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ORIGINAL ARTICLE

Evaluation of Currently Available Laboratory Methods to Detect Terbinafine Resistant Dermatophytes Including a Gradient Strip for Terbinafine, EUCAST Microdilution E.Def 11.0, a Commercial Real-Time PCR Assay, Squalene Epoxidase Sequencing and Whole Genome Sequencing

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ABSTRACT

Background: Terbinafine resistance in dermatophytes is an increasing problem worldwide. Several outbreaks of terbinafine-resistant dermatophytosis are currently occurring in India and surrounding countries, and these recent years, European countries have also been affected by this issue. Currently, antifungal susceptibility testing of dermatophytes is not routinely performed in clinical laboratories.

Objectives: Given the current situation and associated public health concerns, there is an urgent need for accurate and rapid detection of terbinafine resistance in laboratories. Therefore, we evaluated different methods currently available for the detection of terbinafine resistance in dermatophytes.

Methods: Twenty-eight strains previously identified as *T. indotineae/mentagrophytes/interdigitale* were concurrently characterised using terbinafine gradient strips (HiMedia), EUCAST E.Def 11.0 microdilution, the DermaGenius resistance PCR assay (PathoNostics), and SQLE sequencing. These four methods were compared to terbinafine resistance characterisation obtained by whole genome sequencing (WGS).

Results: All four evaluated methods were able to detect terbinafine resistant strains either by showing high MICs (> 0.125 μg/mL) or by detecting SQLE substitutions.

Conclusions: The gradient strips, despite questionable essential agreement with EUCAST E.Def 11.0, can be an easy, fast and cheap method to screen terbinafine resistance among dermatophytes in clinical laboratories. The DermaGenius resistance PCR assay enables rapid detection of the most common substitutions in SQLE associated with terbinafine resistance. However, its inability to precisely determine specific substitutions on SQLE or identify new ones may pose a problem in the future. These limitations can be addressed by using SQLE sequencing or whole genome sequencing (WGS).

1 | Introduction

Dermatophytosis is a common infection affecting the scalp, nails and skin, that is present worldwide. Terbinafine is the first line treatment for dermatophytosis, however resistance to this antifungal becomes an emerging public health concern these recent years. Indeed, an outbreak of terbinafine resistant strains currently occurs in India [1–3]. This outbreak is due to a new species that has been renamed *Trichophyton indotineae* (*T. indotineae*) and which is closely related to *Trichophyton mentagrophytes/interdigitale* but with distinct genomic features [4, 5]. In India, a switch in epidemiology has been observed from *Trichophyton rubrum* to *T. indotineae* as the main etiological agent of dermatophytosis [6, 7]. The current situation in India regarding this dermatophytic infection is nearing epidemic levels. *T. indotineae* is now also spread throughout the globe like in Europe, USA and South America where a lot of different cases of resistant *T. indotineae* have been described [8–14]. *T. indotineae* infection give rise to extended dermatophytoses that are difficult to treat and can become chronic and recurrent [3, 8, 15, 16]. One case of tinea universalis has been recently described with this species showing the potential severity of this infection [17]. The molecular basis of terbinafine resistance is linked to substitutions on squalene epoxidase (SQLE). A lot of different substitutions have been reported worldwide in particular, alterations in the specific amino acid hot spots Leu393, Phe397, Phe415 and His440 of SQLE, with the most frequent one being Phe397Leu and Leu393Phe [1, 10, 18–23]. Regarding the current situation, there is an urgent need to accurately and rapidly identify the dermatophytes at the species level and detect the resistance to terbinafine in clinical laboratories to ensure appropriate management of the patient and avoid an uncontrolled expansion of resistant dermatophytosis. Currently, susceptibility testing of dermatophytes is not routinely performed in clinical laboratories leading to underestimation of resistance rates. The EUCAST E. Def.11.0 microdilution method is fastidious and can only be performed by trained laboratories. That is why, as a National Reference Center for mycoses in Belgium (NRCB), we evaluated in parallel four methods currently available to detect terbinafine resistance on dermatophytes, two of them being commercial devices easy to use by routine laboratories. We compared the minimal inhibitory concentrations (MICs) determined by commercial gradient strips for terbinafine from HiMedia with the EUCAST 11.0 microdilution method for dermatophytes [24, 25]. Then we evaluated a commercial PCR kit DermaGenius resistance from PathoNostics to detect mutations on SQLE. This method has been compared with SQLE sequencing and WGS.

2 | Material and Methods

2.1 | Samples

Twenty-eight strains were selected for this study. Twenty-three of them were from the NRCB collection of the CHU of Liège (renamed BE1-23), while five of them were from the Belgian BCCM/IHEM collection. All strains were molecularly characterised as *T. indotineae* ($n=8$), *T. mentagrophytes* ($n=4$) and *T. interdigitale* ($n=16$) by whole genome sequencing using reference strains. The panel of strains was composed by 21 terbinafine-susceptible isolates and 7 terbinafine-resistant isolates. The strains were subcultured from frozen samples on Sabouraud dextrose agar with gentamicin and chloramphenicol (Biomérieux France) for 5–7 days at 28°C.

2.2 | Gradient Strips

Gradient strips for terbinafine (Terbinafine Ezy MIC Strip, HiMedia, India) were used to determine the minimal inhibitory concentrations (MICs) of the strains. Terbinafine strips presented a MIC range of 0.002–32 μg/mL. Inoculation of medium plates was done with a preparation of a conidial suspension from the culture that was filtered through a sterile filter (11 μm, Merck, Germany). One condition without hyphae filtration was also tested. Then a turbidity of 0.5 McFarland was measured with a spectrophotometer (bioMérieux, France). Conidia were inoculated on RPMI agar medium (Tritium Microbiologie, Netherland). MICs were read after 7 days of incubation at 28°C considering complete inhibition endpoint. For the interpretation, V3.0 EUCAST Ecoff tentative for *T. indotineae* has been used (= 0.125 μg/mL).

2.3 | EUCAST 11.0 Microdilution Method

MICs were determined in parallel by EUCAST E.Def 11.0 microdilution method following previously described instructions [25]. MICs were determined in duplicates for this method. Briefly, inoculum suspensions were filtered through a sterile filter with a pore diameter of 11 μm (Merck, Germany) and diluted 1/10 on sterile distilled water to obtain a final inoculum of $2–5\times10^5$ conidia/mL. Suspension was supplemented with chloramphenicol 2 μL/mL of a stock solution of 50 mg/mL (Merck, Germany) and cycloheximide, 6 μL/mL of a stock solution of 100 mg/mL (Merck, Germany). The final concentration range for terbinafine (after inoculation) was 0.008–8 mg/L. The reading of the MIC50 value was done according to the described recommendations using an automated reading at 490 nm with a Multiscan FC spectrophotometer (ThermoFisher Scientific, USA). For the interpretation, V3.0 EUCAST Ecoff tentative for *T. indotineae* has been used (= 0.125 μg/mL). As concentration range was different for GS (0.002–32 μg/mL) and for EUCAST E. Def 11.0 (0.008–8 μg/mL), interpretation of MIC results and delta calculation was strictly based on MICs values.

2.4 | Squalene Epoxidase Sequencing

Total DNA was extracted from fresh fungal cultures growing in liquid Sabouraud medium (Merck, Germany) using the Maxwell 16 Cell SEV kit (Promega, USA). The PCR amplifying the SQLE gene was performed using the procedure described previously by Yamada et al. [18]. Purification of PCR products was then performed using the ExoSAP-IT PCR Product Cleanup Reagent (ThermoFisher scientific, USA). Bidirectional sequence data were generated after purification using the BigDye terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific, USA). Sequenced products were finally purified using the kit clean Seq Agencourt (Beckman Coulter Life Science, France). The sequencing protocol is fully automated on a StarM (Hamilton, USA) robot. DNA sequencing products were separated by

capillary electrophoresis on 3500XL (24 capillaries) instrument with POP-7 polymer and 3500 Data Collection Software V3.3 (ThermoFisher Scientific, USA). Sequences were edited using Geneious prime (Dotmatics Geneious, New Zeeland). Forward and reverse sequences were then aligned and the consensus sequence was translated on amino acid sequence using the Expasy portal (SIB Bioinformatics Resource Portal, Switzerland). The obtained sequence was then compared to the reference SQLE amino-acid sequence of *T. mentagrophytes* GenBank: BAL48859.1.

2.5 | DermaGenius Resistance Real-Time PCR Assay

DNA extraction was performed from a colony piece of 7 days culture that was scrapped from the Sabouraud medium and immersed into 475 μL of ATL buffer (Qiagen, Germany). A total of 25 μL of proteinase K was then putted in each tube. An overnight incubation was done at 56°C under agitation (900 rpm). DNA extraction was performed using the QIAamp DNA minikit (Qiagen, Germany) following the manufacturer's instructions. The DermaGenius resistance real-time PCR assay (PathoNostics, The Netherlands) was then done according to the manufacturer's instructions. Five microliters of extracted DNA was added to the PCR mix, and a LightCycler 480 II (Roche, Switzerland) was used for amplification and melting curve analysis. Data analysis was done using the 2nd-derivative and Tm-calling function of the LC480 software (version 1.5.1.62 SP2). The species level identification given by the kit was not considered in this study. Melting curve analysis is needed to differentiate between wild-type (WT) and mutant strains. The positive control provided by the manufacturer was used as a standard for the analysis of the melting peaks and was tested on each run with the isolates to determine whether the melting peak show a profile corresponding to a WT strain or to a mutant profile wearing mutations on SQLE. *Trichophyton* spp. with Leu393Phe, Phe397Leu, Leu393Ser, Phe397Ile, Phe397Val amino acid substitutions can be detected as "mutant" by the DermaGenius resistance real-time PCR kit assay. Specific Tm values for wild-type (66°C–69°C) or resistant isolates (55°C–66°C) were obtained in wavelength 465-510 (FAM).

2.6 | Whole Genome Sequencing

DNA extraction was performed using the DNeasy ultraclean microbial extraction kit (Qiagen, Germany). Fungal material was collected and pre-treated using beat beating, heat shock, chemical and enzymatic lysis. Briefly, the fungal culture from liquid Sabouraud was putted into a power bead tube (Qiagen, Germany) filled with power bead solution and SL buffer provided in the DNeasy ultraclean microbial extraction kit. Heat shock was then performed putting the tube 1 min on liquid nitrogen followed by 1 min on water bath at 56°C. A beat beating of the tube was then done for 10 min on a vortex mixer (VWR, USA). These three steps were repeated trice. The DNA was then extracted using the DNeasy ultraclean microbial extraction kit (Qiagen, Germany) following the manufacturer's instructions. DNA yields were quantified using a Nanodrop 1000 spectrophotometer (ThermoFisher Scientific, USA). The extracted DNA were then sequenced by the whole genome shotgun method using the Illumina sequencing (Illumina Inc., USA). A paired-end library was prepared from genomic DNA using the DNA PCR-Free Library Prep tagmentation (Illumina Inc., USA), following the manufacturer instructions. A minimum of 2 μg genomic DNA per sample was used for the library construction. The quantified Illumina paired-end library was used for sequencing with the Illumina NovaSeq6000 platform (Illumina Inc., USA). Quality assessment of raw reads was done using FastQC [26] and MultiQC [27]. Thereafter, raw reads files were automatically pre-processed using Fastp [28]. The cleaned reads resulting from this step were *de novo* assembled using SPAdes [29, 30] with careful option followed by read error-correction running built-in BayesHammer module [31]. Genome assembly completeness was assessed using BUSCO [32] with onygenales_odb10 lineage dataset and MetaEuk gene predictor [33]. Following the recommendations of Jung et al., only genomes with a BUSCO completion greater than 90% were considered for further data analysis [34]. SQLE sequence was extracted from WGS data using tBLASTn with reference BAL48859.1. The 28 assembled genomes were submitted to GenBank and are openly available (Bioproject ID: PRJNA1152509, accession numbers listed in Table A1). Address is as follows: GenBank: www.ncbi.nlm.nih.gov/genbank.

2.7 | Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The project has been approved under N°2022-46.

3 | Results

All 28 strains were submitted to gradient strips and EUCAST E.Def 11.0 microdilution method. Considering the gradient strips with filtration (GSTF), minimal inhibitory concentrations (MICs) of resistant strains were comprised between 0.25 and 8 μg/mL with geometric mean (GM) = 1.1. MICs for susceptible strains were ranged between 0.002–0.008 μg/mL with GM = 0.0023. A condition without filtration of hyphae was also tested (GSTWF). Under this condition, minimal inhibitory concentrations (MICs) of resistant strains were comprised between 0.38 and 2 μg/mL with geometric mean (GM) = 1.07. MICs for susceptible strains were between 0.002 and 0.008 μg/mL with GM = 0.002. Essential agreement between the conditions with and without filtration for gradient strips was of 100% (±2 log2) and of 97.08% (±1 log2).

Considering EUCAST E.Def 11.0., MICs for resistant strains were between 0.5 and 8 μg/mL with GM = 2.97 while MICs for susceptible strains were 0.008–0.03 μg/mL with a GM = 0.014. Essential agreement (±2 log2) between gradient strips (condition GSTF was considered for comparison) and EUCAST E.Def 11.0 was 50% (14/28). The number of isolates with differences of 3, 4, 5 log2 dilutions were 11/28 (39.37%), 2/28 (7.14%) and 1/28 (3.57%), respectively. MICs values were always higher with EUCAST E.Def.11.0 compared to GSTF. 100% of categorical agreement was observed between GSTF and EUCAST E.Def

11.0. The results of minimal inhibitory concentrations determination by gradient strips (GSTF and GSTWF) and EUCAST E.Def 11.0 microdilution method are described in Table 1.

The 28 strains were also characterised with DermaGenius resistance PCR assay. Our results show that 7/7 strains previously defined to be resistant considering MICs values obtained by EUCAST E.Def 11.0 and SQLE WGS sequencing, were detected as "mutant" while 21/21 of strains that were defined to be susceptible by the above cited methods were characterised as "wild-type" (WT) by this PCR assay. This method does not allow to define which substitution is present in SQLE. SQLE sequencing highlighted the substitution F397L in 6/7 R strains and L393F in 1/7 R strains. Moreover, the mutation K276N was detected in two susceptible strains by this method. These last results were concordant with data obtained by WGS considering the extracted SQLE sequence. In this comparison, we could observe that DermaGenius resistance PCR assay did not detect K276N substitution on SQLE. This substitution was not included on the panel of substitutions detected by the PCR kit. The results of DermaGenius Resistance real-time PCR assay, SQLE sequencing and WGS are summarised in Table 2. All strains with MICs $\geq 0.25\,\mu g/mL$ determined by phenotypic methods were wearing SQLE substitutions in positions 393 or 397 and were well detected by the three tested molecular methods. Strains with MICs $< 0.25\,\mu g/mL$ showed a wild type profile for SQLE or were wearing K276N substitutions ($n = 2$). 100% of categorical agreement was observed among GSTF, EUCAST E.Def 11.0, DermaGenius resistance PCR assay, SQLE sequencing and WGS.

4 | Discussion

Currently, only one reference method has been established for testing the drug susceptibility of dermatophytes: the EUCAST E.Def 11.0 method. This method is time-consuming and difficult to implement in non-trained routine laboratories due to the lack of commercial devices available. This study represents one of the first evaluation of combined phenotypic and DNA based methods to detect terbinafine resistance in dermatophytes. We have demonstrated that all evaluated methods were able to detect terbinafine resistant strains either by showing high MICs ($> 0.125\,\mu g/mL$) or by detecting SQLE substitutions. Even though the gradient strips show promising agreement for the screening of dermatophyte susceptibilities, the results of MIC determination should be taken carefully. Indeed, the gradient strips showed a good categorical agreement with EUCAST E.Def 11.0 method, all strains that were predicted to be resistant showed MICs values higher than $0.125\,\mu g/mL$ which is the current defined Ecoff following EUCAST guidelines. Concerning the essential agreement (±2 log2), it has been evaluated to be 50% in our study. This is in accordance to previous results obtained by a French team that also evaluated the commercial gradients strips from HiMedia and observed an essential agreement of 43.0% with a first batch of the gradient strips and 97.1% of essential agreement with a second batch compared to EUCAST E.Def 11.0. Inter batch variability has not been evaluated in our study as a unique batch was used along the evaluation. We observed lower MICs and GM with the gradient strips compared to EUCAST E. Def 11.0 although the incubation time was the same for both methods. The same observation was made by the same French team as lower MICs were also obtained with gradient test compared to EUCAST E.Def 11.0 [35]. In our study, we also tested a condition without hyphal filtration for the inoculum preparation in order to simplify the procedure and we found no significant differences in the MICs obtained as the essential agreement (±2 log2) between both conditions (with and without filtration) was of 100%. Therefore, this additional step can be bypassed, further facilitating the implementation of this method in routine laboratories. Based on these results, we can confidently say that gradient strips are an inexpensive and easy-to-use method for rapidly screening terbinafine resistance among dermatophytes. This method is particularly suitable for low-income countries where resistance rates are higher among dermatophytes. If a resistance is suspected, MIC determination must be further determined by EUCAST E.Def 11.0 microdilution method that remain the sole standardised reference method for MIC determination in dermatophytes.

Regarding DermaGenius Resistance real-time PCR assay, it can detect the most frequent substitutions on SQLE associated with terbinafine resistance currently characterised. This method showed a perfect agreement with high terbinafine MICs ($> 0.125\,\mu g/mL$) observed by EUCAST E. DEF.11.0 and terbinafine gradient strips suggesting that it is an efficient tool to detect terbinafine resistant dermatophytes. Moreover, this PCR assay showed a perfect agreement with SQLE sequencing and WGS to detect SQLE substitutions in positions 393 and 397. This is a rapid method to detect terbinafine resistance as the kit can be used directly on nails, skin and hair samples what can strongly reduce the turnaround time (TAT) to get a result for resistance and significantly improve the patient management and well-being. In contrast, conventional methods can take over a month to determine the minimum inhibitory concentration because the culture process can be slow (1–3 weeks), followed by an additional time for the antifungal test results, which takes around 7 days. One drawback of this PCR kit is that it cannot precisely determine the substitution in SQLE, as it is only identifying a "mutant" or "wildtype" profile. However, this level of determination is adequate for routine laboratories and clinical purposes. Additionally, the method is based on predefined mutations, so new emerging mutations that could become predominant may be missed. The substitutions covered by the kit are the most frequently encountered according to the current epidemiology and previously reported substitutions [1, 18, 21, 22, 36–38]. However, substitutions at other positions have already been linked with terbinafine resistance as in positions 335, 396, 415, 408 and 443 [6, 18, 39] In our study, two strains were wearing the substitution K276N on SQLE. The DermaGenius Resistance real-time PCR assay did not detect this substitution as it was not present on the predefined panel. This substitution was however detected by SQLE sequencing and WGS. This is not currently a real problem of the kit as considering the current literature and the present results, this substitution has not been linked with resistance to terbinafine yet. Indeed, this mutation was already described among susceptible strains to terbinafine from China and France [23, 40]. New substitutions will probably occur in the future which can be a limitation for the use of the DermaGenius Resistance real-time PCR assay. Of course, the problem can be bypassed by a discussion with

TABLE 1 | Minimal inhibitory concentrations (MICs) determination by terbinafine gradient strips with and without filtration and by EUCAST E.Def 11.0.

ID	MIC GSTF (μg/mL)	Interpretation GSTF	MIC GSTWF (μg/mL)	Interpretation GSTWF	Delta GSTF/ GSTWF	MIC EUCAST E.Def 11.0 (μg/mL)	Interpretation EUCAST E.Def 11.0	Delta GSTF/ EUCAST E. Def 11.0
IHEM 2878	1	R	1	R	0	4	R	2log2
IHEM 28379	1	R	1	R	0	4	R	2log2
IHEM 28380	1	R	1.5	R	0.5log2	4	R	2log2
IHEM 28384	1	R	1	R	0	4	R	2log2
IHEM 28388	0.002	S	0.003	S	0.5log2	0.06	S	5log2
BE1	0.004	S	0.003	S	0.5log2	0.03	S	3log2
BE2	8	R	2	R	2log2	2	R	2log2
BE3	0.008	S	0.006	S	0.5log2	0.03	S	2log2
BE4	0.002	S	0.002	S	0	0.016	S	3log2
BE5	0.008	S	0.006	S	0.5log2	0.006	S	3log2
BE6	0.002	S	0.002	S	0	0.016	S	3log2
BE7	0.002	S	0.002	S	0	0.008	S	2log2
BE8	0.002	S	0.002	S	0	0.008	S	2log2
BE9	0.002	S	0.002	S	0	0.008	S	2log2
BE10	0.002	S	0.002	S	0	0.008	S	2log2
BE11	0.002	S	0.002	S	0	0.008	S	2log2
BE12	0.002	S	0.002	S	0	0.008	S	2log2
BE13	0.002	S	0.004	S	1log2	0.03	S	4log2
BE14	0.002	S	0.002	S	0	0.016	S	3log2
BE15	0.002	S	0.002	S	0	0.016	S	3log2
BE16	0.002	S	0.002	S	0	0.016	S	3log2
BE17	1	R	1.5	R	0.5log2	8	R	3log2
BE18	0.002	S	0.002	S	0	0.008	S	2log2
BE19	0.002	S	0.002	S	0	0.016	S	3log2
BE20	0.002	S	0.002	S	0	0.016	S	3log2
BE21	0.002	S	0.002	S	0	0.016	S	3log2
BE22	0.002	s	0.002	s	0	0.03	S	4log2
BE23	0.25	R	0.38	R	0.5log2	0.5	R	1log2

Abbreviations: GSTF = gradient strips terbinafine with filtration, GSTWF = Gradient strips terbinafine without filtration, MIC = minimal inhibitory concentration, R = resistant, S = susceptible.

TABLE 2 | Comparison of results obtained by Squalene epoxidase (SQLE) sequencing, DermaGenius resistance real-time PCR assay and whole genome sequencing (WGS).

ID	SQLE sequencing	DermaGenius PCR 465-510 FAM	WGS
IHEM 2878	F397L	Mutant	F397L
IHEM 28379	F397L	Mutant	F397L
IHEM 28380	F397L	Mutant	F397L
IHEM 28384	F397L	Mutant	F397L
IHEM 28388	WT	WT	WT
BE1	WT	WT	WT
BE2	F397L	Mutant	F397L
BE3	WT	WT	WT
BE4	WT	WT	WT
BE5	K276N	WT	K276N
BE6	WT	WT	WT
BE7	WT	WT	WT
BE8	WT	WT	WT
BE9	WT	WT	WT
BE10	WT	WT	WT
BE11	WT	WT	WT
BE12	WT	WT	WT
BE13	K276N	WT	K276N
BE14	WT	WT	WT
BE15	WT	WT	WT
BE16	WT	WT	WT
BE17	L393F	Mutant	L393F
BE18	WT	WT	WT
BE19	WT	WT	WT
BE20	WT	WT	WT
BE21	WT	WT	WT
BE22	WT	WT	WT
BE23	F397L	Mutant	F397L

Abbreviations: F = amino acid phenylalanine, K = amino acid lysine, L = amino acid leucine, N = amino acid asparagine, SQLE = squalene epoxidase, WGS = whole genome sequencing, WT = wild-type.

the company to add new detected targets. Another limitation of the DermaGenius Resistance real-time PCR assay is that it requires a real-time thermocycler, which is expensive and not accessible to all laboratories. Additionally, the cost of the kit is not suitable for low-income countries, which are the primary regions facing epidemics of resistant dermatophytosis, such as India and its neighbouring countries. Our results are consistent with those obtained on a larger scale by Singh et al. in 2021. In this last study, the DermaGenius Resistance real-time PCR assay has been shown to effectively identify and distinguish wild-type from SQLE mutant isolates harbouring the Phe397Leu and Leu393Phe amino acid substitutions among 97 *Trichophyton* strains. All 40 *T. indotineae* isolates exhibiting the Phe397Leu or Leu393Phe amino acid substitutions previously identified by SQLE sequencing were efficiently characterised as SQLE "mutants" by the DermaGenius Resistance real-time PCR assay [41]. We can then consider that this PCR assay can be useful to rapidly detect terbinafine resistance under the sole condition that the laboratory has the necessary equipment and resources.

5 | Conclusion

In this study, we have described several methods to detect terbinafine resistance among dermatophytes. Gradient strips

show excellent categorical agreement with the EUCAST E.Def 11.0 method and are cheaper and easier to use than the microdilution method in laboratories. However, MICs obtained with this method should be interpreted with caution and confirmed by the EUCAST E.Def 11.0 method if resistance is suspected. The DermaGenius Resistance real-time PCR assay can be used for a rapid response when terbinafine resistance is suspected. For precise determination of substitutions on SQLE, SQLE sequencing or whole-genome sequencing (WGS) can be envisaged.

Author Contributions

Rosalie Sacheli: conceptualization, writing – original draft, methodology, validation, writing – review and editing, software, formal analysis, project administration, data curation, supervision, investigation. **Sabrina Egrek:** investigation, writing – review and editing, supervision, formal analysis. **Khalid El Moussaoui:** investigation, writing – review and editing, software, formal analysis, data curation. **Rajae Darfouf:** investigation, writing – review and editing. **Akole Bahun Adjetey:** investigation, writing – review and editing, formal analysis. **Marie-Pierre Hayette:** conceptualization, writing – review and editing, validation, project administration, data curation, supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in NCBI at https://submit.ncbi.nlm.nih.gov/, reference number PRJNA1152509.

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Appendix A

TABLE A1 | Accession numbers of the strains sequenced by WGS in this study (assembled genomes).

Genbank Accession numbers	Study ID
SAMN43365157	BE1
SAMN43365158	BE2
SAMN43365159	NE3
SAMN43365160	BE4
SAMN43365161	BE5
SAMN43365162	BE6
SAMN43365163	BE7
SAMN43365164	BE8
SAMN43365165	BE9
SAMN43365166	BE10
SAMN43365167	BE11
SAMN43365168	BE12
SAMN43365169	BE13
SAMN43365170	BE14
SAMN43365171	BE15
SAMN43365172	BE16
SAMN43365173	BE17
SAMN43365174	BE18
SAMN43365175	BE19
SAMN43365176	BE20
SAMN43365177	BE21
SAMN43365178	BE22
SAMN43365179	BE23
SAMN43365180	IHEM 28378
SAMN43365181	IHEM 28379
SAMN43365182	IHEM 28380
SAMN43365183	IHEM 28384
SAMN43365184	IHEM 28388