Content available at: https://www.ipinnovative.com/open-access-journals

IP Indian Journal of Clinical and Experimental Dermatology

Journal homepage: www.ijced.org/

Original Research Article

Evaluation of susceptibility range & altered SQLE in co-relation to higher MIC of dermatophytes isolated from increasing altitudes of Uttarakhand

Garima Sharma[1]*, **Daniel Alex Anand**[1]

[1]*Dept. of Biomedical Sciences, Sathybama Institute of Science & Technology, Chennai, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 01-12-2024
Accepted 08-01-2025
Available online 08-02-2025

Keywords:
Disc diffusion test
Microdilution test
MIC
Squalene epoxidase
PK/PD
Cladogram

ABSTRACT

Background: Dermatophytes hyphal fragments accidently deposited on stratum corneum sometimes, in contact with other infected person; cause communicable skin infections particularly, in higher altitudes; due to availability of ample of moisture. Diagnosis specified, primarily by clinical appearance, followed by mass culture of dermatophytes in lab, their identification under microscope & molecular identification by ITS regions.

Problem: Spatial locations of Uttarakhand, Commendable to most mycosis species. Unanticipated number of mycosis patients during monsoon & recalcitrant nature of dermatophytes in consecutive years after 1^{st} infection, grab attention. Study designed to calculate susceptibility range & intensity of modification in pathogen at various altitudes.

Approach: Throughout Monsoon (from July to Nov 2023-24) skin samples from public hospitals of Uttarakhand at Skin-OPDs were collected, mass lab production & subsequently identification; to perceive their appearance time & relationship with increasing height. Standard Disc diffusion & broth dilution tests were carried out to calculate MIC range of frequently used antifungal drugs at public hospitals & modified current available pathogen.

Findings: MIC of Trichophyton, Epidermophyton& Microsporum (5-25μg/ml) for terbinafine, for Itraconazole (0.09-1.5μl/ml) & for Fluconazole (0.03-0.5μl/ml) found much more higher than previously reported. Arthroderma, Blastomycosis & Nanninzzia sp was also isolated from Haldwani region. Species identification confirmed by ITS1 & ITS4 regions of rDNA & squalene epoxidase was also sequenced up to 600bp in a doubt of modification in pathogenic gene of T.mentagrophytes & T.rubrum. F397L, A448T in mentagrophytes & L393F in rubrum found altered by comparatively to control agent.

Conclusion: Initially it looks critical to observe direct output from increased suseptibility range of pathogen but associated in evaluating efficacy of drug,which is contributing in calculating C_{max}/MIC, plasma drug diffusion & retention time ratios,finally their susceptibility range (MIC_{90})for pathogen.This pharmacokinetics guide to choose,combination of drugs & dose schedules

1. Introduction

WHO reports 25% of world population affected by dermatophytes where in India 6.09-27.6% from south & 62.5% (include Himachal Pradesh & Uttarakhand) population from Northreported.[1] Worldwide publication data shows 15838 articles in WOS & 23189 in SCOPUS published[2] during 2019-23 emphasize dermatophytes. Demography of fluctuating altitudes, already observed favorable for most of the dermatophyte as humidity is much more higher in hilly & coastal areas.[3] During Monsoon, Increased no of patients in skin OPD, recalcitrance of infections with long recovery time cause severe immunity damage to Patients in response; prolonged use of

* *Corresponding author*.
E-mail address: garimasharma200@gmail.com (G. Sharma).

https://doi.org/10.18231/j.ijced.2025.004

systemic antifungals like Triazoles, Allylamines & Polyenes frequently prescribed at OPDs. Consistent use of these drugs along with corticosteroids usually improve the pathogen resistance slowly; parallelly damage the host immunity & promote other microbial predators.

2. Objective of Study

Establishing a demographic (with increased height) relation with variation in mycosis species & MIC evaluation for current available, modified pathogen was primary objective of study. In response to increased MIC, pathogenic gene SQLE was multiplied (PCR) & sequenced up to 600bp for any modification in T.mentagrophytes & T.rubrum.

3. Materials and Methods

3.1. Statastical distribution in plan of study

Approximate 1400, 500 & 300 subjects were observed from skin OPD of Doon, Haldwani & Srinagar subsequently with certain microbial skin diseases at public hospitals[4] (Figure 1). Shedding stratum corneum collected from margins of ring worm, for sample collection 410,210 & 110 tinea infected subjects were chosed (from July to Oct 2023-24 approx 50-80 x1 for 5 months) due to precise margins, from subsequent locations. Positive samples in preliminary heat fixed koH slides were 350,170,90 in number, at subsequent locations(Figure 3).[5] Dead skin samples, of patients were collected by sterile blunt scalpel from well-defined margins of roundworm.Subjects were informed & written consent has been taken before collection of samples from group of patients diagnosed with tinea & scabies only, Pregnant,lactating females& 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).

3.2. Sample collection & identification techniques

Skin scrapings collected from OPD was preliminary treated & heat fixed with30% KOH slides & tested under trinocular for confirmation of visible filaments. Specimen found positive, cultured at lab in PDA. Subsequently replicas were plated in variety of media RPMI 1940, SDA, PDB for genus identification under trinocular stained with lactophenol blue.

White cottony Colonies of culture during monsoon in sterilized lab conditions were visible at 28-30^{0}C in autoclave after 7-9 days. As culture fully grown, color variation due to ample of spores formation in several mycosis genera. In fully grown colonies microconidia, macroconidia & spores visibly effective in genus identification, through tissue culture microscope (TC1000) series,at 10x & 22x power magnification.[6] Hyphae,filaments, conidia including spores shape & size of cottony colonies was helpful during identification (Figure 1). Merdinger & divine lipid analysis test was performed for biochemical analysis.

Final confirmation of species identification was done by the ITS regions & Universal primers Forward ITS1-5′-TCCGTAGGTGAACCTGCGG-3, ITS 4 R–5′ -TCCTCCGCTTATTGATATGC- 3′[7] & for Naninnzzia species ITS used was V9G-5'-TTACGTCCCTGCCCTTTGTA-3'[8]& LSU266 5'-GCATTCCCAAACAACTCGACTC-3' followed by sequenced base-pairs compared at NCBI-BLAST global alignments for final confirmation (Figure 4 a).

3.3. Laboratory experimental outlines

Kirbey-Bauer standardized Disk diffusion test with 150mm Petri plate.4mm disc was prepared by watman filter paper after folding it in six folds & followed by dipping in antifungals for almost an hour·Discs were shifted to Petri plate by sterilized tweezers. A single disc was used at a one Petri plate but 2 discs were also used (for growth study only) for study of a combination of drugs as a preliminary test for pathogenic gene modifications. From middle of disc up to 14 mm was marked as zone of inhibition,15-20mm zone of sensitivity, above 22 mm zone of susceptibility was marked. Results were taken manually thrice for each species along with SD calculation[9] (Table 1). Standard Microdilution in 90 well microtiter plate was also performed by std CLSI -EUCAST M-61,Edition-2 AFST method by culturing the spores for 7-10 days in wells & DMSO dissolved antifungals (ratio of 1:9) in two fold microdilution was poured in subsequent wells of each column. Finally, spectrophotometry was performed for each column (Table 2).[10]Comparative analysis was done, with control species for final results. Pure antifungal std powdered form was measured by below formula.

Weight = Volume (μml) X Conc (μg)/ Assay Potency (μgm/ml)

Clustal W (for alingnments) from previously reported & current data to understand that maximum mutations are visible in T.mentagrophytes & T.rubrum so their highest parsimony found to T.indotineae (b).

3.4. AFST outcomes of allylamines, imidazoles & their statistical analysis

3.4.1. Molecular study of increased MIC dermatophytes

PCR was carried out (LT-241-96 wells) in 50μl reaction volumes including 25μl of premix,3μl of DNA template,0.8μM of each primer & DDW added to maintain final volume.[11] Reaction mixtures were preheated to 98^{0}C for 5 min.& then 35 cycles were performed; Initially for 1 min at 96^{0}C, then 68^{0}C for 1 min & 72^{0}C for 1 min. followed by final extension at 72^{0}C for 5 minutes. The PCR

products with appox.2380bp DNA were purified using a minimum elute PCR purification kit. All amplicons were evaluated at 1.5% agarose gel,loaded with ladder. After confirmation all species samples, subsequently loaded For PCR along with 28s rRNA primers & confirmed by sequencing, which primarily confirmed by the presence of successful copies in SYBR green qPCR master mix.[12] Finally, 28s rRNA (ITS1 & 4)primers were used to further confirm species.[13] After species confirmation SQLE (Drsq1 5′-TTGCCAACGGGGTGTAAAG-3′ & Drsq2 5′-GGGCCATCTATAATTCAGACTC-3′) was multiplied by PCR & sequenced up to 600bp for observing any modification by mutated primers.[14]

4. Results

In current study five dermatophyte genera studied with 109 Strains, study was divided in two parts; first cross sectional where Disc diffusion tests & microdilution experiments were performed for confirmation of MIC_{90} range for current available pathogens. Study reveals that Fluconazole found the highest MIC (0.03-0.5μl), followed by Itraconazole (0.2-1.25μl)**,** however Sharma[15] revealed that griseofulvin has lowest MIC followed by terbina fine, Shockingly results were completely contradictory to current study, as Terbinafine found highest MIC range (11.9-21.6μl) followed by clotrimazole in this region. Current study also reveals Itraconazole followed by Fluconazole was most effective in this area as most of genera are sensitive than resistant (table-2). Terbinafine without any combination of drugs were found almost ineffective which was observed as differ from other authors as Salehi[16] & Sharma[15] but supported by Bortoluzi[17] & A. Moreno Sabater[18] studies. Mutations in squalene epoxidase of T.rubrum L393F & F397L,A448T (Figure 5a) in mentagrophytes in rRNA firstly reported from various altitudes of Uttarakhand in current study, were previously also reported from various parts of India[19] & world.[20] Some rarely reported species Blastomycis,Arthroderma & Nanninzzia were also isolated including three common species.However different mutations in SQLE were also reported by Urla[4] & Pavlovic ;but the studies highlighting a major view about mutations that they are leading toward T.indotineae a more advance pathogen.[12] Which can also analyzed (Figure 5b) by the comparative cladograms of previous & current study.

5. Discussion

It is Convinent to observe the impact of increased MIC for improved pathogen resistance but Quantitative evaluation performed through pharmacokinetics & pharmacodynamics by calculating the ratio of maximum serum conc to minimum inhibitory conc Cmax/MIC & the ratio of area under plasma conc vs time curve AUC/MIC ratios**.**The duration of dosing interval that plasma conc

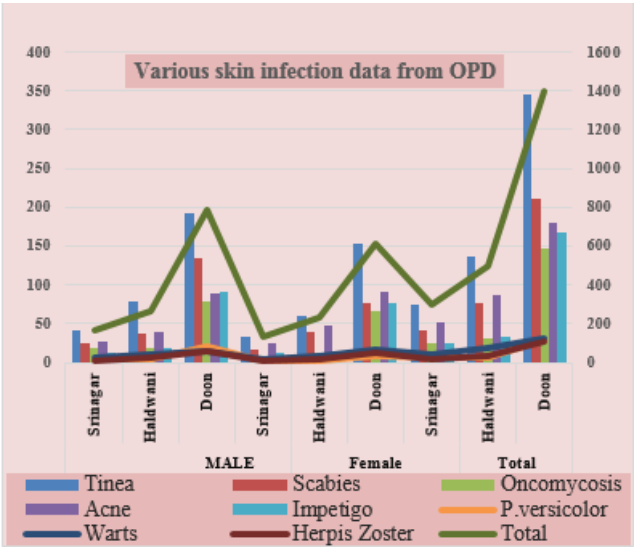

Figure 1: Above graphical presentation explaining the no of various communicable derma infections, carrying patients of both gender reach public hospitals explaining their epidemiology at all three altitudes (Srinagar, Haldwani & Dehradun) of Doon valley.

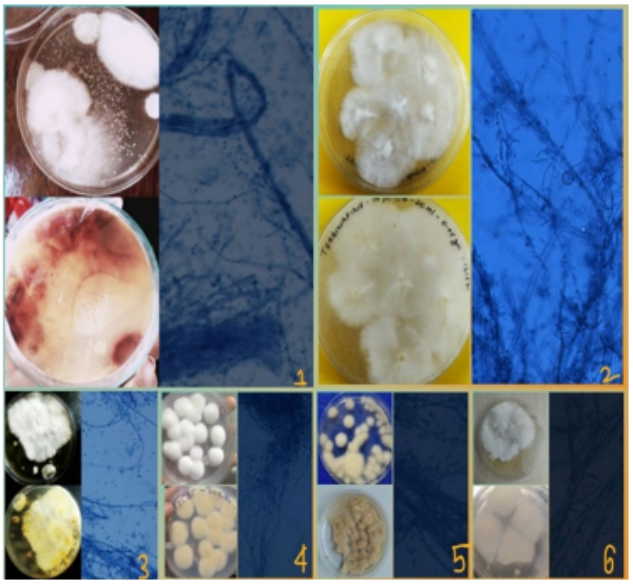

Figure 2: Dermatophytesisolated from Doon Tricophyton rubrum (**1**) Microsporum (**2**) T.mentagrophytes (**3**) Arthroderma pure white cottony colonies initially (**4**) which will impart brown to black pigment on reverse side, Epidermophyton (**5**), T.behminae fluffy colonies conidiophores visible under ultramicroscope

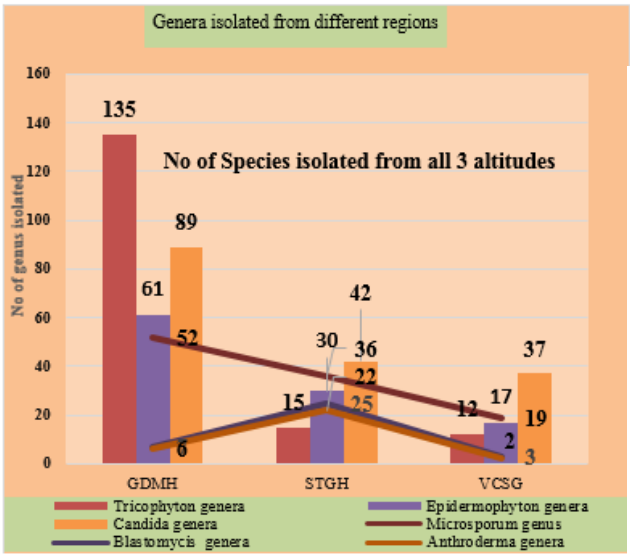

Figure 3: In left, no. of data collected from all three public hospitals GDMC (Govt Doon hospital Dehradun),STGH (Susila Tiwari hospital) Haldwani & VCSG (Veer Chandra Singh Garhwali hospital)Srinagar represented in a graphical form.

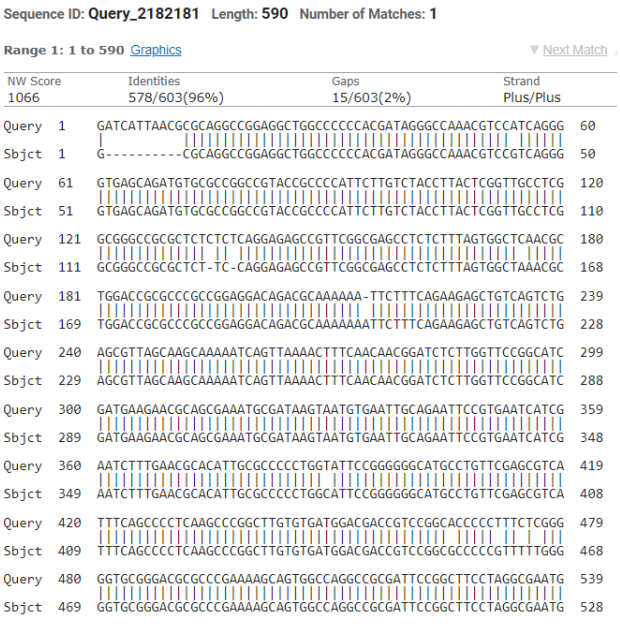

Figure 4: Comparison of isolated & sequenced ITS regions at global alignments of NCBI-BLAST, sequenced basepairs found96% similar to T.benhamiae acession no.NR_103705 Species identification was performed similarly as other species found 92-98% resemblance to isolated dermatophytes,

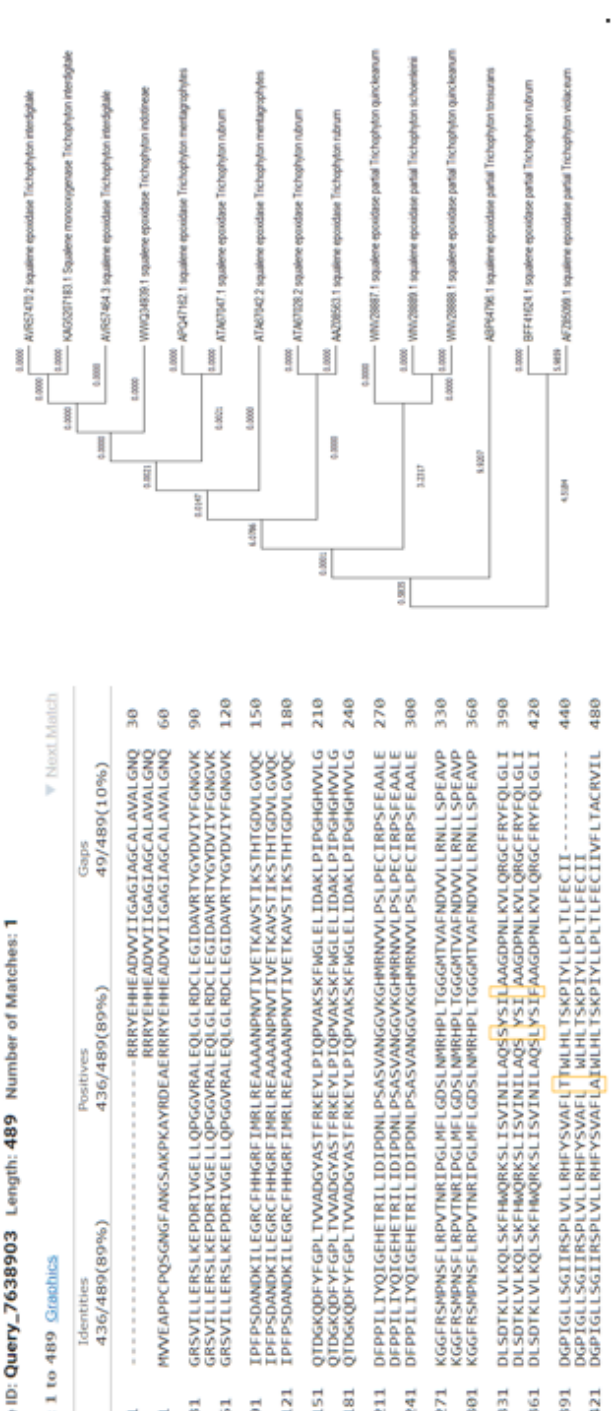

Figure 5: Mutations were visible in all three aa at NCBI-BLAST global alignments duringcomparison; **a:** Cladogram prepared by MEGA 11 & Clustal W (for alingnments) from previously reported & currentdata to understand that maximum mutations are visible in T.mentagrophytes& T.rubrum so their highest parsimony found to T.indotineae(**b**).

Table 1: Discdiffusion test performed as to find out the break point against anti fungals asdrugs crosses the zone of Inhibition 14mm), Zone of sensitivity (15-20mm) & Zone of susceptibility (22mm & above) confirmation results of drugs at MLN

		Inhibition Zone Diameter								
	Genera	**Itraconazole**		**Fluconazole**		**ketaconazole**		**Clotrimazole**		**Terbin**
1.	T.mentagrophytes	16.8 ±0.05	S	21.8 ±2.87	S	11.6 ±0.67	S	13.8 ±0.87	S	0.9
2.	T.rubrum	14.2 ± 0.89	S	18.6 ±5.68	S	8.8 ±0.34	S	12.9 ±0.43	S	0.4
3.	E.flocossum	13.8 ±0.96	R	13.6 ±1.54	S	2.5 ±0.57	R	8.9 ±0.32	R	0.8
4.	M.canis	12.4 ±0.67	S	15.3 ± 4.5	S	7.6 ±0.34	R	11.9 ±0.22	S	0.6
5.	Nannizzia gypsea	2.5 ±0.25	R	6.7 ±3.2	R	3.2 ±0.12	R	4.2 ±0.23	R	0.9
6.	Arthroderma sp	3.8 ±0.57	R	5.9 ±2.3	R	2.2 ±0.12	R	2.8 ±0.12	R	0.2

Table 2: Above data in tabulated form was collected from patients from Almora reached at various govt hospitals, Susila Tiwari govt hospital haldwani &Veer chand singh Garhwali govt hospital srinagar (Sri) & regional patients indicating the MIC range Observed during experiment. M*-mean was taken after 5 times reading collection in spectrophotometer

Species	**ItraconazoleMIC_{90} (μg/ml) 0.09-1.5(M)***				**FluconazoleMIC_{90} (μg/ml) 0.03-0.5(M)***				**TerbinafineMIC_{90} (μg/ml) 5-25 (M)***				**KetoconazoleMIC_{90}(μg/ml) 0.09-1.0(M)***				**ClotrimazoleMIC_{90} (μg/ml) 0.5-3.0(M)***			
	Hal	**Alm**	**Sri**	**SD**	**Hal**	**Alm**	**Sri**	**SD**	**Hal**	**Alm**	**Sri**	**SD**	**Hal**	**Alm**	**Sri**	**SD**	**Hal**	**Alm**	**Sri**	**SD**
T.mentagrophyte	0.52	0.58	0.48	±0.06	0.16	0.16	0.15	±0.028	11.5	11.9	12.2	±0.680	0.48	0.42	0.38	±0.050	1.2	1.6	2.7	±0.776
T. rubrum	0.60	0.68	0.57	±0.043	0.22	0.20	0.16	±0.032	12.0	12.6	12.8	±0.598	0.72	0.65	0.56	±0.080	1.5	1.7	1.5	±0.115
E. flocossum	0.30	0.28	0.25	±0.051	0.28	0.25	0.18	±0.086	14.0	13.5	14.5	±0.752	0.62	0.52	0.46	±0.080	0.9	1.2	0.8	±0.208
N. gypseum	0.62	0.56	0.46	±0.115	0.32	0.35	0.26	±0.052	13.5	13.8	11.0	±0.648	0.52	0.48	0.37	±0.077	1.8	2.1	1.8	±0.173
M. audii	0.86	0.82	0.72	±0.063	0.38	0.36	0.22	±0.08	12.0	13.6	12.7	±0.328	0.68	0.62	0.53	±0.075	2.0	2.4	2.0	±0.230

exceeded MIC;So PK/PD index helpful in scheduling doses, according to regional environmental conditions & consequently modify the combination of drugs with higher impact & low immune damage. To override current modified pathogen it is significant to find out the MIC range & PK/PD index (i.e-time of drug infusion in serum & its residing time, to maximize the impact at target organ) guiding practitioners to keep the maximum herd immunity as it is a communicable infection.

Except MIC, modified pathogenic genes will also be helpful in establishing a phylogenetic relationship, as in current study; a single species reported, from various regions of India & world, where SQLE gene modifications has been observed by using NCBI FASTA global alignment, MEGA X & CLUSTAL W among various genera of common species. Alignments & Cladogram of T. mentagrophytes & T.indotineae (individually & Collectively) was constructed along with current study data, it was observed that T.mentagrophytes (90% parsimony) closely related to T.indotineae & a slow evolution of T.mentagrophytes & T.rubrum toward T.indotineae observed in this area also. Modified versions of dermatophytes need a time to time dose modification, otherwise long-term use of antifungals & compromised immunity is clearly visible by secondary infections in population.

6. Conclusion

As the altitude increases resistance found increased, T.mentagrophytes & M.canis both were found modified & inclined toward T.indotineae. Changes in drug shedules in response to higher MIC range found crucial to minimize the infection as drug retains in serum for longer period of time & improve the mortality rate of colonies on skin. Changes in combination of drug can improve the condition by mimizing the MDR effect by misleading the pathogen.

7. Limitations

To find out the current updated status of modified SQLE of dermatophytes in this area which will be helpful for practitioners.

8. Conflicts of Interest

There was no source of funding for current study.

9. Source of Funding

None.

References

1. Keshwania P, Kaur N, Chauhanj, Sharma G. Superficial dermatophytosis across: the world's population potential benefits from Nanocarrier-Based therapies & rising challenges. *ACS Omega*. 20235;8(35):31575–99.
2. Ortiz B, Manuel G, Juan RT, Bush M, John SG, Fontecha G, et al. Global Insights and Trends in Research on Dermatophytes and Dermatophytosis: A Bibliometric Analysis. *Mycoses*. 13803;67(10):e13803. doi:10.1111/myc.13803.
3. Vanapalli S, Turpati NR, Gopal KVT, Krishnam PV. A Clinico-mycological, Antifungal Drug Sensitivity and Therapeutic Study of Extensive Dermatophytosis in Coastal Andhra Pradesh. *Indian Dermat Online J*. 2022;13(6):747–53. doi:10.4103/idoj.idoj_143_22.
4. Urla S, Verma BS, Graser Y, Ali AM, Hatami M, Schaller M, et al. Trichophyton indotineae—An Emerging Pathogen Causing Recalcitrant Dermatophytoses in India and Worldwide—A Multidimensional Perspective. *J Fungi*. 2022;8(7):757. doi:10.3390/jof8070757.
5. Moskaluk AE, Woude SV. Current Topics in Dermatophyte Classification and Clinical Diagnosis. *Pathogens*. 2022;11(9):957. doi:10.3390/pathogens11090957.
6. Uhrlaß S, Mey S, Storch S, Witting F, Koch D, Kruger C, et al. Nannizzia incurvata as a rare cause of favus and tinea corporis in Cambodia and Vietnam. *Indian J Dermatol Venereol Leprol*. 2021;87(4):515–21.
7. Fonseca LAV, Araújo MAS, Silva DMW, Maranhão F. ITS-RFLP optimization for dermatophyte identification from clinical sources in Alagoas (Brazil) versus phenotypic methods. *J Infect Dev Ctries*. 2022;16(11):1773–7.
8. Ortiz B, Manuel G, Juan RT, Bush M, John SG, Fontecha G, et al. Global Insights and Trends in Research on Dermatophytes and Dermatophytosis: A Bibliometric Analysis. *Mycoses*. 13803;67(10):e13803. doi:10.1111/myc.13803.
9. Sacheli R, Hayette MP. Antifungal resistance in dermatophytes: Genetic considerations,clinical presentation & alternative therapies. *JFungi(Basel)*. 2021;7(11):983. doi:10.3390/jof7110983.
10. Arendrup MC, Kahlmeter G, Guinea J, Meletiadis J. How to: perform antifungal susceptibility testing of microconidia-forming dermatophytes following the new reference EUCAST method E.Def 11.0, exemplified by Trichophyton. *Clin Microbiol Infect*. 2021;27(1):55–60.
11. Farmazi S, Motamedi, Ali RM, Ansari S, Didehar M. A simple multiplex polymerase chain reaction assay for rapid identification of the common pathogenic dermatophytes: Trichophyton interdigitale, Trichophyton rubrum, and Epidermophyton floccosum. *Current Med Mycol*. 2021;7(2):1–7.
12. Pavlovic MD, Marzouk S, Bećiri L. Widespread dermatophytosis in a healthy adolescent the first report of multiderug resistant T.indotineae infection in UAE. *Acta Dermatovenerol Alp Pannonica Adriat*. 2024;33(1):53–5.
13. Brumster A, Hipler UC, Uhrlaß S, Nenoff P, Singal A, Verma SB, et al. Indian Trichophyton mentagrophytes squalene epoxidase ergl double mutants show high proportion of combined fluconazole and terbinafine resistance. *Mycoses*. 2020;63(11):1175–80.
14. Sharma S, Maheshwari M, Thakur R, Sah SP, Chauhan S. Antifungal susceptibility testing of five antifungal agents against clinically isolated dermatophyte species from a tertiary care hospital of northern india. *J Cin Diagn Res*. 2022;16(3):36–42.
15. Salehi Z, Shams-Gahfaroki M, Abyaneh MR. Molecular Epidemiology, Genetic Diversity, and Antifungal Susceptibility of Major Pathogenic Dermatophytes Isolated From Human Dermatophytosis. *Front Microbiol*. 2021;12:643509. doi:10.3389/fmicb.2021.643509.
16. Bortoluzi P, Prigitano A, Sechi A, Germiniasi F, Esposto MC, Romanò L, et al. Report of terbinafine resistant Trichophyton spp. in Italy: Clinical presentations, molecular identification, antifungal susceptibility testing and mutations in the squalene epoxidase gene. *Mycoses*. 2023;66(8):680–7.
17. Sabater AM, Normand AC, Bidaud AL, Cremer G, Foulet F, Brun S, et al. Terbinafine Resistance in Dermatophytes: A French Multicenter Prospective Study. *J Fungi (Basel)*. 2022;8(3):220. doi:10.3390/jof8030220.
18. Kumar P, Ramchandran S, Das S, Bhattacharya SN. Insights into Changing Dermatophyte Spectrum in India Through Analysis of

Cumulative 161,245 Cases Between 1939 and 2021. *Mycopathologia*. 2023;188(3):183–202.
19. Russo G, Trellu LT, Frontao L, Ninet B. Towards an Early Clinical and Biological Resistance Detection in Dermatophytosis: About 2 Cases of Trichophyton indotineae. *J Fungi (Basel)*. 2023;9(7):733. doi:10.3390/jof9070733.
20. Pavlovic MD, Marzouk S, Beciri L. Widespread dermatophytosis in a healthy adolescent: the first report of multidrug-resistant Trichophyton indotineae infection in the UAE. *Acta Dermatovenerol Alp Pannonica Adriat*. 2024;33(1):53–5.

Author's biography

Garima Sharma, PhD Scholar https://orcid.org/0000-0002-8251-4798

Daniel Alex Anand, Associate Professor https://orcid.org/0000-0002-5842-5365

Cite this article: Sharma G, Anand DA. Evaluation of susceptibility range & altered SQLE in co-relation to higher MIC of dermatophytes isolated from increasing altitudes of Uttarakhand. *IP Indian J Clin Exp Dermatol* 2025;11(1):26-32.