



Multilocus Phylogenetic Study Defining Resistance by Biofilms, Metalloprotease and SQLE of Dermatophytes Isolated from Various Altitudes of Uttarakhand

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ABSTRACT

Background: Treatment failure of multiple cases becomes a source of attention toward mycosis infections at Uttarakhand hills. Antifungals found almost ineffective against dermatophytes in several cases and their pathogenic genes (*i.e* SQLE), enhance drug failure due several kinds of genetic modifications. Due to a biogeography of this area prevalence of humidity is favorable climatic conditions for dermatophytes and consistent use of antifungals as well as OTC medicines became a cause of recalcitrance of pathogen.

Methods: Monsoon is a favorable climate of dermatophyte in which they grow vigorously, so during this seasons (July-Oct 2024) almost 550 samples (all 5 locations) of dead stratum corneum from margins of tinea rashes were collected, heat fixed with 10% KOH (potassium hydroxide) + DMSO (di-methyl sulfonic acid). Presence of filaments confirmed under trinocular and sent for culture at PDA (potato dextrose agar). Fungal species identified under trinocular and confirmation was done through ITS1 and 4 regions (Internal transcribe spacer). Disc diffusion along with 90 well microdilution CLSI M61, ed.2 (Clinical and standard laboratory institute) was performed to analyse AFST (Antifungal susceptibility test). Finally SQLE and MEP1-5 was multiplied, sequenced and biofilm was also analyzed of higher MIC (minimum inhibitory conc) of dermatophytes.

Result: MIC of *Trichophyton*, *Epidermophyton* and *Microsporum* (5-25 µg/ml) for terbinafine, for Itraconazole (0.09-1.5 µg/ml) and for Fluconazole (0.03-0.5 µg/ml) found much more higher than previously reported data. Point mutations previously reported were observed in SQLE (squalene epoxidase). MEP-3 (metalloprotease) was prominently found in all the genera followed by MEP-5 and MEP-4 was in least amount in most of the genus isolated from this area. Biofilms deplete by increasing conc (0.2-1.0) of SS14 in microtiter wells; found more effective with SS14, R-70, when studied under trinocular and spectroscopically.

Key words: ECM (Extracellular matrix), Metalloprotease (MEP), Minimum inhibitory conc (MIC), RLSS14 (Rhamnolipid produced by a strain *P. aeruginosa*), Squalene epoxidase (SQLE).

INTRODUCTION

Dermatophytes hyphal fragments spread unknowingly by being deposited on the stratum corneum, in contact with other infected humans and animals (Shivraju *et al.*, 2025) become a cause of communicable skin infection, particularly at higher altitudes. Due to the availability of ample of moisture (humidity) dermatomycosis recalcitrance after first infection becomes common over the last few years (Nanavare *et al.*, 2024). According to Centre for drug control and Prevention (CDC) report 2024 emergence of antifungal-resistant *T. indotineae* as a cause of genital lesions and possible acquisition through sexual contact. CDC was also spreading awareness through SAW (STI awareness week) during Sep 16-24, 2024 with a theme of antifungal resistance, which 'in itself' striking how mycosis spreading throughout the world (Graph 1). Dermatophytes slowly screening worldwide, branching in 7 clades with genera, *Trichophyton*, *Epidermophyton*, *Microsporum*, *Naninzzia*, *Paraphyton*, *Lophophyton*, *Arthroderma*. Last 15 years of dermatophytes susceptibility data indicating the continuous appraisal of MIC in subsequent years in several genera and for some specific antifungals.

Objective

To observe recalcitrance of dermatomycosis infections in this area, trials were carry out, through three different ways

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Primarily dermatophyte collection and predominance Secondly their resistance through MIC (Amin *et al.*, 2025) calculation (90 well micro titre). Third factor including biofilms, pathogenic genes (*i.e* SQLE) and MEP multiplication for any mutations and quantitative predominance to confirm the most influential factors in dermatophyte resistance (Sharma *et al.*, 2022).

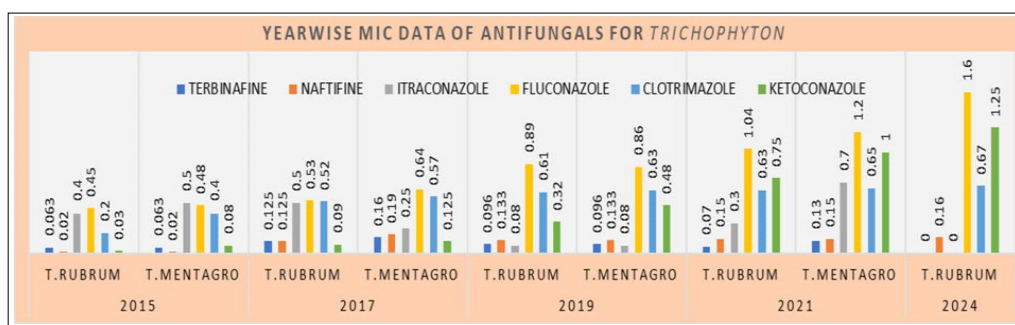
MATERIALS AND METHODS

Total 550 isolates during monsoon (July 2023-Oct 2024) were collected from all five locations of govt hospitals from

different altitudes. Dead stratum corneum collected by sterile blunt scalpel from well-defined margins of tinea, in sterilized bottles (Vanapalli *et al.*, 2022). These were primarily heat fixed in KOH and DMSO at a microscope slide, then observed under tissue culture microscope (TC1000) series at 10× and 22× power magnification. Samples observed positive cultured at PDA in aseptic conditions at 28°C. Subjects were informed and written consent has been taken, before collection of samples from a group of patients diagnosed with tinea and scabies only. Pregnant, lactating females and kids (up to 14 yr) were excluded from study to follow Helsinki guidelines for medical research. Ethic clearance was taken from IHEC from SIST Chennai. (Ref no 210/IRB-IBSEC/SIST 13 oct 2022).

Dermatophyte Identification and MIC Evaluation

Genera identification was performed after culturing the mycosis at 28°C in aseptic conditions at several culture medias *i.e.* PDA, SDA, RPMI 1640 *etc.* as mycosis impart several pigments at selective media which helpful in identifying dermatophytes macroscopically, followed by filaments from grown culture stained with cotton blue helpful in identification under microscope through microconidia, macroconidia, spore size and shapes *etc* (Moskaluk *et al.*, 2022) (Fig 1). Final confirmation of species was performed through molecular identification. Subsequently antifungal susceptibility range was also observed by standard Disc diffusion (Hong *et al.*, 2025) and CLSI M61 ed2, 90 well microdilution with two fold increasing conc. for



Graph 1: Representing susceptibility range rising year wise reported by various authors globally for all three classes of antifungals, here specifically two species were taken to draw graph because both have the highest rate of SQLE mutations.

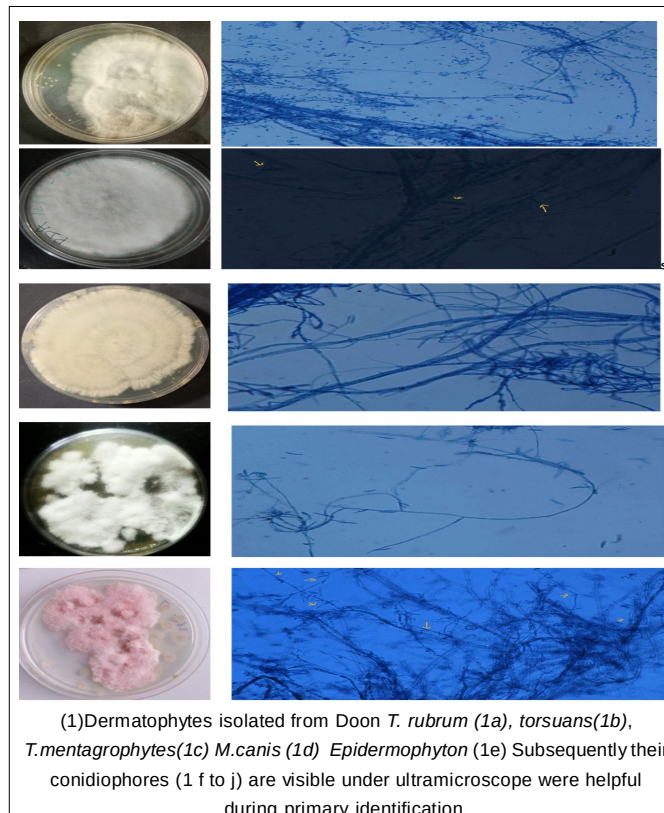


Fig 1: Patient with ringworm.

all the species (Arendrup *et al.*, 2021). Data results were taken five times and finally mean was estimated, to conclude the susceptibility range against all three groups of antifungals (azoles, allylamines and polyenes) (Pavlovic *et al.*, 2024) for current isolated pathogen from various altitudes (Table 1).

Biofilms Study of Isolated dermatophytes

Isolated strains of this region *T. mentagrophyte*, *T. rubrum*, *E. floccosum* and *M. canis* were used to study the biofilms also. Column purified SS14 was collected from *P. aeruginosa* cultured at selective substrates in media (*i.e* glucose, glycerol and manitol) and observed for inhibition of biofilms produced at specific (*i.e* PDA) media by dermatophytes (Anthi *et al.*, 2023). Isolated and identified strains were cultured at PDA and spores were transferred in RPMI - 1640 at conc. of 2×10^5 CFU (colony forming unit) in 96 well microtiter plate. Suspension was cultured at 27°C for 72 hrs along with control cells without RL-SS14, Then culture diluted up to 2 fold (max up to 5 fold finally) in each subsequent well by SS14, R-70 (Conc) overnight, then filamentous cells and ECM was stained using safranin and crystal violet.

Crstal-Violet and safranin staining for biofilm demarcation

Staining of cell wall and extracellular membrane of dermatophyte was performed using safranin and quantification using UV-spectrophotometry at 470 nm. Subsequently wells were washed by PBS to detach unbound cells so wells left with ECM and biofilms only; which were finally stained with 120 μ l of 0.2% Crystal Violet for 20 minutes (Suparna *et al.*, 2020). Surplus stain was removed by using 120 μ l of 80% ethanol (used as de-stainers). Finally suspension was taken separately for UV spectrum study and peaks were visible at 580 nm. Biofilms were also visible under trinocular to observe density of different strains. This is a physical study by which a researcher can observe the impact of enzyme on biofilms.

$$\text{Biofilm formation (\%)} = \frac{\text{OD (treated)}}{\text{OD (Control)}} \times 100$$

Biofilm formation

According to CLSI guidelines 96 well microtiter plates were used to study biofilm density to observe its role in resistance of dermatophytes. Spores were collected from *T. mentagrophytes*, *T. rubrum*, *E. floccosum*, *M. canis* and cultured in 96 well microtiter plate for 144 hrs. in RPMI 1640 at 28°C. Microtiter wells were siphoned off without moving and static cells were swiped by PBS (Phosphate buffer saline). RLSS14, R-70 at different conc, added to the media. (two-fold conc with each subsequent well up to 5-fold) up to 24 hrs at 28°C, then stained with safranin and CV, as described above. Subsequently, UV Spectroscopy was performed, including control samples; Both were comparatively analyzed under trinocular also for different stains Biofilm with increased concentration of RLSS 14, along with CV stain kept for 24 hours. Spectrophotometry could be used

Table 1: Above data in tabulated form was collected from patients from reached at various govt hospitals, from increasing altitudes, regional patients indicating the MIC range (in μ g/ml) Observed during experiment. M $\bar{g}$ -mean was taken after 5 times reading collection in spectrophotometer.

Species	Itraconazole MIC ₉₀						Fluconazole MIC ₉₀						Terbinafine MIC ₉₀					
	Doon	Srinag	Haldw	Almora	Pithora	SD	Doon	Srinag	Haldw	Almora	Pithora	SD	Doon	Srinag	Haldw	Almora	Pithora	SD
<i>T. mentagrophyte</i>	0.43	0.52	0.41	0.53	0.57	± 0.061	0.15	0.16	0.16	0.18	0.21	± 0.021	9.5	10.9	11.2	12.0	12.7	± 1.01
<i>T. rubrum</i>	0.57	0.60	0.63	0.68	0.69	± 0.045	0.16	0.20	0.22	0.22	0.23	± 0.024	10.3	10.5	11.8	11.6	12.9	± 0.945
<i>E. floccosum</i>	0.25	0.30	0.28	0.27	0.29	± 0.017	0.18	0.23	0.25	0.28	0.28	± 0.037	14.0	13.5	14.5	13.7	14.2	± 0.354
<i>Nanninzia gyp</i>	0.46	0.56	0.52	0.58	0.59	± 0.047	0.26	0.32	0.36	0.33	0.37	± 0.038	13.5	12.8	13.3	12.3	13.0	± 0.416
<i>M. audii</i>	0.64	0.67	0.69	0.73	0.79	± 0.052	0.22	0.24	0.26	0.27	0.29	± 0.024	12.0	13.6	13.7	14.2	14.6	± 0.886

to observe its density. For trinocular study, wells were decanted and washed with PBS and then kept in 2.5% glutaraldehyde in 1M PBS (pH 7.5) at 40°C overnight to remove the fixative. Samples were dehydrated with increased ethanol conc (10µl to absolute) and mounted at carbon tape under tissue culture microscope (TC1000) series at 10x and 22x power magnification. This was an experimental study to conclude a data to validate the physical study under microscope.

Diminutive study of biofilm hampering using rhamnolipid

Study was performed under Scanning electron microscope for biofilm and ECM recovered from different conc.of RL-SS14 along with antifungals (Anthi *et al.*, 2023). Comparative analysis of different substrates used to grow *P.aeurognosa* and its impact on diminishing biofilm of dermatophytes was also observed. At two fold conc. (up to 10 fold) MIC was tabulated as below up to which visibility of ECM was drastically reduced.

AFST range analysis of current isolated pathogen

Standard CLSI M-61,Ed-2,Std. procedure for antifungals was performed by using 90-microwell titer plate (Patel *et al.*, 2023). Two-fold of antifungals conc, increased with each subsequent row of wells ,was poured with a multichannel pipette. Standard MTCC-DBT strains were used as controlled pathogen and std antifungals powders dissolved with 0.1% conc., of DMSO to make the final con 100 µl, was calculated by formula mentioned below (Gupta *et al.*, 2025). RPMI-1640 media was used along with fungal spores culture for 8 to 14 days then a spectrophotometer was used to take the readings of media density(as cell death increases media became opaque). Peaks given for controlled was comparatively analyzed with isolated genera, which helpful in finding out the susceptibility range of current isolated pathogen for different antifungal classes (Brumster *et al.*, 2020). Experiments were performed at least 3-5 times to calculate geometric mean so accurate results could be achieved (Table 2).

Weight = $\frac{\text{Volume } (\mu\text{ml}) \times \text{Conc } (\mu\text{g})}{\text{Assay potency } (\mu\text{gm/ml})}$

Molecular confirmation of species and pathogenic genes multiplication

Fresh culture incubated at 27°C for 7-14 days at PDA was used for DNA extraction and multiplication. DNA was extracted by Phenol/Chloroform/Isoamyl alcohol as well as by the QUIGEN-KIT method (Farmazi *et al.*, 2021). Qualitative DNA was multiplied by PCR which was carried out (LT-241-96 wells) in 50 µl reaction volumes including 25 µl of premix, 3 µl of DNA template,0.8 µM of each primer and double-distilled water(DDW) added to maintain final volume (Bortoluzi *et al.*, 2023). Reaction mixtures were preheated to 98° for 5 min. and then 35 cycles were performed; Initially for 1 min at 96°C, then 68°C for 1 min and 72°C for 1 min followed by a final extension at 72°C for

Table 2: Explaining about the MIC₅₀ at specific altitude and its range in combination to RL decreases consistently with increased quantity,the test was performed on the basis of previous tests explaining the highest impactful drug at specific location.

Genera	RL	Itra	RL+I	Genera	Fluconaz	RL + F	Genera	Terbinafin	RL+T	Genera	Ketac	RL+T	Genera	clotri	RL+C
<i>E.Flocossum</i>	0.2	0.30	0.27	<i>T.menragrophytes</i>	0.16	0.12	<i>T.menragrophytes</i>	11.9	10.8	<i>T.menragrophytes</i>	0.42	0.39	<i>E.flocossum</i>	0.9	0.7
<i>T. Menragrophyte</i>	0.4	0.52	0.47	<i>T.rubrum</i>	0.22	0.19	<i>T.rubrum</i>	12.6	11.9	<i>N.gypsea</i>	0.48	0.46	<i>T.rubrum</i>	1.2	0.97
<i>T. Rubrum</i>	0.6	0.60	0.54	<i>E.flocossum</i>	0.28	0.23	<i>N.gypsea</i>	13.8	12.9	<i>E.flocossum</i>	0.53	0.49	<i>N.gypsea</i>	1.7	1.59
<i>N. Gypsea</i>	0.8	0.58	0.53	<i>M.audii</i>	0.29	0.23	<i>E.flocossum</i>	14.8	12.9	<i>T.rubrum</i>	0.72	0.68	<i>T.menragrophytes</i>	2.7	2.53
<i>M. Audii</i>	1.0	0.79	0.67	<i>N.gypsea</i>	0.38	0.32	<i>M.audii</i>	14.6	12.5	<i>M.audii</i>	0.87	0.82	<i>M.audii</i>	3.1	2.97

5 minutes. The PCR products with approx. 2380 bp DNA was purified using a minimum elute PCR purification kit (Fonseca *et al.*, 2022). All amplicons were evaluated at 1.5% agarose gel, loaded with ladder initially, then sequencing was performed for result analysis. Species confirmation by Universal Forward and reverse primers ITS1-5' -TCCGTA GGTGAACCTGCGG-3', ITS4-5' -TCCTCCGCTTATTGATATGC-3' and for Naninnzzia species ITS used was V9G-5'-TTACGTCCCTGCCCTTTGTA-3' (Kano *et al.*, 2022) and LSU266 5' -GCATTCCCAACAACCTCGACTC-3'. Finally, species were confirmed at NCBI FASTA global alignments followed by ITS multiplication and sequencing (Kumar *et al.*, 2023). After confirming species by ITS, accordingly pathogenic genes were multiplied and sequenced *i.e* Squalene monooxygenase (Drsq1 5'-TTGCCAACGGGGTGTAAG-3' and Drsq2 5'-GGGCCATCTATAATTTCAGACTC-3') was primarily multiplied in a suspect of any mutation followed by metalloprotease (MEP 1-5), multiplied to find out the most frequently found qualitative MEP (Table 2) in response to mutation (Ortiz *et al.*, 2024). Finally, sequencing was performed by the Sanger method, followed by analysis of data at FINCH TV software and comparative analysis at NCBI BLAST and global alignment at FASTA. Most similar base pair alignments were identified and finally their cladograms were prepared to connect their close relatives (Pavlovic *et al.*, 2024) (Fig 3a and b).

Composing Primer and Quantitative PCR for metalloprotease

Metalloprotease (1to5) were also observed during the current study, to understand their quantitative role play in resistance of dermatophytes, so lipolyzed primers in nuclease-free water up to 120 pmol/l were provided by BARCODE PVT LTD. Bengaluru. The Primers used here to multiply were previously reported by several authors (Hasshoosh *et al.*, 2024). 20 p/mol of primers mixed with 80 ml of nuclease-free water and stored at -20°C for use. A PCR tube, strip of 8 with 100 µl capacity, was used for reaction. It contains 10 µl of mastermix, 1 µl of primer, 3 µl of DNA, 6 µl of nuclease-free water, which makes the volume up to 20 µl. Mastermix containing SYBR green by G-biosciences so during the multiplication reaction, should be visible, which provides advanced info that the qualitative multiplication of

MEP was taking place. Results were studied by electrophoresis to observe fragment length and primarily to confirm the multiplication, followed by qubit spectrophotometer by following the formula. Then pool the samples equimolar to a final conc of 10nM, measure the pool with a tape station D1000 measurement Fig 2b.

Molar concentration [nM] =

$$\frac{(\text{PCR 2 concentration [ng/}\mu\text{l]})}{660 [\text{g/mol}] \times (\text{fragment length [bp]})} \times 10^6$$

RESULTS AND DISCUSSION

In the current study Itraconazole found highest impactful drug as it is having lowest MIC₉₀ 0.59 µg/ml and terbinafine MIC₉₀ 14.6 µg/ml was highest and least impactful followed by fluconazole MIC 0.37 µg/ml, in this area. (Amin *et al.*, 2025), (Oguz *et al.*, 2024) and (Gibas *et al.*, 2023), (Sabater *et al.*, 2023) studies supporting itraconazole, fluconazole and terbinafine (Russo *et al.*, 2023) susceptibility range subsequently. However some studies have contrary results to current study as (Vernekar *et al.*, 2024) reported , 64 µg/ml MIC range for fluconazole. (Sharma *et al.*, 2022) also reported up to 32 µg/ml for fluconazole, 0.5 µg/ml for itraconazole and up to 0.5 µg/ml for terbinafine which is comparatively very less than current study. Secondary factors like, Biofilms depleted with increased conc of rhamnolipids and in combination of tabulated antifungals.

Biofilms play a vital role in controlling infection as ramnolipid supplied with combination of antifungals, MIC reduced to normal range, supported by a study at Assam (Suparna *et al.*, 2020). Along with these factors, metalloprotease study also clearly indicating that MEP-3 was found more prominent in most of the isolated genera isolated from this area , MEP-2, MEP-1 , MEP-4 found in in descending quantitative amount (Fig 2a), which also supported by a study in Iraq (Hasshoosh *et al.*, 2024) and opposed by a study in china where MEP 4 and 5 were found least pathogenic (Zhang *et al.*, 2014). So metalloprotease interpreting their involvement in resistance of dermatophytes are just like markers in a molecular study, It indicating that the most prominent MEP in specific species and could be manage through ; An allosteric enzyme inhibitors, at skin,

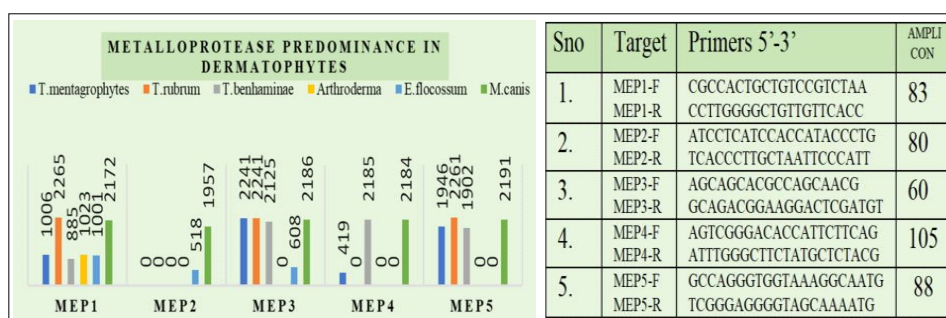


Fig 2a and b: Primers (a) used for RT PCR (Q-PCR) to detect the presence of MEP1-5 genes in species isolated from doon hills. Result Showing no of BP mentioned below is an approx. Idea by finding weight of DNA on agarose gel (Dash represents either the weights less than 0.5 KD or not visible).

along with antifungals (Deng *et al.*, 2023). In the current study, SQLE gene mutations L393F and F397L, A448T were also observed (Fig 3b) leading mentagrophytes and rubrum more close to *T. indotineae* clearly visible in cladogram (Fig 3a) however all three mutations were previously reported from various location of India (Kumar *et al.*, 2023) and worldwide (Keshwania *et al.*, 2023).

There is no direct application of calculating MIC but Quantitative evaluation leads pharmacokinetics and pharmacodynamics by calculating the ratio of maximum serum conc to minimum inhibitory conc Cmax/MIC and the ratio of area under plasma conc vs time curve AUC/MIC ratios^[9,10]. The duration of dosing interval that plasma conc exceeded MIC; So PK/PD index helpful in scheduling doses, according to regional environmental conditions and consequently modify the combination of drugs with higher impact and low immune damage (Urla *et al.*, 2020). To override current modified pathogen it is significant to find out the MIC range and PK/PD index (*i.e.* time of drug infusion in serum and its residing time, to maximize the impact at target organ) guiding practitioners to keep the maximum herd immunity as it is a communicable infection (Oguz *et al.*, 2024). Biofilm study have a direct influence to observe the epidemiological cutoff accurately to differentiate between wild and modified species (Vernekar *et al.*, 2024).

Summary

Globally, almost 48 Single and double mutations reported in Squalene epoxidase of dermatophytes. K276 N, L393F, L393S, F397L, F397I, Q408L, F415S, F415L, F415V, L437P, H440T, S443P, A443P, A448T *etc.* were the most frequent mutations reported globally till now. From India, only 4 types of SQLE gene mutations (393^{Leu}, 397^{Phe}, 415^{Phe}, 440^{His}) were reported most frequently (Verma *et al.*, 2020) which are very common in India (Kano *et al.*, 2022). *T. mentagrophyte* and *T. rubrum*, showing maximum parsimony to *T. indotineae*. Mutations were indicating somewhere resistance toward antifungal drugs (Ortiz *et al.*, 2024). Along with mutations several supporting factors are there which are responsible for resistance of dermatophytes as biofilm play a huge role in pathogen resistance which is clearly visible through RL experiments. Another major clue of resistance come through M36 family of metalloproteases which help to degrade keratin. Different genera have a different predominated metalloprotease gene, so their study helpful to target the M36 by antifungals. To override current modified pathogen, it is significant to find out the MIC range and PK/PD index (*i.e.* time of drug infusion in serum and its residing time, to maximize the impact at target organ) guiding practitioners to keep the maximum herd immunity (Deng *et al.*, 2023) as it is a communicable infection.



Fig 3a and b: Cladogram showing here close relationship of pathogenic genes SQLE of *T. mentagrophytes* and *T. rubrum* (3a) and commonly found three mutations observed there in SQLE gene aa (3b).

Limitations

To find out the current updated status of modified SQLE of various dermatophytes in this area which will be helpful for practitioners. As well as find out the role play of biofilms and metalloprotease (MEP) in antifungal resistance. Among all three angles which is the most impactful for resistance and up to which levels combination of drugs ,enzymes can manage the situation.

CONCLUSION

Mutations in SQLE, Biofilms penetrance in skin layers and patterns of metalloprotease contribute in resistance of dermatophytes; these factors more or less impactful among each other due to several favourable climatic conditions.

Interpretation

Three different methods used here to establish a resistance pattern of current pathogen and their comparative analysis was performed to find out the most impactful factor in resistance of pathogen.

Ethic clearance

Ethic clearance was taken from IHEC from SIST Chennai. (Ref no 210/IRB-IBSEC/SIST 13 oct 2022). Separately attached.

Conflict of interest

There is no direct or indirect funding/Conflicts of interest for the current study.

REFERENCES

- Amin, N., Shenoy, Manjunath, M., Pai, V. (2025). Antifungal susceptibility of dermatophyte isolates from patients with chronic and recurrent dermatophytosis. *Indian Dermatology Online Journal*. **16(1)**: 110-115.
- Anthi, M.M., Samaras, K., Vyzantiadis T.A. (2023). Dermatophytic biofilms: Characteristics significance and treatment approaches. *J. Fungi (Basel)*. **9(2)**: 228.
- Arendrup, M.C., Kahlmeter, G., Guinea, J., Meletiadis, J. (2021). How to : Perform antifungal susceptibility testing of microconidia-forming dermatophytes following the new reference EUCAST method E.Def 11.0, exemplified by *Trichophyton*, *Clinical Microbiology and Infection*. **27(1)**: 55-60.
- Bortoluzzi, P., Prigitano, A., Sechi, A., Boneschi, V., Germiniasi, F. (2023). Report on terbinafine resistance *Trichophyton* spp in Italy; Clinical presentations, molecular identification, antifungal susceptibility testing and mutations in SQLE gen, *Mycosis(MDTPFD)*. **66**: Iss8.
- Brumster, A., Uhrli, H.S., Nenoff, P., Singal, A., Shyam, B.V., Peter, E., Wiegand, C. (2020). Indian *Trichophyton mentagrophytes* squalene epoxidase erg1 double mutants show high proportion of combined fluconazole and terbinafine resistance. *WileyMycosis*. **63**: 1175-1180.
- Deng, R., Wang, X., Ruoyu, Li. (2023). Dermatophyte infection : From fungal pathogenicity to host immune responses; *Frontiers in immunology*. doi: 10.3389/fimmu.2023.1285887.
- Farmazi, S., Motamedi, Ali R.M., Ansari, S., Didehar, M. (2021). A simple multiplex polymerase chain reaction assay for rapid identification of the common pathogenic dermatophytes: *T. interdigitale*, *T. rubrum* and *E. floccosum*, *Current Med Mycol*. **7(2)**: 1-7
- Fonseca, L.A.V., Araujo, M.A.S., Silwa, D.M.W. (2022). ITS_RFLP Optimization for dermatophytes identification from clinical sources in Alagoas(Brazil) versus phenotypic method. *The Journal of Infection in Developing Countries*. **16(11)**: 1773-1777.
- Gibas, C.F.C., Mele, J., Patterson, H.J., Sanders, C.J. (2023). Terbinafine resistant dermatophytes and the presence of *T.indotineae* in North America. *ASM Journals/Journal of Clinical Microbio*. **68**: 8.
- Gupta, A.K., Sushmita, Nguyen, H.C., Liddy, A., Vasiliki, E., Tong, W. (2025). Terbinafine resistance in *Trichophyton rubrum* and *Trichophyton indotineae* : A literature review. *MDPI Antibiotics*. **4(5)**.
- Hasshoosh, H.Q., Yaseen, A.M., Al-araji. (2024). Determination of metalloprotease genes in a few dermatophyte species and its relationship with antifungal relationship. *Journal of Communicable Diseases*. **56(1)**: 127-134.
- Hong, J., Sylvester, W., Alhassan, A., Sharma, B., Amandi, V., Kamashi, K. (2025). Retrospective analysis of antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* from clinical samples of dogs in Grenada ,West indies. *Indian Journal of Animal Research*. **59(1)**: 136-140. doi: 10.18805/IJAR.B-5310.
- Kano, R., Noguchi, H., Hiruma, M. (2022). A deletion mutation in amino acid sequence of squalene epoxidase in terbinafine resistant *Trichophyton rubrum*. *J.of Infection and Chemotherapy*. **28(6)**: 741-44. 9
- Keshwania, P., Kaur, N., Chauhan, J., Sharma, G. (2023). Afzal. Superficial dermatophytosis across: The world's population potential benefits from Nanocarrier-Based therapies and rising challenges. *ACS Omega*. **8(35)**: 31575-31599.
- Kumar, P., Ramchandran, S., Das, S., Bhattacharya, S.N. (2023). Insights in to changing dermatophyte spectrum in India through analysis of cumulative 165,245 cases between 1939 and 2021. *Mycopathologia*. **188**: (183-202).
- Moskaluk, A.E., Woude, S.V. (2022). Current Topics in Dermatophyte Classification and Clinical Diagnosis. *Pathogens*. **11(9)**: 957.
- Nanavare, A.S., Sakhare, M.P., Siddiqui, M.F.M.F., Chepte, S.D., Whagamare, R.N., Rathod, P.R., Mane, P.M., Yeotikar. P.V. (2024). Epidemiological and clinico therapeutic studies on dermatophytosis in cattle at prabhani. *Indian Journal of Animal Research*. doi: 10.18805/IJAR.B-5125.
- Oguz, D., Direkel, S., Aksan, B., Kulanki, S., Aksoy, S. (2024). *In vitro* antifungal susceptibility of dermatophytes : A single center study from eastern black sea region of Turkey. *ERMRS*. **28**: 2144-2154.
- Ortiz, B., Manuel, G., Juan, R.T., Bush, M., John, S.G. (2024). Fontecha G. Global insights and trend in research on dermatophytes and dermatophytosis : A Bibliometric Analysis. *Mycosis*. **67(10)**: e13803.
- Patel, M.A., Pandey, A., Patel, S., Mohapatra, S.K., Chauhan, H., Sharma, K., Prajapati, B.K., Chandel, B.S. (2023). Prevalence and characterization of antimicrobial resistance pattern of ESBL producing *E.coli* isolated from poultry in Banaskantha district ,India. *IJAR*. **93(6)**: 566-571.

- Pavlovic, M.D., Marzouk, S., Beciri, L. (2024). Widespread dermatophytosis in a healthy adolescent the first report of multidrug resistant *T. indotineae* infection in UAE. *Acta Dermatovenereol Alp Pannonica Adriat.* **33(1)**: actaapa.4. PMID: 38347717.
- Russo, G., Trelu, L.T., Frontao, L., Ninet, B. (2023). Toward an early clinical and biological resistance detection in dermatophytosis about two cases of *T. indotineae*. *J. Fungi (Basel)*. doi: 10.3390/jo 9070733.
- Sabater, A.M., Normand, A.C., Bidaud, A.L. (2023). Terbinafine resistance in dermatophyte: A French multicentre prospective study. *Journal of fungi, MDPI*.
- Sharma, S., Maheshwari, M., Thakur, R. (2022). Antifungal susceptibility testing of five antifungal agents against clinically isolated dermatophyte species from a tertiary care hospital of northern india. *Journal of Clinical and Diagnostic Research*. **16(3)**: 36-42.
- Shivraju, S., Maiti, K.S., Mohan, D., Kalaiselvan, E., Sunil, K.S.P., Banu, A.S., Kumar, N. (2025). The larval stage of *Taenia taeniaeformis* in wister albino rats with an illustration of biochemical and histopathological changes. *Indian Journal of Animal Research*. **59(2)**: 321-325. doi: 10.18805/IJAR.B-4728.
- Suparna, S., Siddharth, N.B., Arijit, B., Deka, S. (2020). Ramnolipid biofilm exhibit antibiofilm activity against the dermatophytic fungi *Trichophyton rubrum* and *T. mentagrophytes*. *Biotechnology Reports*. **27(2020)**: e00516
- Urla, S., Mey, S., Storch, S., Witting, F., Koch, D., Kruger, C., Nenoff, P. (2020). *Naninzzia incurvate* as a rare cause of favus and *T. corporis* in Cambodia and Vietnam. *IJDVL*. IP. **91(36)**: 220138.
- Vanapalli, S., Turpati, N.R., Gopal, K.V.T., Krishnam, R.P.V. (2022). A Clinico-mycological, Antifungal Drug Sensitivity and Therapeutic Study of Extensive Dermatophytosis in Coastal, Andhra Pradesh. *Indian Dermatology Online Journal*. **13(6)**: 747. doi: 10.4103/idoj.idoj_143_22.
- Verma, B.S., Panda, S., Nenoff, P., Singhal, A., Rudramurthy, M.S., Uhrlass, Das, A., Bisherwal, K., Shaw, D., Vasani R. (2020). The unprecedented epidemic like scenario of dermatophytosis in India:III. Antifungal resistance and treatment options. *Indian Journal of Dermatology Venereology and Leprology*. **87(3)**: 1-7. doi 1025259/IJDVL-303-20.
- Vernekar, P.P., Shweta, R., Pavitra, A., Kikeri, N. (2024). Clinicomycological study of dermatophyte infection in patients attending dermatology outpatient department in a tertiary care hospital. *Clinical Dermatological Review*. page 120-26.
- Zhang, X., Wang, Y., Chi, W., Shi, Y., Chen, S., Lin, D., Jin, Y. (2014). Metalloprotease genes of *T.mentagrophytes* are important for pathogenicity. *Med Mycology*. **52(1)**: 36-45.