Easy and rapid screening method to detect the resistance to terbinafine in dermatophytes

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Introduction

- Dermatophytes=Filamentous fungi
- Dermatophytosis= ***ring worm***
- Infect the skin, hair and nails
- Most common causes of skin disease (20-25% population).
- **Anthropophilic**, **zoophilic** or geophilic
- Clinical presentation depends of immunological response of the host and the **dermatophyte species**

20-25%
→ Superficial mycosis

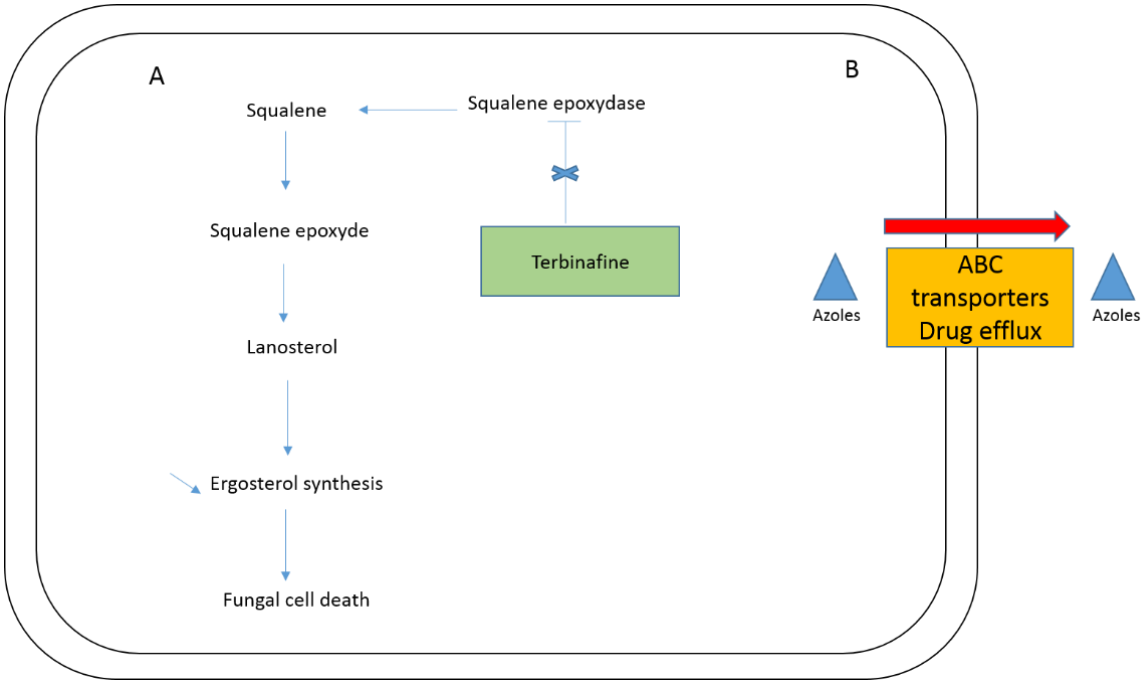
Mostly dermatophytes

Terbinafine resistance

- Terbinafine resistant dermatophytoses caused by *Trichophyton rubrum (T. rubrum)* or *Trichophyton indotineae (T. indotineae)* ➔global public health issue
- Phenomenon spreading and particularly important in endemic areas such as **India**
- **Extended dermatophytoses difficult to treat**

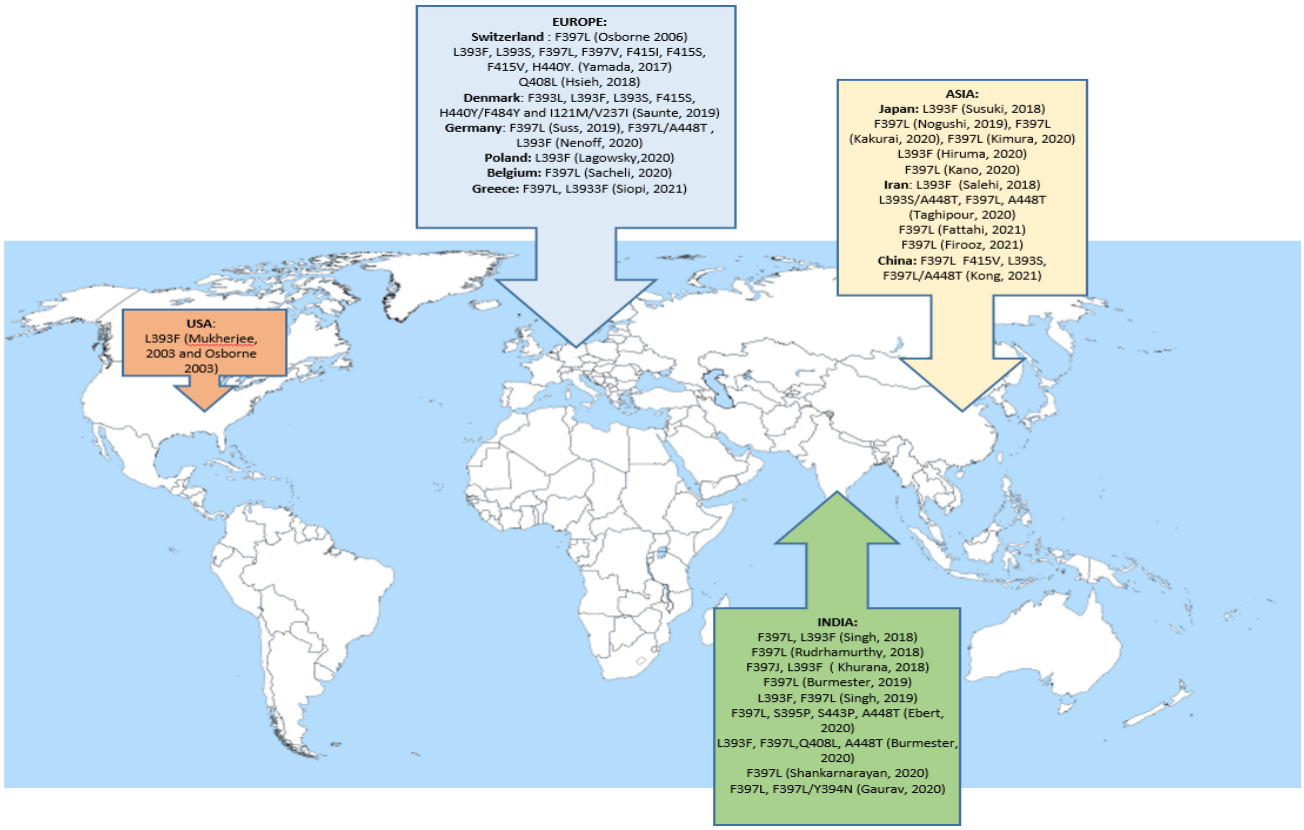
Verma et al, *Indian J Dermatol Venereol Leprol 2021*

Terbinafine resistance



Sacheli et al, 2021, *J. of Fungi*

SQLE mutations

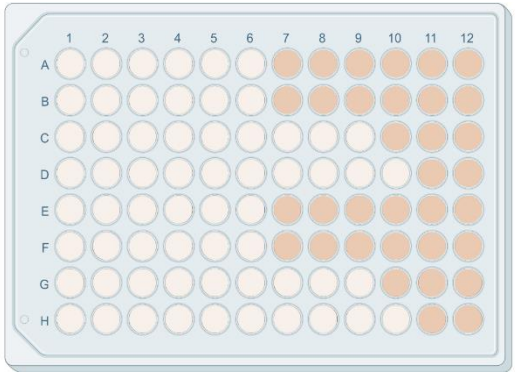


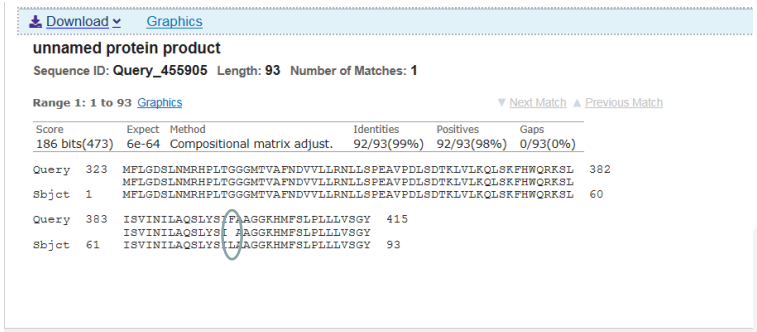
Sacheli et al, 2021, *J. of Fungi*

5 strains with F397L and 1 strain with L393F substitution at **CHU of Liège**

Aim of the study

- Design a **rapid method** for the **screening** of resistances to terbinafine in dermatophytes
- Preceeding confirmations tests
  - Eucast E.Def.11.0
  - SQLE PCR/sequencing




Download Graphics

unnamed protein product

Sequence ID: Query_455905 Length: 93 Number of Matches: 1

Range 1: 1 to 93 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
186 bits(473)	6e-64	Compositional matrix adjust.	92/93(99%)	92/93(98%)	0/93(0%)

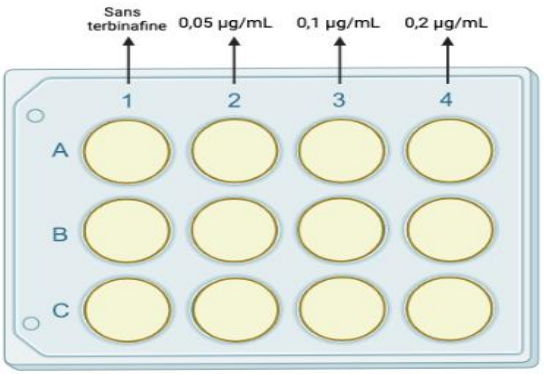
```
Query  323  MFLGDSLNMRHPLTGGGMTVAFNDVVLLRNLLSPEAVPDLSDTKLVLKQLSKFHWQRKSL  382
            MFLGDSLNMRHPLTGGGMTVAFNDVVLLRNLLSPEAVPDLSDTKLVLKQLSKFHWQRKSL
Sbjct  1    MFLGDSLNMRHPLTGGGMTVAFNDVVLLRNLLSPEAVPDLSDTKLVLKQLSKFHWQRKSL  60

Query  383  ISVINILAQSLYSFFAAGGKHMFSLPLLLVSGY  415
            ISVINILAQSLYSF AAGGKHMFSLPLLLVSGY
Sbjct  61   ISVINILAQSLYSFLAAGGKHMFSLPLLLVSGY  93
```

Procedure

Agar plates preparation

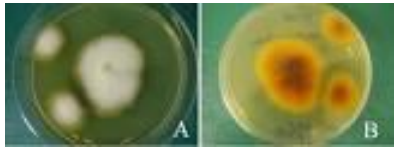
- **Medium** :Sabouraud dextrose medium +1.5% agar +0/0.05/0.1/0.2 µg/ml TERB
- Make a dermatophyte suspension of 0.5 McFarland
- 25µl of the suspension in each well
- Incubation for 4 days, at 30°C



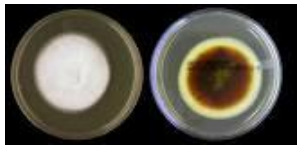
➔R suspected if culture growth +++ at least in well 0.1µg/ml

Strains selected

- 35 *T. rubrum*/*T. indotineae* strains tested (Arendrup et al, JAC 2020)
  - 15 resistant to TERB
  - 20 susceptible to TERB

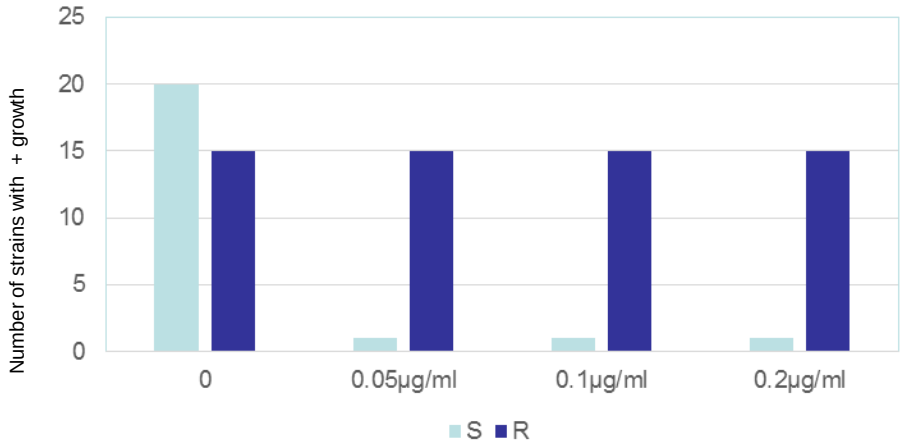


T. indotineae (Kano et al, 2020)



T. rubrum *(Mokobi, 2021)*

Results

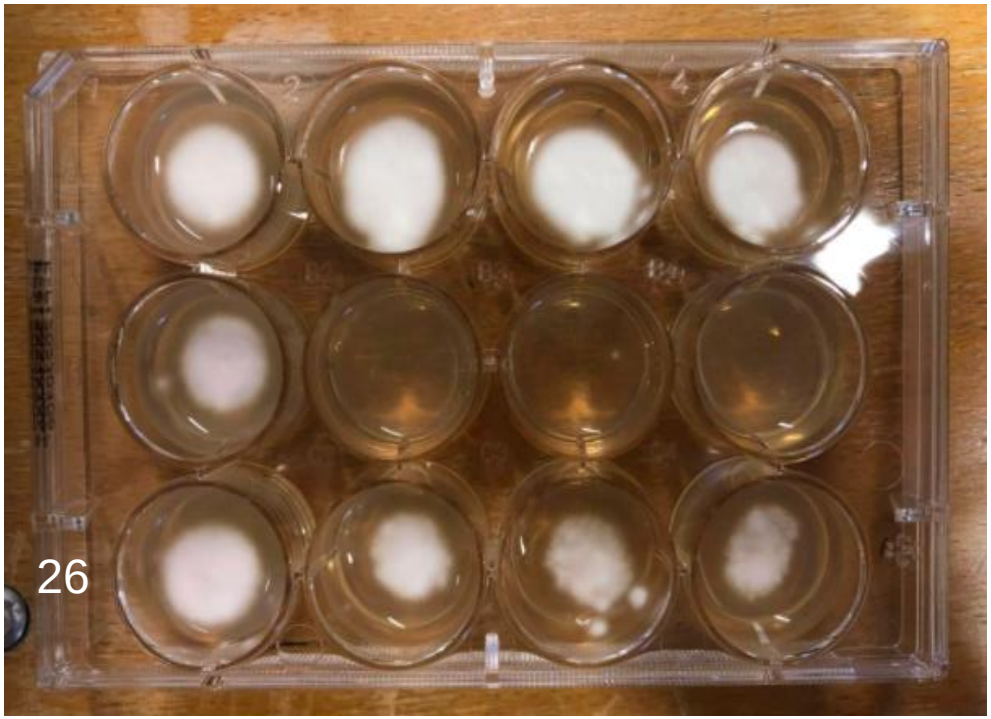


Sensitivity : 100 %
Specificity : 95 %

⇒ False positive : strain n° 26 (MIC : 0,06 µg/mL, SQLE WT by WGS)

⇒ Increasing N° of false positive with longer incubation

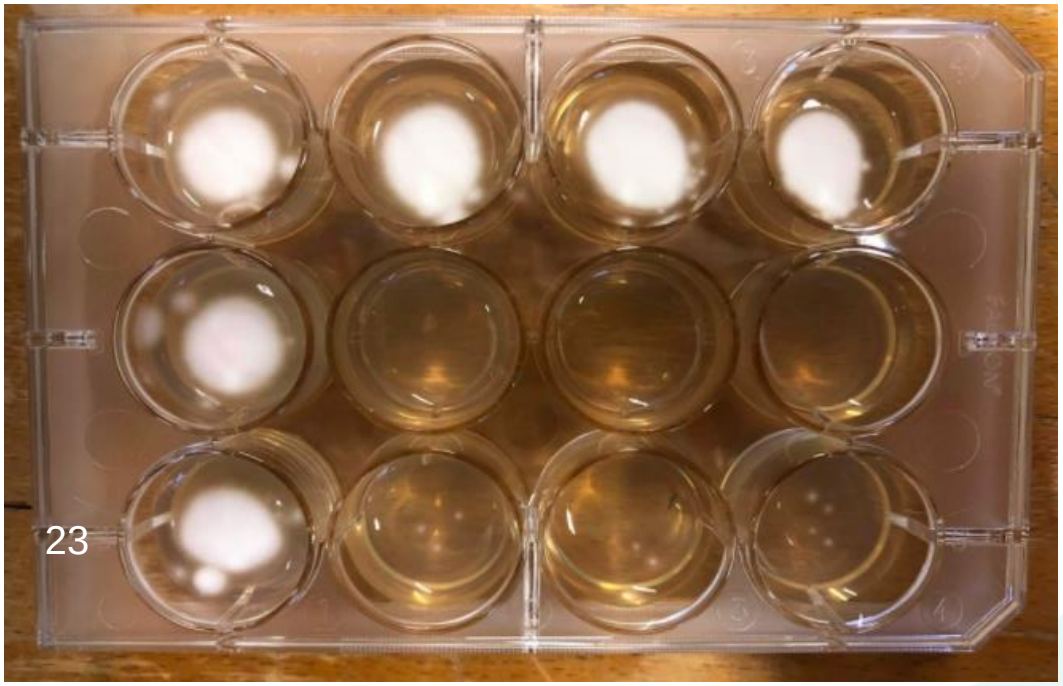
Lack of specificity



⇒False positive : strain n° 26 (MIC : 0,06 μg/mL, SQLE WT by WGS)

Residual growth not considered +

- Small colonies but growth in surface < 50% of the positive CTRL ➔ NEGATIVE



Discussion screening method

Positive points

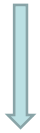
- Easy to use
- Rapid results in 4 days
- Not expensive
- Sensitivity 100 %

Negative points

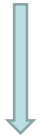
- Less specificity
- MICs are not precisely determined

Proposed algorithm

Screening method on all
T. indotineae/rubrum



If positive growth on well
0.1µg/ml TERB ➔ *Microdilution*
Eucast E.Def.11.0



If MIC >0.1 µg/ml ➔ *SQLE*
PCR/sequencing

➔Overview of the occurrence of TERB R in your laboratory
AND **switch** to alternative treatment if necessary

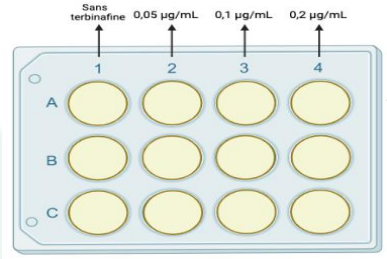
Alternative therapies

Dermatophyte species	Clinical presentation	Alternative therapy	Reference
T.mentagrophytes	Disseminated tinea corporis	Itraconazole 200mg/day for 2-3 weeks +topical eberconazole	Hsieh et al, 2019 (17)
T.mentagrophytes	Tinea, corporis, tinea cruris	Itraconazole +ciclopirox	Burmester et al, 2019 (14)
T. mentagrophytes VIII	Extended tinea corporis	Topical miconazole and later ciclopirox	Suss et al, 2019 (25)
T. mentatgrophytes/interdigitale	Extensive tinea corporis	Itraconazole 100mg/day and topical luliconazole	Kimura et al, 2020 (35)
T. interdigitale	Extensive tinea corporis	Itraconazole 100mg/day	Kakurai et al, 2020 (34)
T. mentagrophytes VIII	29 cases of tinea corporis	Recommended Itraconazole 200mg/day for 4-8 weeks	Nenoff et al, 2020 (26)
T. mentagrophytes VIII	Extended tinea corporis from the groin	Voriconazole 200mg/day	Fattahi et al, 2021 (38)
T. mentagrophytes VIII	Tinea pedis	2 successive itraconazole pulse therapy	Fattahi et al, 2021 (38)
T. mentagrophytes VIII	Extended tinea corporis from the groin	Itraconazole 100mg/day for 4 weeks	Fattahi et al, 2021 (38)
T. mentagrophytes VIII	Extended tinea corporis from the groin	Voriconazole 200mg/day	Fattahi et al, 2021(38)

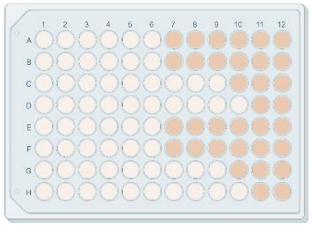
Sacheli et al, 2021, *J. of Fungi*

Terbinafine resistance national study 2022

- **Sciensano** will send an information email to all Belgian labs
- All Belgian laboratories can send to the NRC (CHU Liège), all strains of ***T.indotineae/mentagrophytes/interdigitale*** **isolated from skin** (Excl. foot skin) between 1st April 2022-April 2023
- Phenotypical and genotypical characterisation of R to TERB in all received strains



Screening method



Eucast E.Def.11.0

SQLE mutations

Don’t hesitate to participate!

Thank you for your attention

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