogenetic approach by including all avaliable global isolates. Thirdly, we evaluated the antifungal susceptibility profiles of these Chinese clinical isolates, as well as characterizing the potential molecular mechanisms of resistance using genomic and quantitative PCR approaches.

## Materials and methods

A total of 25 Chinese clinical isolates of *T. indotineae* were identified between 2021 and 2024. The isolates were collected from 3 hospitals across 3 provinces: Chinese Academy of Medical Sciences Hospital of Dermatology (Jiangsu Province, n = 23), Dermatology Hospital, Southern Medical University (Guangdong Province, n = 1), and Xijing Hospital (Shaanxi Province, n = 1). Since drug resistant *T. indotineae* has been considered to be originated from India, 6 Indian *T. indotineae* isolates were included in the investigation (Table 1). Species identification was performed using internal transcribed spacer (ITS) rRNA sequencing, followed by antifungal susceptibility testing (AFST). WGS and qPCR were conducted to investigate the molecular features of the isolates. All 31 isolates were sequenced using Illumina platform. To improve genome assembly and to reduce the number of smaller-size contigs, two *T. indotineae* isolates were further sequenced using PacBio platform (Supplementary 1 Table 1). Detailed procedures for DNA extraction, WGS, AFST and the defined threshold for resistance, qPCR methodologies, and statistical analyses are described in Supplementary 2. This study was approved by the ethics committee of the Institute of Dermatology, Chinese Academy of Medical Science and Peking Union Medical College (grant number2022-KY-037).

To trace the origin of Chinese isolates and to explore their genetic relationships among global isolates, we incorporated publicly available paired-end raw sequencing reads from 150 *T. indotineae* strains (retrieved up to December 2, 2024) from the National Center for Biotechnology Information Sequence Read Archive, including the recent samples from Rhodes et al. [16]. In total, 181 strains were analysed from 9 countries across Asia, Europe, and North America: China (n = 25), India (n = 39) [16, 17], Canada (n = 57) [16, 18], the USA (n = 14) [15], the UK (n = 25) [16], France (n = 18) [16], Japan (n = 1) [16], Ireland (n = 1) [16], and Singapore (n = 1) [19]. The samples were obtained from skin scrapings of patients with dermatophytosis from 2018 to 2024, except for 3 strains isolated from Canis lupus familiaris (Supplementary 1 Table 2). The SNP-based phylogenetic analysis was performed using both maximum likelihood and Bayesian inference methods to infer the global phylogeny of *T. indotineae* as well as the potential origins and clonal clusters of the Chinese isolates. Methodological details regarding SNP analysis, resistance-associated gene mutation screening, and copy number variation (CNV) analysis are provided in Supplementary 2.

## Results

### *Genomic features and conservation of T. indotineae*

The genome assemblies of 25 Chinese *T. indotineae* isolates ranged from 22.09–22.62 Mb, comprising 69–106 scaffolds, similar to the Indian source strains, which ranged from 22.15–22.28 Mb with 78–111 scaffolds. The PacBio genome assemblies for strains D04782 and D03895 were further optimized to 24 and 11 scaffolds, respectively. In these haploid dermatophytes, 6,803–7,179 protein-coding genes and 5,303–5,770 tandem repeat sequences were predicted (Table 1).

**Table 1.** Genome assembly statistics of 31 *T. indotineae* isolates in this study.

| Isolates | 25 isolates of Chinese source | 6 isolates of Indian source |
|---|---|---|
| Total assembly size (Mb) | 22.09–22.62 | 22.15–22.28 |
| Scaffolds | 69–106 (24)* | 78–111 (11)* |
| Scaffold N50 (Mb) | 0.61–1.70 (4.52)* | 0.73–1.17 (3.64)* |
| GC content (%) | 48.58–48.76% | 48.67%—48.71% |
| Protein coding genes | 6803–7179 | 6813–7151 |
| Gene/Genome (%) | 51.10–51.90% (63.65%)* | 51.00—51.70% (63.83%)* |
| Tandem repeat | 5355–5414 | 5,303–5,770 |
| Tandem repeat/Genome (%) | 1.14–1.16% (0.85%)* | 1.15–1.16% (0.92%)* |
| Ploidy | Haploid | Haploid |

*The strains' genomes were further assembled by PacBio sequencing.

We analysed the SNP differences among these 181 isolates, including 150 isolates retrieved from NCBI. Although they come from 9 countries on 3 continents, the genome of *T. indotineae* was highly conserved compared to other species. A total of 3,133 SNPs were identified in the genomes of 181 isolates, significantly fewer than the previously reported numbers for *T. rubrum* (24,740 SNPs) and *T. interdigitale* (22,568 SNPs) [20], and also much smaller than other multi-drug-resistant yeasts, such as *C. auris* (236,900 SNPs) [21] and *C. haemulonii* (6,907 SNPs) [22]. Pairwise SNP differences ranged from 7 to 595 bp (median 165) among the international isolates, and from 16 to 433 bp (median 119) among the Chinese and international isolates. However, genetic polymorphism within the Chinese isolates was lower, ranging from 7 to 152 bp (median 21). Additionally, mating type loci characterization revealed that all 181 strains had MAT1-2-1, encoding a high mobility group (HMG) domain protein. The presence of a single mating type in these isolates strongly suggests that *T. indotineae* reproduces mainly clonally [20].

## Four phylogenetic groups identified among the global isolates

Phylogenetic tree generated based on 3,133 SNPs using both maximum likelihood (Figure 1) and Bayesian inference methods (Supplementary 3 Figure 1), revealed that *T. indotineae* could be divided into four major groups. The tree inferred that 10 isolates (5.52%) belonged to Group I, 42 (23.20%) in Group II, 102 (56.35%) in Group III, and 26 (14.36%) in Group IV. There was one divergent sample not belonging to any group. Isolates in these 4 groups were from different geographic regions, with sampling dates spanning from 2018 to 2024. To provide a quantitative measure of genetic variation within the *T. indotineae* population, we calculated the genome-wide nucleotide diversity ($\pi$). The $\pi$ value of whole population was 0.00058. The estimated $\pi$ was the lowest for Group III (0.0000096), and slightly lower for Groups I and IV (0.000027 and 0.000031, respectively), while Group II exhibited the higher diversity ($\pi = 0.0024$). Both Group II and III had a wide geographical distribution, however, they exhibited significant differences in genetic diversity. Notably, isolates from India nested in all groups and some were early reported based on available records. Meanwhile, most early reports of *T. indotineae* infections in Europe and North America had links to travels to the Indian subcontinent, primarily India and Bangladesh [23, 24]. The genomic epidemiological survey suggested that the spread of *T. indotineae* was likely to be originated from South Asia [16], with direct or indirect introduction events and cross transmission chains contributing to its worldwide dissemination.

## Clonal spread and regional clustering in China

Twenty-four isolates from China were classified into Group III, except that strain D06109 was assigned to Group II, of which was isolated from a young Indian male with tinea cruris in Guangdong Province. Alarmingly, 22 Chinese drug-resistant isolates from the same region in Jiangsu Province clustered closely together in one cluster (Cluster A), with SNP differences ranging from 7 to 36 bp (median 19) (Figure 2). This suggested either close contact transmission or multiple immediate infections from a common source. The SNP differences of these 22 isolates were also lower than the average pairwise SNP difference (98 bp) among all isolates in Group III, indicating the recent emergence of resistant *T. indotineae* locally, and their ongoing local transmission within easterm China. Interestingly, Cluster A was highly related to a sample from Japan (SRA accession number ERR13913792), with SNP differences ranging from 19 to 63 bp (Figure 2). Moreover, strain D04226, isolated in 2021 from a young Chinese male with tinea corporis in Anhui Province, was closely related to two Indian isolates (ERR13913778 and ERR13913771), with SNP differences ranging from 32 to 36 bp. Likewise, strain D06109 was most closely related to the Indian isolates D03902 and D03945, with SNP differences of only 16–21 bp. Strain D06009 from Shaanxi Province, isolated from a male patient who developed tinea corporis after returning from a trip to Malaysia, was most closely related to the isolate from the USA (SRR27198739). Consequently, Chinese isolates were genetically close not only to South Asia but also to Japanese and American

**Table 2.** The Pattern of CYP51B copy number variation in 27 azole-resistant *T. indotineae* strains sequenced in this study, and corresponding minimum inhibitory concentrations of four azoles.

| Pattern | Types | Duplicated segments (kb) | No. *CYP51B* copy | No. isolates | Country | Minimum inhibitory concentration (mg/L) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Fluconazole | Itraconazole | Posaconazole | Voriconazole |
| Tandem gene duplications | I | 2.4 | 6–11 | 24 | China | 64– ≥ 128 | 0.2–0.5 | 0.25–0.5 | 0.25–0.5 |
| | II | 7.3 | 13 | 2 | India | 32 | 0.25 | 0.25–0.5 | 0.5 |
| | III | 21.0 | 5 | 1 | China | 32 | 0.25 | 0.5 | 1 |

strains, implying that *T. indotineae* strains might have not only originated through importation from India but also through unknown indirect transmissions from other countries.

## *Highly resistant to terbinafine and mutations of SQLE in T. indotineae*

Based on the literature and published data [15-19], among the 181 isolates studied, 69.06% (125/181) exhibited resistance to terbinafine (MICs > 0.125 mg/L). The proportion of resistance varied across different groups, with Group III showing the highest resistance rate at 88.24% (90/102), while Group II had the lowest, with only 28.57% (12/42) of strains resistant (Figure 3(A)). The resistance rate to terbinafine also differed between countries, ranging from 33.33% to 92.00% (Figure 3(B)). Terbinafine resistance was most challenging in China, where all native isolates (92%, 23/25) were resistant, and the vast majority of strains had a MIC ≥128 mg/L. Among the 6 sequenced Indian isolates, only one has been identified as resistant to terbinafine (MIC = 32 mg/L). Among the terbinafine-resistant strains, the most common mutation in *SQLE* was Phe397Leu (81.6%, 102/125), followed by Leu393Ser (6.4%, 8/125), Leu393Phe (1.6%, 2/125),

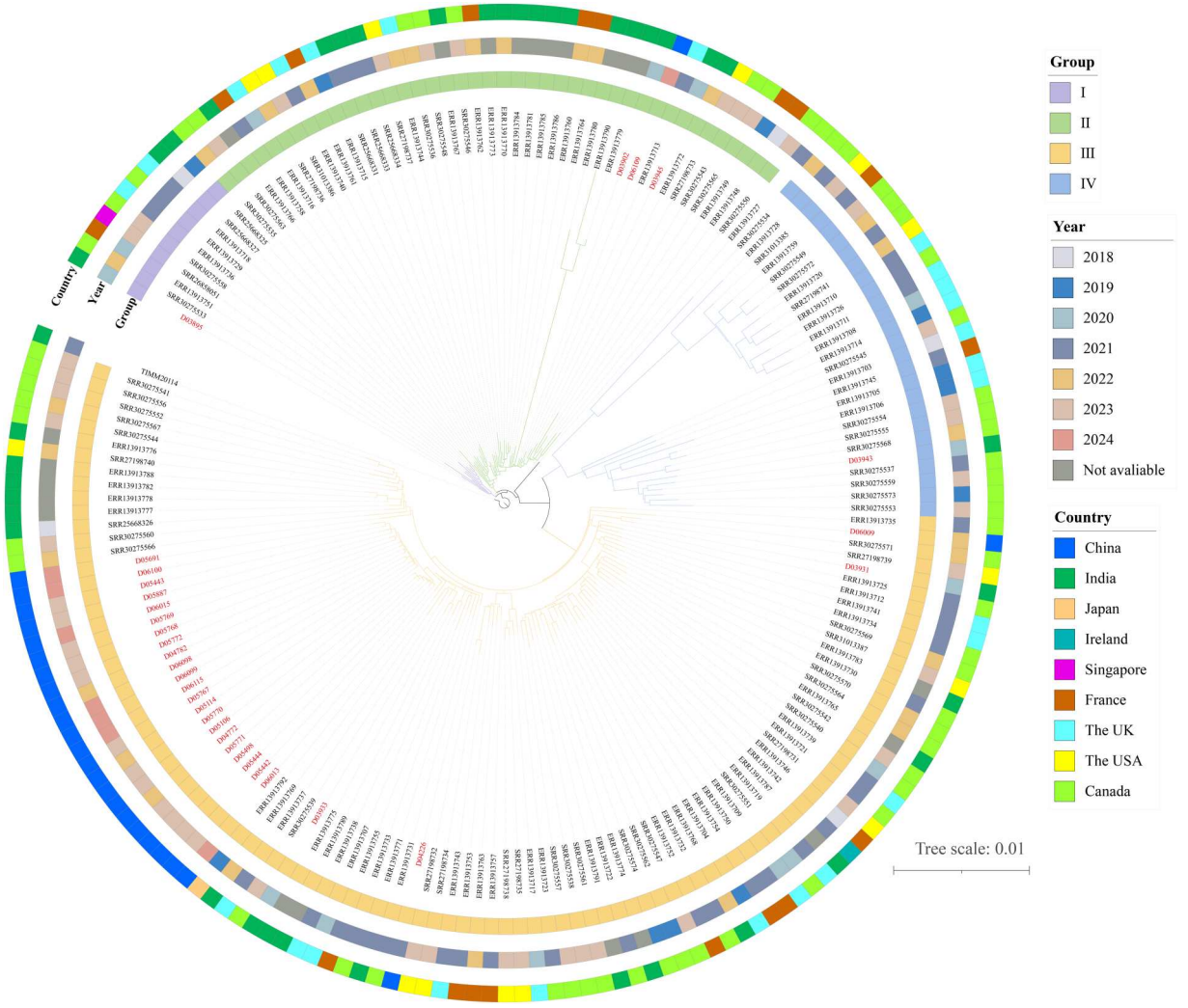


**Figure 1.** Phylogenetic relationships between *T. indotineae* in China and international isolates based on maximum likelihood analysis of SNPs from 181 genomes. The tree was rooted with *T. indotineae* TIMM20114, and revealed the presence of four phylogenetic groups. Scale bar showing the number of substitutions per nucleotide. Samples are overlaid with the year and country of collection.

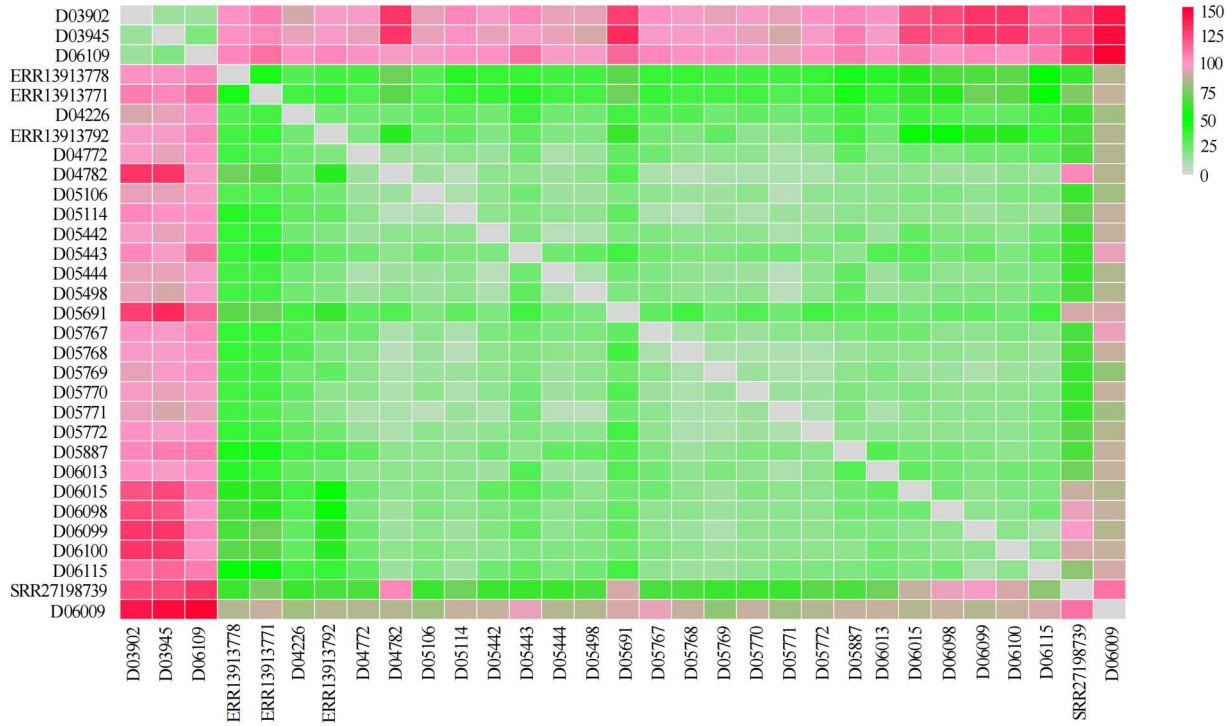


**Figure 2.** Pairwise genetic distance of Chinese *T. indotineae* isolates and closely related international strains estimated from genomic SNPs.

and Phe397Leu/Tyr414Leu (0.8%, 1/125). Additionally, 9.6% of the isolates (12/125) did not exhibit *SQLE* missense mutations, suggesting the possibility of other resistance mechanisms (Figure 3(C)). We also discovered mutations in *SQLE* in 25.45% (14/55) terbinafine-sensitive isolates (Figure 3(D)), including Ala448Thr (16.36%), Ser395Pro (5.45%), His440Tyr (1.82%), and Phe415Cys (1.82%). Therefore, the detection of specific *SQLE* mutations, particularly Phe397Leu, is useful in predicting terbinafine resistance in *T. indotineae*.

## Rise of resistance to multiple azoles in *T. indotineae* and potential mechanisms

Among the *T. indotineae* isolates with azoles susceptibility, 68.09% (32/47) were resistant to fluconazole (MICs range 32 – ≥ 128 mg/L), 27.91% (24/86) resistant to itraconazole (MICs range 0.5 - 2 mg/L), 3.49% (3/86) to voriconazole (MICs range 2 - 4 mg/L), and 52.94% (18/34) to posaconazole (MICs = 0.5 mg/L). Azole-resistant strains were predominantly identified in Group III, with a few strains in Groups I and II. No azole resistance has been observed in Group IV (Supplementary 1 Table 2).

Due to the limited AFST data, we focused on 31 *T. indotineae* strains sequenced in our study. Of 25 Chinese isolates, all were resistant to fluconazole; 72% (18/25) were resistant to both fluconazole and itraconazole; 68% (17/25) were resistant to both fluconazole and posaconazole; 44% (11/25) were resistant to fluconazole, itraconazole, and posaconazole; and 72% were multidrug resistant, showing resistance to terbinafine, azoles, and griseofulvin. In contrast, among the 6 Indian isolates, only one strain was resistant to fluconazole, while another exhibited resistance to two azoles.

The genome of strain TIMM20114 was used as a reference sequence to identify missense mutations in 14 genes associated with azole resistance. Among the *CYP51B* gene of azole-resistant isolates, 85.19% (23/27) and 3.70% (1/27) possessed GLY443Glu and Tyr444His mutations, respectively. Among the 4 azole-sensitive isolates, each contained a single mutation in *CYP51B* gene: GLY443Glu, Gly443Arg, and Tyr444His. Therefore, these mutations in *CYP51B* were likely not associated with azole resistance. Thirteen additional resistance-related genes also did not detect any missense mutations linked to azole resistance phenotypes (Supplementary 1 Table 3).

## Tandem gene duplication-mediated *CYP51B* CNVs associated with azole resistance

We further performed CNV analysis on these 31 *T. indotineae* strains to investigate their potential as one of the resistance mechanisms. Of note, only the *CYP51B* gene in the 27 fluconazole-resistant isolates showed an average sequencing depth elevation greater than 9 times (average depth 9.45 ± 1.60) vs 4 azole-susceptible isolates (average depth 1.12 ± 0.10), suggesting the occurrence of notable CNV events ($p < 0.001$). Azole-resistant isolates displayed tandem

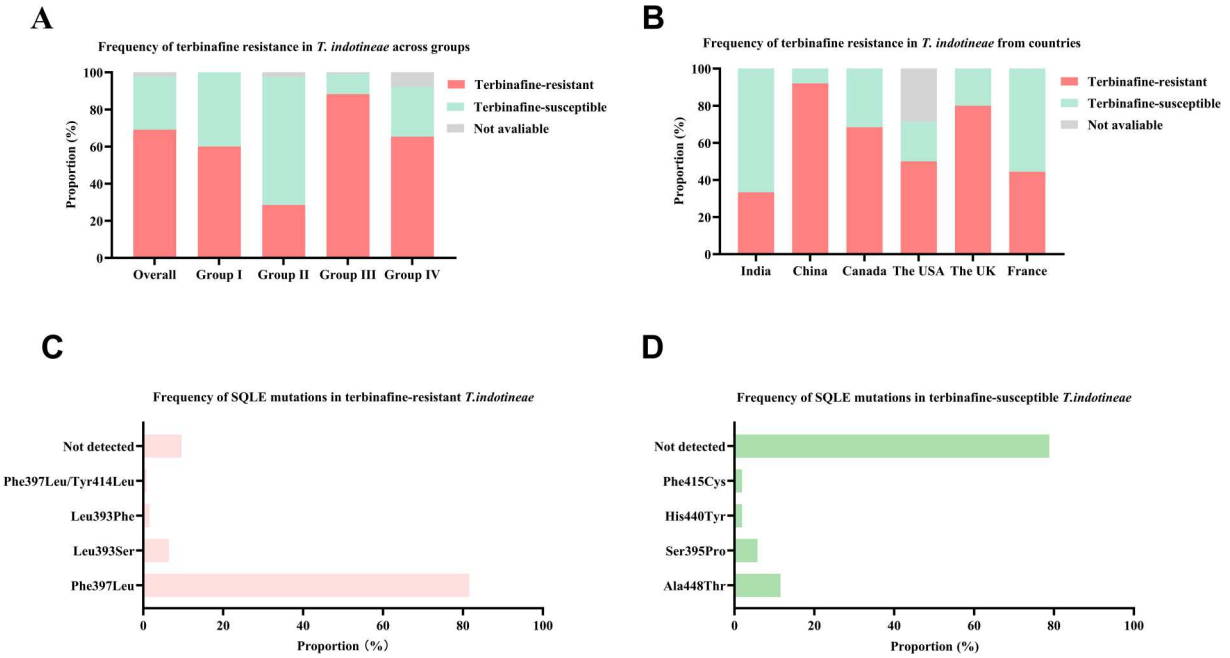


**Figure 3.** Terbinafine resistance and frequency of missense mutations in squalene epoxidase (*SQLE*) in *T. indotineae* isolates. (A) The terbinafine resistance rates of *T. indotineae* within the four groups. (B) Terbinafine resistance rates of *T. indotineae* from different countries (for countries > 10 isolates). (C) Frequency of *SQLE* mutations in *T. indotineae* resistant to terbinafine. (D) Proportion of *SQLE* mutations and those without mutations in *T. indotineae* sensitive to terbinafine. Ala, Alanine; Cys, Cysteine; His, Histidine; Leu, Leucine; Phe, Phenylalanine; Pro, Proline; Ser, Serine; Thr, Threonine; Tyr, Tyrosine.

gene duplication-mediated copy number increases ranging from 6 to 13, compared to the sensitive strains with a single copy number (Table 2). Based on the size of *CYP51B* duplicated segments, the 27 strains could be classified into three types: Type I, consisting of 24 Chinese isolates with 2.4 kb of repetitive sequences; Type II, consisting of 2 Indian isolates with 7.3 kb of repetitive sequences; and Type III, consisting of one imported strain in China from abroad with 21.0 kb of repetitive sequences (Figure 4). In addition, CNV analysis revealed that out of the 7 strains from Canada, 2 itraconazole-resistant strains contained Type I duplication, the other 5 strains carried Type II, but none exhibited resistance to either itraconazole or voriconazole [18]. More interestingly, Type I was exclusively found in Group III, whereas Type II was only present in Group II.

### *High expression of CYP51B, MDR2, and MDR3 in azole-resistant T. indotineae*

The expression levels of *CYP51A*, *CYP51B*, *MDR1*, *MDR2*, *MDR3*, and *MFS2*, were compared between azole-resistant group (AZR, n = 25) and azole-susceptible group (AZS, n = 3) using qPCR. Compared to AZS group, AZR group showed a significant increase in the expression of *CYP51B*, *MDR2*, and *MDR3* (Figure 5(A–C)), while no significant differences were observed for *CYP51A*, *MDR1*, and *MFS2* (Figure 5(D–F)). The average expression levels of *CYP51B*, *MDR2*, and *MDR3* in AZR group were 10.45, 3.49, and 3.31 times higher than those in the AZS group, respectively. The increased expression of *CYP51B* could be correlated with the 6 - 13 folds increase in *CYP51B* copy number in the AZR group strains.

## Discussion

In recent years, the emergence and spread of *T. indotineae*, a pathogen of difficult-to-treat dermatophytosis, has posed an increasing threat to public health. The discovery of this pathogen beyond its initial report in Indian subcontinent highlights its global dissemination [1]. In this study, we investigated the molecular epidemiology of *T. indotineae* strains worldwide, which represented the largest and most geographically diverse WGS study of *T. indotineae*. Four genetic groups (I-IV) were recognized among 181 isolates from China, India, Canada, the USA, the UK, France, Japan, Ireland, and Singapore, with Groups II and III being predominant. The total number of SNPs in the 22.16 Mb genome of *T. indotineae* was only 3,133 (< 0.01%), which is much fewer than in the similarly sized genomes of *T. rubrum*, *T. mentagrophytes*, *and T. interdigitale* [20, 25]. The SNP differences between *T. indotineae* and *T. mentagrophytes* (> 88, 034 bp) and *T. interdigitale* (> 78, 802 bp) were also great [19]. The lack of geographical and temporal clustering of strains within each group ruled out the presence of region-specific strains [18].

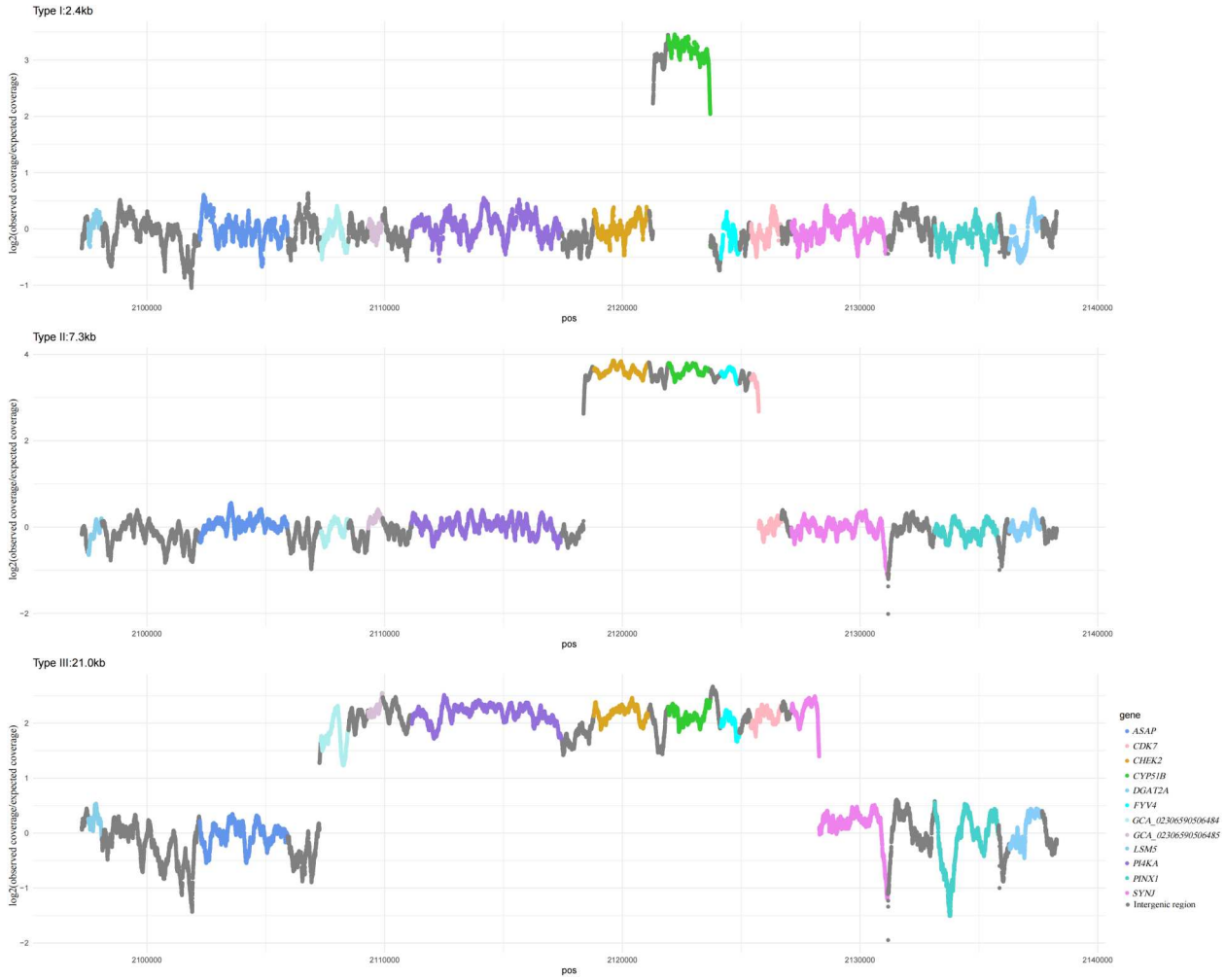


**Figure 4.** Illustrations showing three types of copy number variation in the pattern of tandem gene duplications. Each spot represents the sequence depth for one bp on the scaffold.

Since 2022, *T. indotineae* isolates have been increasingly reported in France, the UK, the USA, and Canada, consistent with the relaxation of COVID-19 restrictions [18]. Many reported cases had travel histories to the Indian subcontinent. The transition of *T. indotineae* from being initially reported in India as the cause of refractory tinea corporis-cruris to triggering outbreaks has led to a significant epidemiological shift in dermatophytosis over the past few years [8]. The large population in India, coupled with the inappropriate use of corticosteroid-antifungal agents, may have created an environment conducive to the emergence and spread of this highly resistant fungus [7]. The phylogenetic tree indicated that Indian isolates were present in all phylogenetic groups, with earlier isolation dates, suggesting Indian strains were closely related to isolates from other countries. The increased people movement has likely exacerbated the spread of this pathogen, bringing significant challenges to clinical management of dermatophytosis.

The genetic diversity and antifungal resistance profiles of isolates in different groups had variations. Group III has the lowest genetic diversity but relatively higher resistance to terbinafine and azoles. The low diversity could suggest a stronger tendency for vertical transmission, where mutations and resistance loci might be passed to the next generation through clonal reproduction [26]. The accumulation of resistance traits, such as the Phe397Leu mutation in *SQLE* gene and increased copy numbers of *CYP51B* gene, might indicate that these resistance-related genes have been propagated and selected within this group. The absence of sexual reproduction [27], as suggested by the presence of a single MAT-1-2 mating type, could further support the clonal propagation. Selective pressure from antifungals could have driven the emergence and evolution of the resistance traits, resulting in the relatively high resistance observed in Group III.

*T. indotineae* has also been increasingly isolated in eastern China, particularly between July 2022 and September 2024, when 22 isolates from Jiangsu province were found to cluster together within Group III. Nevertheless, the number of *T. indotineae* infections may be underestimated, as some Chinese provinces lack cases data, likely due to the limited accuracy of conventional methods and the unavailability of AFST for

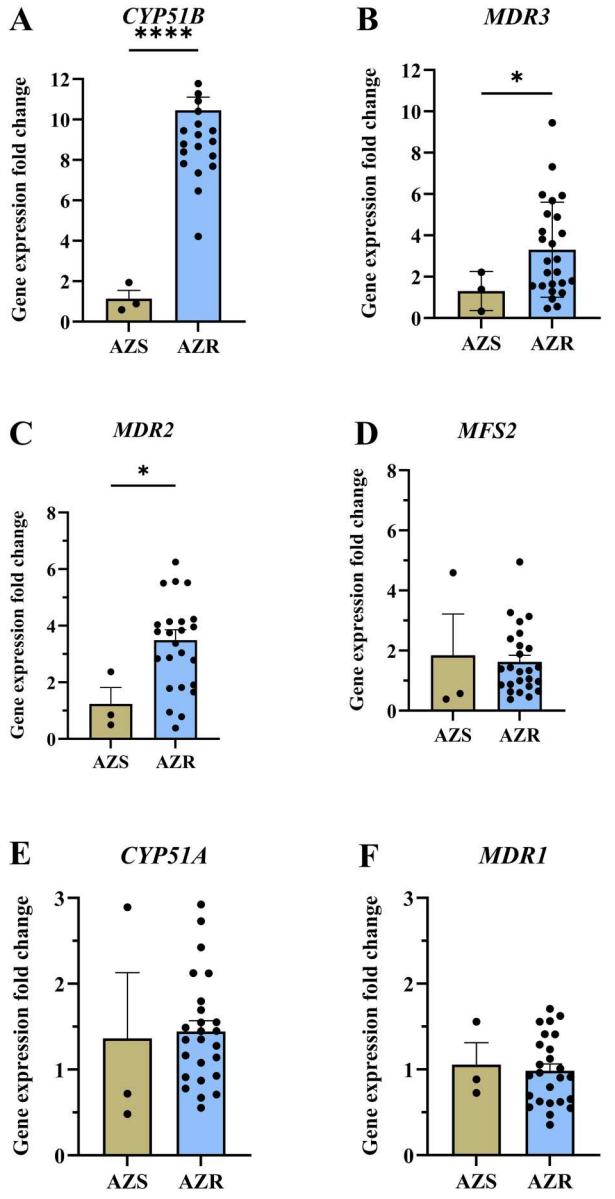


**Figure 5.** The gene expression levels of *CYP51A*, *CYP51B*, *MDR1*, *MDR2*, *MDR3*, and *MFS2* in *T. indotineae* isolates from the azole-resistant group (AZR) and the azole-susceptible group (AZS) were assessed by qPCR, using *GADPH* as the reference. Gene expression levels were displayed using bar charts, with individual data points overlaid as dot plots. Error bars represent the standard error of the mean (SEM) for each group. Statistical analysis was performed using *t*-tests and non-parametric tests. * denotes $P < .05$, and **** denotes $P < .0001$.

dermatophytes in most hospitals. The pairwise SNP differences between these 22 isolates ranged from 7 to 36 bp, which is markedly lower than the average SNP difference observed among Group III strains. Given their shared geographic distribution, a small window of isolation dates, and similar resistance phenotypes, we proposed that these isolates represented a regional clonal cluster, designated Cluster A. Based on this, we suggested that a paired SNP difference of ≤ 36 bp between strains with similar epidemiological backgrounds could serve as a criterion for identifying clonal clusters of *T. indotineae*. Similar studies have shown that SNP differences can be an effective indicator for identifying transmission clusters. Chow et al. [28] used an SNP difference of 12 bp as a cut-off value for defining outbreaks of *C. auris* infections in the USA, and Chen et al. [22] suggested that SNP differences of ≤ 50 bp could define clonally transmitted infections of *C. haemulonii*. Considering the clonal nature and contact transmission of *T. indotineae*, it is crucial to be vigilant about its potential threat in China.

*T. indotineae* is very different from other well-known dermatophytes, due to its resistance to multiple antifungals. 69.06% of *T. indotineae* isolates were resistant to the first-line antifungal agent terbinafine, with Group III global strains showing even higher resistance. Among the resistant strains, 81.6% harboured point mutation, Phe397Leu in *SQLE* gene, followed by Leu393Ser, Leu393Phe, and Phe397Leu/Tyr414Leu. Homology modelling of *SQLE* in *T. indotineae* indicated that Leu393 and Phe397 formed parts of the hydrophobic site housing the naphthalene portion of terbinafine, while Ala448 was located outside the terbinafine-binding pocket [15, 29]. So missense mutations in Leu393 and Phe397 residues can lead to elevated terbinafine MIC values, but Ala448 variation does not affect terbinafine susceptibility [15, 30].

Azole resistance in *T. indotineae* is being highlighted, though the mechanism of resistance remains to be fully explored [31]. Studies from India [32] and Canada [18] reported that 25% and 23.7% of isolates, respectively, which exhibited decreased susceptibility to itraconazole and voriconazole. Meanwhile, Chinese isolates also showed high resistance to azoles, with all strains resistant to fluconazole and most exhibiting cross-resistance to itraconazole and posaconazole. Point mutations in azole resistance-related genes were screened, and although GLY443Glu mutation was identified in the *CYP51B* gene, it was not specific to azole-resistant strains, with functional studies showing no impact on resistance [33].

Increased copy numbers of the *ERG11* (*CYP51* homologues) have been found to mediate high levels of fluconazole resistance in *C. auris* [34], *C. tropicalis* [35], and *C. parapsilosis* [36]. Recently, it has been reported that elevated copy numbers of *CYP51B* (mainly Type II) are associated with decreased susceptibility to itraconazole in Indian isolates of *T. indotineae* [33]. Differently, our analysis of Chinese isolates revealed that tandem gene duplication-mediated increases in *CYP51B* copy numbers (predominantly Type I) were observed in all fluconazole-resistant strains compared to sensitive strains.

However, not all of these resistant strains exhibited cross-resistance to itraconazole, posaconazole, or voriconazole. Furthermore, azole-resistant strains from Canada did not consistently exhibit *CYP51B* CNV [18].

qPCR analysis revealed that expression level of *CYP51B*, *MDR2*, and *MDR3* was significantly higher in azole-resistant strains than the sensitive strains. The higher copy number of *CYP51B* could lead to gene overexpression, which played a key role in mediating azole resistance in *T. indotineae*. However, the observed cross-resistance to other azoles might be due to other factors, such as the overexpression of efflux pump genes (e.g. *MDR2*, *MDR3*), which likely contributed to the overall resistance phenotype.

Effective management strategies are crucial for treat this difficult-to-diagnose fungal infection. We have developed a Chinese expert consensus on the management of resistant dermatophyte infections [37]. Early identification and resistance testing, along with the rational use of antifungals and avoidance of corticosteroids, are key to improving cure rates and controlling *T. indotineae* infections. Current clinical evidence recommends itraconazole (0.2 - 0.4 g daily for 4 - 8 weeks) as the preferred treatment for refractory *T. indotineae* infections, with the option to adjust the dosage or treatment duration if needed [38]. Establishing international collaborations for resistance monitoring is also essential for tracking the global spread of resistant strains and guiding treatment decisions [39].

This study has limitations. Although our study included the largest collection of *T. indotineae* isolates from China through the resistance surveillance network, all 25 isolates were obtained from only three provinces, with the majority from Jiangsu. Meanwhile, two retrospective studies have reported three imported cases of *T. indotineae* infections in China, including two in Indian patients [40] and one in a Chinese resident returning from abroad [41]. Additionally, AFST revealed that most *T. indotineae* isolates had elevated MICs to azoles, indicating reduced susceptibility. When assessing azole resistance-associated gene expression via qPCR, the azole-susceptible group was limited to three available samples, leading to inevitable intra-group variability. Moving forward, ongoing strain collection and expanded genomics-based surveillance with broader geographical sampling will be essential.

In conclusion, our molecular epidemiological investigation revealed the clonal transmission pattern of *T. indotineae* isolates in China and their genetic relationship to the global collection. The increasing multi-drug resistance, particularly to terbinafine and azoles, position *T. indotineae* as an emerging and serious global threat. Several questions regarding *T. indotineae* remain unresolved: for instance, what is the expansion pattern, and human-to-human transmission pathway of resistant clones? What are the specific environmental or clinical factors contributing to its resistance? We advocate for international collaboration in the surveillance and research on the resistance mechanisms to implement effective management measures.

## Data availability statement

The raw sequencing reads are available from the NCBI under the BioProject accession number PRJNA1157579.

## Acknowledgments

Two Chinese *T. indotineae* strains were collected by the China Antifungal Resistance Dermatophytes Surveillance (CARDS) Network. We would like to thank Hongfang Liu, Dermatology Hospital, Southern Medical University, Guangzhou, Guangdong Province; Meng Fu and Yanyang Guo, Xijing Hospital, Xi'an, Shaanxi Province. We also express our gratitude to Prof. Sybren de Hoog (Radboud University Medical Centre, the Netherlands) for his contribution to strains information and resources, and helpful discussion in studying *T. indotineae*. We also thank Junhui Li and Ye Tao (Shanghai Biozeron Biotechnology Co., Ltd, China) for their assistance in bioinformatics training, Illumina and PacBio sequencing.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This work was supported by the National Key Research and Development Program of China, #1 [grant number 2022YFC2504804 and 2022YFC2504800]; CAMS Innovation Fund for Medical Sciences, #2 [grant number CIFMS, 2021-I2M-1-039]; Jiangsu Provincial Medical Key Laboratory, Jiangsu Province Capability Improvement Project through Science, Technology and Education, #3 [grant number ZDXYS202204]; Double-Innovation Doctor Program of Jiangsu Province, #4 [grant number JSSCBS20221924].

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