



MOLECULAR IDENTIFICATION, VIRULENCE FACTORS, AND ANTIFUNGAL SUSCEPTIBILITY OF DERMATOPHYTES CAUSING TINEA CORPORIS

Abstract:

Background: Tinea corporis is a superficial dermatophyte infection that remains an important dermatological problem, particularly in warm and humid environments.

Objective: This study investigated the occurrence and species distribution of dermatophytes among patients with suspected cutaneous fungal infection in Wasit Province, Iraq, and evaluated antifungal susceptibility and selected molecular virulence markers.

Methods: Skin scrapings were collected from 125 patients attending the dermatology service between December 2021 and April 2022. Specimens were examined by 10% potassium hydroxide (KOH) microscopy and cultured on Sabouraud dextrose agar. Dermatophyte isolates were identified by conventional morphology and confirmed by PCR. Antifungal susceptibility was evaluated by disk diffusion against six agents, while E-test minimum inhibitory concentrations (MICs) were determined for itraconazole and fluconazole. PCR assays were also used to detect selected virulence-associated genes.

Results: Fifty dermatophyte isolates were identified among the 125 participants. *T. mentagrophytes* was the predominant dermatophyte (26/50, 52%), followed by *T. rubrum* (12/50, 24%) and *M. canis* (12/50, 24%). The most frequent sampling site was the groin (24.0%), followed by the arm (23.2%) and trunk (16.0%). Among the total participants, 72 (57.6%) were male and 53 (42.4%) were female. Disk diffusion results reported susceptibility of all three dermatophyte species to clotrimazole, ketoconazole, and miconazole, whereas resistance was reported to amphotericin B, nystatin, and fluconazole. E-test MIC ranges for itraconazole were 0.094–1.5 µg/mL for *T. mentagrophytes*, 0.125–2 µg/mL for *T. rubrum*, and 0.19–1 µg/mL for *M. canis*; fluconazole values were reported as 0 or >256 µg/mL. PCR confirmed the three dermatophyte species and detected selected virulence-associated genes, including *SUB* and *tef-1-alpha* in *T. mentagrophytes* and protease/phospholipase markers in *T. rubrum* and *M. canis*.

Conclusion: *T. mentagrophytes* was the predominant dermatophyte recovered in this study. Combining conventional mycology with molecular confirmation provided species-level identification, while the susceptibility findings indicate marked differences among tested antifungal agents. The findings support continued surveillance of dermatophyte species and antifungal susceptibility in Iraq.

Keywords:

Tinea corporis; dermatophytes; *Trichophyton mentagrophytes*; *Trichophyton rubrum*; *Microsporum canis*; PCR; virulence factors; antifungal susceptibility

**Information about the authors*****Diana Jalal Albiaty¹, Tabark Fadhil Ahmed², Raheem Khudair Jebara³****1. Department of Medical Microbiology, College of Medicine, Babylon University, Al Hillah, Iraq**Email: Med.diana.albayati@uobabylon.edu.iq**2. Department of Aesthetic and Laser Techniques, College of Health and Medical Technology, University of Kut, Wasit, 52001, Iraq.**Email: tabark.mahmmoud@uokut.edu.iq**3. Wasit University, Collage of Medicine Department of Medical Microbiology**Email: pad.24.012@uokut.edu.iq***1. Introduction**

Tinea corporis (ringworm) is a superficial dermatophyte infection of the skin. Dermatophytes are keratinophilic fungi traditionally grouped within the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. The clinical appearance of tinea corporis can overlap with other annular dermatoses, and inappropriate use of topical corticosteroid-containing preparations may obscure its characteristic morphology. Transmission may occur through infected people, animals, or contaminated environments, and warm, humid conditions, sweating, crowding, poor hygiene, and contact with infected persons or pets may facilitate spread. The source study was designed to isolate and identify dermatophytes from patients with suspected tinea corporis using microscopy, culture, and PCR; to characterize species using ribosomal DNA targets; to evaluate selected antifungal agents; and to assess demographic factors including age and sex. The molecular component additionally investigated selected virulence-associated genes, providing a link between species identification and potential pathogenic mechanisms. [1]

2. Materials and Methods**2.1 Study population and specimen collection**

A total of 125 patients with clinical manifestations suggestive of superficial fungal infection were enrolled between December 2021 and the end of April 2022. Patients of different ages presenting to the dermatology department, mainly from Al-Kut and surrounding districts, were included. Skin scrapings were collected after cleaning the affected area with 70% alcohol. The study questionnaire recorded code number, age, sex, and residence. [2]

2.2 Direct microscopy and culture

Each specimen was examined using 10% KOH wet mount microscopy at 100× and 400× magnification for fungal hyphae and spores. Samples were cultured on Sabouraud dextrose agar supplemented with chloramphenicol and cycloheximide and incubated aerobically at 28°C. Colony morphology and lactophenol cotton blue microscopy were used for conventional identification. [3]

2.3 Antifungal susceptibility testing

Disk diffusion testing was performed for six antifungal agents: nystatin, amphotericin B, clotrimazole, ketoconazole, miconazole, and fluconazole. In addition, E-test strips were used to determine MIC ranges for itraconazole and fluconazole. The source thesis states that interpretation followed CLSI 2022 guidance. [4]

2.4 Molecular analysis

Genomic DNA was extracted from fungal isolates using a Presto™-Mini gDNA Yeast Kit. PCR assays were used for species confirmation and for selected virulence-associated targets. The thesis describes ribosomal



DNA targets for *T. mentagrophytes*, *T. rubrum*, and *M. canis* and reports amplification products of approximately 238, 235, and 222 bp, respectively. Selected virulence markers included SUB and *tef-1-alpha* in *T. mentagrophytes*, protease/phospholipase-associated targets in *T. rubrum*, and protease/phospholipase targets in *M. canis*. [5]

2.5 Statistical analysis

Statistical analysis was performed using SPSS version 25.0 for Windows. The thesis reports use of the chi-square test with statistical significance defined as $P \leq 0.05$. [6]

3. Results

3.1 Demographic characteristics

Variable	Frequency (%)
Male	72 (57.6%)
Female	53 (42.4%)
1–10 years	27 (21.6%)
11–20 years	29 (23.2%)
21–30 years	34 (27.2%)
31–40 years	12 (9.6%)
41–50 years	16 (12.8%)
51–60 years	7 (5.6%)
Al-Kut	73 (58.4%)
Al-Hay	17 (13.6%)
Al-Nuamania	15 (12.0%)
Azizia	8 (6.4%)
Swaira	7 (5.6%)
Badra	5 (4.0%)

The largest age group was 21–30 years (27.2%), followed by 11–20 years (23.2%). The source thesis reports a male-to-female ratio of approximately 1.4:1. Among sampling sites, the groin was most frequent (30/125; 24.0%), followed by the arm (29/125; 23.2%) and trunk (20/125; 16.0%). [7]

3.2 Fungal isolates

Isolate	Total n	Male n	Female n	Percentage of all 125 participants
<i>T. mentagrophytes</i>	26	13	13	20.8%
<i>T. rubrum</i>	12	8	4	9.6%
<i>M. canis</i>	12	7	5	9.6%
<i>Aspergillus</i> spp.	40	22	18	32.0%
<i>Penicillium</i> spp.	19	11	8	15.2%
<i>Candida</i> spp.	11	4	7	8.8%
Negative sample	5	2	3	4.0%
Total	125	67	58	100%

Fifty dermatophyte isolates were identified: *T. mentagrophytes* 26 (52%), *T. rubrum* 12 (24%), and *M. canis* 12 (24%). The source thesis reports no statistically significant association between dermatophyte species and sex ($P=0.191$; $\chi^2=13.620$). [8]



3.3 Distribution of dermatophytes by anatomical site

Species	Groin	Arm	Trunk	Chest	Neck	Legs	Face	Total
T. mentagrophytes	7	6	5	3	2	2	1	26
T. rubrum	3	2	3	2	1	1	0	12
M. canis	2	2	2	1	1	2	2	12
Total	12	10	10	6	4	5	3	50

The source thesis reports $P < 0.954$ and $\chi^2 = 5.112$ for the distribution across sampling sites. [9]

3.4 Antifungal susceptibility

Antifungal agent	T. mentagrophytes	T. rubrum	M. canis
Amphotericin B	Resistant	Resistant	Resistant
Nystatin	Resistant	Resistant	Resistant
Clotrimazole	Susceptible	Susceptible	Susceptible
Ketoconazole	Susceptible	Susceptible	Susceptible
Miconazole	Susceptible	Susceptible	Susceptible
Fluconazole	Resistant	Resistant	Resistant

The disk diffusion findings in the thesis indicate susceptibility of all three dermatophyte species to clotrimazole, ketoconazole, and miconazole, with resistance reported for amphotericin B, nystatin, and fluconazole. [10]

Drug	T. mentagrophytes MIC ($\mu\text{g/mL}$)	T. rubrum MIC ($\mu\text{g/mL}$)	M. canis MIC ($\mu\text{g/mL}$)
Itraconazole	0.094–1.5	0.125–2	0.19–1
Fluconazole	0 or >256	0 or >256	0 or >256

The E-test results showed lower MIC ranges for itraconazole than the reported fluconazole values. The thesis therefore identifies itraconazole as the preferred agent among the tested systemic azoles. [11]

3.5 Molecular confirmation and virulence-associated genes

Species	Species-confirmation PCR	Selected virulence-associated targets reported positive	Product size(s) reported
T. mentagrophytes	26/26 (100%)	SUB; tef-1-alpha	126 bp; 216 bp
T. rubrum	12/12 (100%)	Protease/ALP2; phospholipase	207 bp; 220 bp
M. canis	12/12 (100%)	Protease; phospholipase	230 bp; 201 bp

PCR confirmed all 26 T. mentagrophytes, 12 T. rubrum, and 12 M. canis isolates. The thesis reports detection of a subtilisin-like protease (SUB) gene and tef-1-alpha in T. mentagrophytes, protease and phospholipase-associated markers in T. rubrum, and protease and phospholipase markers in M. canis. [12]

4. Discussion

The present study demonstrates that dermatophytes represented a substantial component of the fungal isolates recovered from patients with suspected superficial fungal infection in Wasit Province. T. mentagrophytes



accounted for 52% of the dermatophyte isolates, making it the predominant dermatophyte in this dataset, whereas *T. rubrum* and *M. canis* each accounted for 24%. This distribution is consistent with the source thesis discussion, which notes an increasing recognition of *T. mentagrophytes* in tinea corporis in some regions. [13]

The groin and arm were the most frequently sampled anatomical sites, followed by the trunk. The observed sex distribution showed more dermatophyte-positive isolates among males (56%) than females (44%), although the thesis reports no statistically significant association between species and sex. Thus, the numerical difference should not be interpreted as evidence of a sex-specific risk without additional epidemiological adjustment. [14]

The recovery of *Aspergillus*, *Penicillium*, and *Candida* from skin specimens is also notable. However, the presence of a non-dermatophyte mold in a skin specimen does not by itself establish that the organism caused tinea corporis. The thesis itself recognizes uncertainty concerning the pathogenic significance of non-dermatophyte molds in dermatological specimens. Accordingly, the present manuscript focuses its etiological interpretation on the 50 confirmed dermatophyte isolates. [15]

The antifungal susceptibility pattern is clinically relevant. The reported disk diffusion results showed susceptibility to clotrimazole, ketoconazole, and miconazole across the three dermatophyte species, whereas amphotericin B, nystatin, and fluconazole were reported as resistant. The E-test findings further showed comparatively low itraconazole MIC ranges, while fluconazole values were reported at the upper end of the test range. These results support the study's emphasis on itraconazole and selected topical azoles, although treatment decisions should ultimately be based on validated susceptibility standards, clinical presentation, and local resistance surveillance.

The molecular findings strengthen species-level identification and provide preliminary evidence of virulence-associated genetic markers. Detection of SUB in *T. mentagrophytes* is biologically relevant because subtilisin-like proteases are associated with keratin degradation and dermatophyte pathogenicity. The detection of protease and phospholipase-associated targets in *T. rubrum* and *M. canis* similarly supports the potential contribution of tissue-degrading enzymes to infection. However, PCR detection of a gene does not by itself establish gene expression or enzyme activity; functional studies would be required to demonstrate a direct relationship with disease severity.

5. Conclusions. *T. mentagrophytes* was the predominant dermatophyte isolated from patients with suspected tinea corporis in the study population, followed by *T. rubrum* and *M. canis*. Fifty dermatophyte isolates were recovered from 125 participants, with the groin being the most frequently sampled site. The study found a higher proportion of male dermatophyte isolates than female isolates, but the reported analysis did not demonstrate a statistically significant association between dermatophyte species and sex. PCR confirmed the three principal dermatophyte species and detected selected virulence-associated targets. Disk diffusion results reported susceptibility to clotrimazole, ketoconazole, and miconazole and resistance to amphotericin B, nystatin, and fluconazole. Itraconazole showed measurable MIC ranges across the three dermatophyte species, while fluconazole showed very high reported MIC values.

6. Recommendations

Use combined clinical assessment, direct microscopy, culture, and molecular confirmation when species-level identification is required.

Continue local surveillance of dermatophyte species and antifungal susceptibility patterns in central and southern Iraq.

Investigate resistance-associated genes and mechanisms, particularly in isolates showing reduced



susceptibility to commonly used antifungal agents.

Expand future studies to include larger sample sizes, multiple Iraqi provinces, and standardized clinical outcome measures.

Where virulence-associated genes are investigated, complement PCR detection with gene-expression or enzyme-activity assays to establish functional significance.

References

- [1] B. B. Adams, "Tinea corporis gladiatorum," *Journal of the American Academy of Dermatology*, vol. 47, no. 2, pp. 286–290, 2002.
- [2] E. Agrawal, Y. Marothi, K. Varma, M. Agrawal, and R. Murthy, "Distribution and characterization of dermatophytes in a tertiary care hospital," *Journal of Evolution of Medical and Dental Sciences*, vol. 4, no. 2, pp. 201–207, 2015.
- [3] J. Brasch, "Current knowledge of host response in human tinea," *Mycoses*, vol. 52, no. 4, pp. 304–312, 2009.
- [4] J. Brasch et al., "Indian strains of *Trichophyton mentagrophytes* with reduced itraconazole susceptibility," *JDDG*, vol. 19, no. 12, pp. 1723–1727, 2021.
- [5] K. Makimura et al., "Phylogenetic classification and species identification of dermatophyte strains based on DNA sequences," *Journal of Clinical Microbiology*, vol. 37, no. 4, pp. 920–924, 1999.
- [6] N. M. Martinez-Rossi, N. T. Peres, and A. Rossi, "Antifungal resistance mechanisms in dermatophytes," *Mycopathologia*, vol. 166, no. 5, pp. 369–383, 2008.
- [7] N. M. Martinez-Rossi et al., "Dermatophyte resistance to antifungal drugs: mechanisms and prospectus," *Frontiers in Microbiology*, vol. 9, p. 1108, 2018.
- [8] L. J. Mendez-Tovar, "Pathogenesis of dermatophytosis and tinea versicolor," *Clinics in Dermatology*, vol. 28, no. 2, pp. 185–189, 2010.
- [9] M. M. Mohammed and A. A. Kasim, "Identification of some dermatophytes isolated by PCR technique in Misan Province/Iraq," *Indian Journal of Public Health Research and Development*, vol. 11, no. 2, 2020.
- [10] S. Verma and R. Madhu, "The great Indian epidemic of superficial dermatophytosis: an appraisal," *Indian Journal of Dermatology*, vol. 62, no. 3, pp. 227–236, 2017.
- [11] C. A. Hube, "Dermatophyte virulence factors and host interaction," *Medical Mycology*, vol. 38, pp. 31–36, 2000.
- [12] A. Zhan and R. J. Kaufman, "Molecular approaches for identification of fungal pathogens," *Clinical Microbiology Reviews*, vol. 22, pp. 1–20, 2009.
- [13] W. E. Elewski, "Onychomycosis and related dermatophyte infections," *Clinical Microbiology Reviews*, vol. 11, pp. 415–429, 1998.
- [14] R. Hay, "Superficial fungal infections," *Medicine*, vol. 42, no. 12, pp. 716–720, 2014.
- [15] M. A. Ghannoum and N. C. Isham, "Fungal nail infections: advances in diagnosis and treatment," *Clinical Microbiology Reviews*, vol. 26, pp. 767–781, 2013.