Trichophyton indotineae: A Collaboration between Clinicians, Clinical, and Public Health Laboratories is Vital to Prevent the Spread of this Drug-Resistant Fungal Pathogen

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Abstract

Background: Widespread outbreaks of tinea in South Asia are currently driven by *Trichophyton indotineae*, which readily spreads from person to person and is frequently resistant to antifungals, including terbinafine (TRB), a drug commonly used to treat tinea. Recently, *T. indotineae* has been detected in patients from New York City (NYC), USA. Given its anthropophilic spread and multidrug resistance, there is an urgent need to understand the prevalence, spread, and drug-resistance profile of *T. indotineae* to improve patient outcomes.

Methods: Cultures from skin scrapings of NYC patients suspected of *T. indotineae* infection were submitted to the Wadsworth Center, NYSDOH. For species-level identification, isolates were grown on urea agar for ten days at 30°C, and agar color change was scored visually. For species confirmation, DNA extracted from these isolates was subjected to PCR of the internal transcribed spacer (ITS) region followed by Sanger sequencing and a BLAST search. Antifungal susceptibility testing (AFST) was performed using broth microdilution with a commercially prepared panel of antifungals (ThermoFisher) and an in-house prepared 2-fold dilution series of TRB and griseofulvin (GRF). Whole genome sequencing (WGS) was performed on all confirmed *T. indotineae* isolates. CLC Genomics Workbench 23.0.3 was used to determine isolate relatedness, identify single nucleotide polymorphisms (SNPs), and detect mutations in squalene epoxidase (SQLE), the cellular target of TRB. A structural model of *T. indotineae* SQLE was generated using Alphafold and Swiss-Modeler, and TRB docking was performed using Quickvina2 with Autodock Vina.

Results: A total of 11 cases of *T. indotineae* aged three to 64 years, with a median age of 39, including five females and six males from NYC were part of this investigation. ITS sequencing and lack of color change on urea agar confirmed all isolates as *T. indotineae*. AFST revealed that most (80%) of the *T. indotineae* isolates were TRB resistant, and 50% had high minimum inhibitory concentrations (MIC) to azoles. In patients, TRB treatment, in general, was not effective, but GRF and a combination of azoles were effective. WGS analysis revealed that *T. indotineae* isolates from NYC were distinct from *T. indotineae* from India. Also, SNP analysis indicates that *T. indotineae* spread among close contacts, but there have also been independent introductions. Modeling of SQLE revealed that F397 and L393 participate in a hydrophobic binding pocket for the naphthalene moiety of TRB, and mutations of F397L or L393S likely abrogated TRB binding, resulting in drug resistance.

Conclusions: In refractory tinea infections, dermatophytes resembling *T. mentagrophytes* should be identified using molecular and urea agar culturing methods and have AFST performed. Furthermore, WGS can inform isolate relatedness during outbreaks and identify mutations in the genes of antifungal targets. To prevent the spread of *T. indotineae*, a strong collaborative effort is needed between clinicians and laboratories, both clinical and public health, to tackle drug-resistant *T. indotineae*.

Introduction

An epidemic of anthropophilic, antifungal resistant dermatophytosis is currently plaguing South Asia [1-3]. The causative agent for these infections is predominantly fungi that are members of the *Trichophyton (T.) mentagrophytes/interdigitale* species complex [1, 2, 4]. A 2020 study of dermatophytes across India, revealed that more than three quarters of cases of dermatophytosis were caused by *T. mentagrophytes genotype VIII* [5], which has since been declared a separate species, *T. indotineae* [6]. TRB is a common drug of choice for clinicians to treat dermatophytic infections [4, 6-8]. However, the widespread abuse of readily available topical corticosteroid creams, generally containing clobetasol or another steroid mixed with antifungal and/or antibacterial agents, has led to the rapid rise in TRB resistant infections [2, 3, 5, 9]. *T. indotineae* continues to spread around the world due to global migration and travel [1], and has recently been identified in two NYC patients, representing the first reported instances of this dermophyte in the USA [10]. Since the first report of USA cases, we have received several more isolates from NYC patients suffering from *T. indotineae* infections. Here we summarize the collaborative efforts of clinical and laboratory findings of 11 cases of *T. indotineae*.

Acknowledgements

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Dermatophyte Species Identification

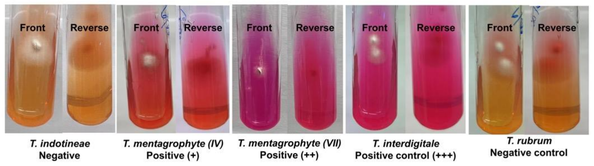

Figure 1: Species determination by urease assay
Using a sterile needle, mold was inoculated onto a urea agar slant containing phenol red and incubated for ten days at 30°C. Isolates expressing urease raise the agar's pH, causing a color shift from yellow to pink.

T. indotineae *did not change the color of the medium, while other* Trichophyton *species did, providing presumptive ID.*

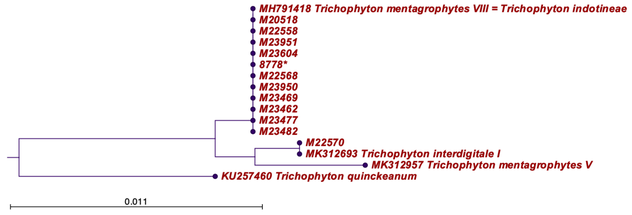

Figure 2: Species determination by ITS sequencing
DNA from suspected *T. indotineae* isolates was extracted using a QIAamp DNA Mini Kit, followed by ITS PCR and Sanger sequencing. The sequences were aligned in CLC Genomics Workbench, and a phylogenetic tree was generated using a neighbor-joining algorithm with a Jukes-Cantor nucleotide substitution model and 1,000 bootstrap replicates. The scale bar indicates the number of substitutions/changes per nucleotide.

Clustering of T. indotineae *isolates with the reference* T. indotineae *strain (MH791418) confirmed the molecular ID.*

Whole-Genome Sequencing Analysis

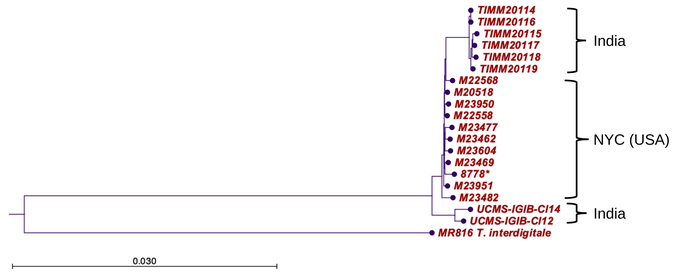

Figure 3: K-mer analysis of *T. indotineae* isolates
A phylogenetic k-mer tree was constructed in CLC Genomics Workbench using whole genome assemblies of the 11 NYC isolates of *T. indotineae* and eight publicly available genome sequences of *T. indotineae* in GenBank. K-mers with a prefix of ATGAC and a length of 16 on either strand were included. The scale bar for branch length indicates the level of similarity in k-mer distribution among isolates.

T. indotineae *isolates from the USA make a unique cluster that falls between two clusters of* T. indotineae *isolates from India.*

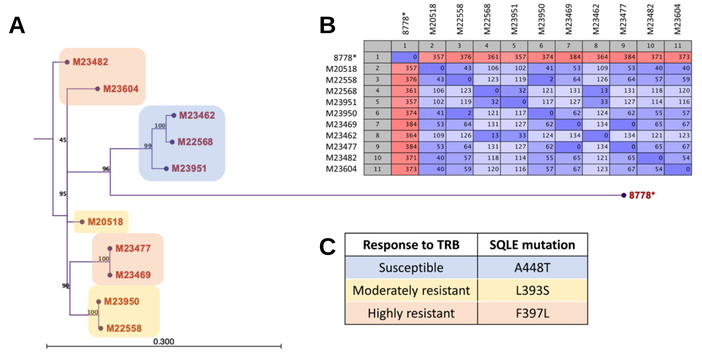

Figure 4: Relatedness of *T. indotineae* isolates

T. indotineae isolates were aligned to the reference strain TIMM20114, and SNPs were identified. (**A**) Maximum likelihood tree using a Juke-Cantor nucleotide substitution model and 1,000 bootstrap replicates. Scale bar indicates the number of substitutions/changes per nucleotide, (**B**) SNP matrix of isolates, (**C**) Shaded boxes with isolate susceptibility to TRB and mutations present in SQLE.

SNPs of 0-2 are indicative of transmission among close relatives, while SNPs of 32-373 among the other isolates are likely indicative of independent introductions. The majority of isolates had either the L393S or F397L mutations in SQLE, which correlate with TRB resistance.

Antifungal Susceptibility Testing

Sample Name	MIC values (µg/mL)												SQLE mutations
	AMB	AND	CAS	MCF	PSC	VRC	FLC	ITC	ISA	KTC	GRF	TRB	
M22568	0.5	0.015	0.015	0.015	0.5	4	32	0.25	8	1	2	<0.0039	A448T
M23951	0.5	0.015	0.015	0.015	0.06	0.25	16	0.12	1	0.12	4	<0.0039	A448T
M23462	1	0.015	0.015	0.015	0.12	1	32	0.12	4	0.5	4	<0.0039	A448T
M20518	0.5	0.015	0.015	0.015	0.06	0.25	16	0.06	1	0.12	4	1	L393S
M22558	0.5	0.015	0.015	0.015	0.12	0.5	32	0.12	4	0.5	4	0.5	L393S
M23950	0.5	0.015	0.015	0.015	0.25	1	32	0.25	4	0.5	4	0.5	L393S
M23469	0.5	0.015	0.015	0.015	0.5	2	64	0.5	8	1	4	>128	F397L
M23477	0.5	0.015	0.015	0.015	0.25	2	16	0.25	8	1	4	>128	F397L
M23482	0.5	0.015	0.015	0.015	0.06	0.25	16	0.06	0.5	0.25	2	32	F397L
M23604	0.5	0.015	0.015	0.015	0.12	0.25	16	0.12	1	0.5	4	128	F397L
8778*	-	-	-	-	-	-	-	-	-	-	-	-	F397L
ECV[2]						≥0.25		≥0.5				≥0.2	
ECV[5]						≥0.125		≥0.25				≥8	
ECV[11]						≥0.25	≥32	≥0.5		≥0.5	≥64	≥8	

Table 1: Antifungal Susceptilitiy Test (AFST)
Minimum inhibitory concentration (MIC) for each antifungal drug was determined by the broth microdilution method after 96 hrs of growth at 35°C. The MIC for azoles, echinocandins, TRB, and GRF were defined as the lowest concentration of drug that inhibited >90% of the growth as compared to the growth in the drug free control. The MIC for amphotericin B was defined as the lowest concentration at which 100% of growth was inhibited. AMB – amphotericin B, AND – anidulafungin, CAS – caspofungin, MCF – micafungin, PSC – posaconazole, VRC – voriconazole, FLC – fluconazole, ITC – itraconazole, ISA – isavuconazole, KTC – ketoconazole, GRF – griseofulvin, TRB – terbinafine. Known epidemiological cut-off values (ECV) for drugs against *T. indotineae* are indicated. The MIC for 8778* could not be determined as this culture was not saved in the Mycology Culture Collection.

All T. indotineae *isolates were resistant to VRC, 80% were resistant to TRB and ~50% were resistant to other azoles. SQLE F397L correlated with high TRB resistance, while L393S correlated with moderate TRB resistance.*

SQLE Modeling and TRB Docking

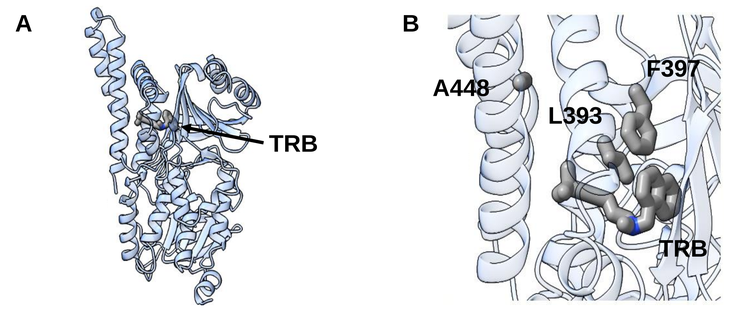

Figure 5: Impact of SQLE mutations on TRB binding
(A) The Alphafold model of squalene epoxidase (SQLE) from *T. mentagrophytes* (A0A289ZCP0) was used to model the TRB binding site in *T. indotineae* SQLE using template-based homology modeling with Swiss-Modeler. (B) Commonly mutated SQLE residues A448, L393, and F397 are shown as opaque sticks. Since A448 lies outside of the TRB binding pocket, mutation at this residue does not impact TRB binding or lead to resistance. In contrast, mutations at L393 and F397 likely disrupt TRB binding resulting in resistance.

Isolates with the SQLE mutation L393F had moderate and F397L had high resistance to TRB, respectively. These mutations likely disrupt the hydrophobic binding pocket of the aromatic naphthalene group of TRB leading to resistance.

Conclusions

- *Trichophyton indotineae* has emerged in the USA and is rapidly spreading.
- We report 11 cases of *T. indotineae* from NYC. The patients were aged three to 64 years, with a median age of 39; there were five females and six males.
- Clinical laboratories can utilize a urease test for provisional ID of *T. indotineae*; however, ITS sequencing is required for final species confirmation.
- *In-vitro* AFST revealed that most *T. indotineae* isolates were resistant to TRB and VRC, and ~50% were resistant to other azoles. They were susceptible to GRF, echinocandins, and AMB.
- All patients were successfully treated with GRF, KTC and a combination of azoles.
- The WGS analysis revealed that the NYC *T. indotineae* isolates are distinct from *T. indotineae* isolates from India.
- SNP analysis suggests *T. indotineae* transmission through close contacts as well as independent introductions.
- Modeling of TRB binding to SQLE provides mechanistic insight as to how the mutations L393F and F397L lead to TRB resistance.
- A strong collaborative effort is needed between clinicians and laboratories, both clinical and public health, to tackle the spread of drug-resistant *T. indotineae.*

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