

Molecular detection of subtilisin and fungalsin genes and antifungal susceptibility of *Microsporum canis* from human and animal dermatophytosis

Nada Salih Hadi ^{1*}, Hanadi Abdulqader Jasim ¹, Doooha Khalil Alhamdi ²

¹ Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq

² Department of Medicine, College of Medicine, University of Basrah, Basrah, Iraq

Article info

Received: 12 March 2026

Received in revised form: 06 May 2026

Accepted: 07 May 2026

Published online: 12 May 2026

Keywords

Dermatophytosis
Antifungal susceptibility
Virulence genes
Phylogenetic analysis
Microsporum canis

* Corresponding author:

Nada Salih Hadi

Email: nada.saleh@uobasrah.edu.iq

Reviewed by:

Sonu S. Nair

Indian Veterinary Research Institute, Izatnagar,
Bareilly, UP, India

Abstract

Microsporum canis is a zoonotic dermatophyte responsible for dermatophytosis in both animals and humans, whereas *Arthroderma otae* represents its teleomorphic state. Considering the increasing incidence of dermatophytosis, emerging antifungal resistance and limited availability of related reliable data in Iraq, this study aimed to evaluate the antifungal susceptibility patterns of *Microsporum canis* and to molecularly characterize selected virulence-associated genes and to assess the genetic relatedness and evolutionary relationships among the isolates. A total of 250 clinical samples were collected from dermatophyte lesions and screened, from which 57 molecularly confirmed isolates (40 *M. canis* and 17 *A. otae*) were obtained. The presence of Subtilisin (SUB1-3) and Fungalsin (MEP1-5) genes was assessed using both previously published and newly designed specific primers. Additionally, antifungal susceptibility testing of isolates using the broth microdilution method was performed against commonly used antifungal agents. The results revealed that, except for MEP2 and MEP5, all isolates of *M. canis* and *A. otae* carried SUB2, SUB3, MEP1, and MEP3, with no significant difference between human and animal isolates ($p \geq 0.05$). However, SUB1 and MEP4 showed variable distribution. Antifungal susceptibility analysis revealed significant differences among tested agents ($p = 0.006$). Terbinafine exhibited the highest antifungal activity, whereas fluconazole exhibited the lowest, suggesting reduced susceptibility. These results demonstrate the widespread distribution of key protease enzyme-coding genes in dermatophyte isolates and their variable antifungal profiles in humans and animals. Although the present findings support the integration of molecular characterization with antifungal susceptibility testing, the presence of these genes alone does not confirm their functional role in pathogenicity; therefore, further functional and epidemiological investigations are required to clarify their significance.

This is an open access article under the CC Attribution license (<http://creativecommons.org/licenses/by/4.0/>)

1. Introduction

Previously, seven genera of dermatophytes were recognized. As with other fungal families, species nomenclature has undergone continuous changes as the field of mycology has switched from morphology- and clinically based classification to molecular approaches. However, with advancements in phylogenetic analysis, a total of nine genera have been identified: *Trichophyton*, *Epidermophyton*, *Nannizzia*, *Paraphyton*, *Lopophyton*, *Microsporum*, *Arthroderma*, *Ctenomyces*, and *Guaromyces* (de Hoog et al. 2017). Dermatophytes not only cause different clinical forms of dermatophytosis in animals but can also be transferred to humans (Zafar et al. 2023). In particular, *Microsporum canis* is a zoophilic dermatophyte of medical importance, primarily transmitted from domestic animals such as cats and dogs to humans. Although it is not the most predominant dermatophyte, it remains the main etiological agent of zoonotic dermatophytosis (Vite-Garín et al. 2024). The prevalence of *M. canis* varies geographically, with higher rates reported in regions with close human-animal contact (Seyedmousavi et al. 2018; Moriello 2014). Clinically, infection is associated with a range of manifestations. Signs can range from mild scaling and alopecia to severe inflammation with pustules and invasion of the dermis via the hair follicles, developing into Majocchi's granuloma (Moskaluk and VandeWoude 2022). While the clinical expression of dermatophytosis depends on both the characteristics of the host and the characteristics of the causative fungi (Dubljanin et al. 2024), the severity of infection largely depends on the release of particular extracellular proteolytic

enzymes that promote the penetration of dermatophytes into the host keratinized tissue (Dubljanin et al. 2024; Burchacka et al. 2022) and the enzymes secreted depend on the type of lesions and dermatophyte species involved (Cafarchia et al. 2012). These enzymes, including endoproteases and exoproteases, cause keratin hydrolysis to facilitate fungal invasion, colonization, and initiation of disease (Martinez 2023). While endoproteases disrupt internal bonds of polypeptides, exoproteases can only target the polypeptide bonds at the N- or C-terminus. The production of these proteases has been associated with increased disease severity (Moskaluk and VandeWoude 2022). Among these enzymes, subtilisins (SUB) and fungalsin (MEP) are the vital factors in dermatophytes associated with their virulence. Subtilisins are serine proteases and fungalsins are metalloproteases, both of which have been implicated in dermatophyte virulence in previous *in-vivo* experiments (Brouta et al. 2002; Naeimipour et al. 2021). Their expression during infection is vital for the growth, pathogenicity, and virulence of dermatophytes; and they are of special interest in the development of targets for possible diagnostic and therapeutic applications (Burchacka et al. 2022). Apart from the role of virulence factors in disease severity, the emergence of antifungal-resistance in clinical dermatophyte isolates leads to failure of effective treatment with recalcitrant infections. Therefore, *in vitro* antifungal susceptibility testing is required for effective management with favourable clinical outcomes (Katirae et al. 2021).

Despite the biological and clinical importance of the virulence-associated genes discussed above, molecular data regarding their

distribution in dermatophytes remains limited. Though previous studies in Iraq have reported on virulence-associated genes and antifungal susceptibility of human-derived *M. canis* isolates, corresponding data for animal-derived isolates are lacking. Owing to the zoonotic nature of *M. canis*, this study extends the investigation to animal cases to provide a wider epidemiological view. Therefore, the present study was conducted in Basrah, Iraq to investigate the distribution of subtilisin (SUB1–3) and fungalsin (MEP1–5) genes in *M. canis* and its teleomorph *A. otae*, from both human and animal dermatophytosis cases, in addition to evaluating antifungal susceptibility patterns.

2. Materials and Methods

2.1. Source of strains and their identification

A total of 250 clinical samples, including skin scrapings and plucked hair were collected from human and animal sources, yielding 57 molecularly confirmed isolates, comprising 40 *M. canis* and 17 *A. otae*. Initial identification of isolates was based on morphological characteristics and was subsequently confirmed at the molecular level by sequencing of the internal transcribed spacer ITS region. Human samples were obtained from hair and skin lesions of patients admitted to the outpatient clinic of Basrah Teaching Hospital, AlSadr Teaching Hospital, and other private clinics. Whereas, animal samples were collected from hair and skin lesions of animals of different ages, sex, and breeds from veterinary hospitals and clinics located across different regions of Basrah, Iraq. Suspected lesions were disinfected with 70 % ethyl alcohol to eliminate surface contaminants. Skin scales were scraped near the edges of the skin lesions using a blunt scalpel. Damaged hair roots were collected using sterile forceps. The specimens were placed on a grease-free glass slide, treated with 2-3 drops of a mixture of 10% Potassium hydroxide (KOH) with 40% Dimethyl sulphoxide (DMSO), covered with a cover slip, and examined after being kept for 5-8 minutes (Mohamed et al. 2016). All specimens, irrespective of KOH mount findings, were cultured on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM) supplemented with Chloramphenicol and Cycloheximide. After 1 to 2 weeks of incubation at 25±2 °C, microscopic examination was performed using Lactophenol Cotton Blue (LCB) staining. Species identification was confirmed by sequencing the amplified DNA fragments at the National Instrumentation Center for Environmental Management (NICEM) (http://nicem.snu.ac.kr/main/?en_skin=index.html). Sequence alignment and analysis were conducted using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>)

2.2. Primer design and PCR amplification reaction

Primers targeting subtilisin (SUB1) and fungalsin (MEP2, MEP4 and MEP5) genes were adopted from a previously published study (Lemsaddek et al. 2010). While primers targeting subtilisin (SUB2 and SUB3) and fungalsin (MEP1 and MEP3) genes were designed in this study with sequence data retrieved from the National Center for Biotechnology Information (NCBI) database. OLIGO primer analysis software was used to create primers from conserved regions of homologous genes in dermatophytes. To determine the efficiency and specificity of each designed primer, Primer-BLAST, available at <http://www.ncbi.nlm.nih.gov>, was used. The characteristics of each primer used in the present study are summarized in (Table 1). DNA was

Table 1. Primers used in the polymerase chain reaction (PCR) technique for the detection of SUB and MEP genes in *M. canis* and *A. otae*

Gene	Primers (5' → 3')	Amplicon size (bp)
MEP1	F- ATGCACGGCCTCTTGCTTG R- ACAGCTCGGCCAGCGAGGCT	153
MEP2	F- CTTGGGCGTTGCTGGTCAC R- TGTCGAAGTTGTTGGTGTAGCC	1900
MEP3	F- CTCCACCGCCACCAAGCTTG R- ATCGATGTCAAGACCGTGAG	133
MEP4	F- TGATACCAGCGCCTACTTC R- TTAAGGATGGTAAAGTCATTGC	1200
MEP5	F- CGAGCTGTTGATCTGAATG R- TTGAGGCTGGTGAATAG	1650
SUB1	F- TATCTCTCTTGGGCTGTGTCC R- GTTCCAGAATCTGCCGAAGGTC	1540
SUB2	F- CAGCCTGAGCCACTGCGTG R- ACGGTCTCGACTCCGCTGGCG	173
SUB3	F- CGACTATGTTGAGCATGAC R- GTTGATATCAATACCAGTG	181

extracted from suspected dermatophyte isolates according to the protocol of the Wizard Genomic DNA purification kit (Promega/USA). The quality and concentration of extracted DNA were evaluated using a NanoDrop spectrophotometer (Thermo Scientific, USA). PCR amplifications were carried out using a Thermal cycler (Bio Rad, USA), in a final reaction volume of 25 µL containing 13 µL of Taq master mix (Promega /USA), 1 µL of each primer, and 3 µL of extracted DNA. PCR cycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 1 min, and extension at 72 °C for 45 s, with a final extension step at 72 °C for 10 min. The PCR products were resolved by agarose gel electrophoresis (1% w/v) at 90 V for 1 h and visualized under UV light after staining with ethidium bromide.

2.3. Phylogenetic analysis

In order to assess the degree of genetic relatedness among the isolates, multiple sequence alignments of the ITS region were performed using the MEGA (version 11) software program. Phylogenetic tree analysis was conducted using the Neighbor-Joining method. The robustness of the tree was evaluated by bootstrap analysis with 1000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Bootstrap values greater than 50% are shown next to the tree branches. Evolutionary distances were computed using the Kimura 2-parameter model and expressed as the number of base substitutions per site. This analysis included a total of 25 nucleotide sequences. All positions were considered (noncoding region) and ambiguous positions were removed using the pairwise deletion option. *Microsporium audouinii* was used as an outgroup to root the tree.

2.4. Antifungal susceptibility

Antifungal susceptibility testing was performed using the modified broth microdilution method based on the Clinical and Laboratory Standards Institute (CLSI) M38 guidelines for filamentous fungi (CLSI 2017). Sterile, flat-bottomed disposable 96-well micro-dilution plates were used for all experiments. Five rows of wells were assigned for testing five antimicrobial agents against each isolate, including four antifungal drugs (terbinafine, itraconazole, fluconazole, and griseofulvin), supplied as powders (sigma-Aldrich, USA), and one antibacterial drug, minocycline (locally manufactured). This study included minocycline as a non-conventional agent to assess its potential

adjunct effect, as combined topical formulations containing minocycline and antifungal agents are increasingly used in current clinical practice to improve therapeutic effects. Stock solutions of antimicrobial agents were prepared at a concentration of 4 mg/mL (4000 µg/mL). Working solutions were subsequently obtained by appropriate dilution in Roswell Park Memorial Institute (RPMI 1640) medium to achieve a starting concentration of 4 µg/mL. Two-fold serial dilutions were prepared to obtain final concentrations ranging from 4 to 0.0019 µg/mL across the wells. Initially, 198 µL of sterile RPMI 1640 broth was dispensed into the first well of each row, while 100 µL was added to the remaining wells. Two microliters of each antimicrobial stock solution were added to the first well, followed by two-fold serial dilutions achieved by transferring 100 µL sequentially from the first well to subsequent wells up to the twelfth well. After mixing, 100 µL was discarded from the final well to maintain equal volumes. An equal volume (100 µL) of fungal inoculum, previously prepared and standardized spectrophotometrically to achieve a turbidity equivalent to approximately 0.5 McFarland standard, then further diluted in RPMI broth to obtain a final inoculum concentration of 1×10^3 to 3×10^3 CFU/mL, was added to all wells. For each plate, two drug-free controls were included: a sterility control containing 200 µL RPMI broth alone and a growth control containing 200 µL RPMI broth with fungal inoculum. The plates were sealed and incubated at 25 °C. Optical density (OD) was measured using a microplate spectrophotometer at a wavelength of 630 nm after 4 days of incubation. Flat-bottom 96-well plates were used to allow uniform optical density measurement across wells, ensuring consistent spectrophotometric reading of fungal growth. Spectrophotometric reading was used as an alternative approach to provide a quantitative assessment of fungal growth inhibition and to improve objectivity in MIC determination. Minimum inhibitory concentrations at 50% and 90% (MIC₅₀ and MIC₉₀) were defined as the points at which there was 50% and 90% inhibition of growth, respectively, compared with the growth control.

2.5. Statistical Analysis

Data obtained in the present study were analyzed statistically using SPSS software (version 26). To compare variables between the groups, Chi-square test and Fisher's exact test were used. Antifungal susceptibility data were analyzed using the Kruskal-Wallis test to compare differences among the tested agents and post hoc comparisons were performed using Dunn's test. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

The pathogenesis of *M. canis* is determined by the presence of virulence factors and host susceptibility, which jointly contribute to their pathophysiological mechanisms linked to fungal survival on host tissues and the appearance of skin lesions (Aneke et al. 2021). The clinical presentation of dermatophyte infection in human samples in the present study appeared as a scaly annular lesion with inflammation of the skin, as well as circular scaly alopecic patches on the scalp (Fig. 1). Similar patterns were observed in animal samples, indicating comparable pathogenic behavior across different hosts (Fig. 2). Based on the macroscopic appearance using dermatophytes test medium, the growing colonies were white in color and produced alkaline metabolites which caused a color change of the medium from yellow to red. Whereas, on Sabouraud dextrose agar, colonies exhibited rapid growth and were characterized by a cottony and fluffy hairy with



Fig. 1. Skin lesions in patients with dermatophytes:

- (A) Erythematous scaly annular plaque with an active advancing border at the gluteal area
- (B) Erythematous scaly annular plaque with an active border on the face
- (C) Multiple erythematous scaly annular macules and patches on the trunk and submammary area
- (D) Single patch of hair loss with erythema and overlying gray scales

abundant aerial mycelium. With aging, the center of the colony became buff colored, while the reverse side showed a yellow to orange pigmentation. The stained smears with lactophenol cotton blue (LCB) revealed fungal elements with characteristic fusiform, multi-celled rough-walled macroconidia bearing a terminal knob. Additionally, pyriform to clavate microconidia were occasionally observed (Fig. 3). These findings concurred with those reported by (Midgley et al. 1997) who described similar morphological features for dermatophyte identification. Molecular identification based on the BLAST result of sequenced samples in the present study revealed that 40 isolates (35.09%) were identical to reference sequence of *M. canis*, while 17



Fig. 2. Dermatophyte infection in animals:

- Fungal infection of a goat: on the lower leg (A), on the face (B)
- Ringworm in sheep: scaly alopecia with grayish wart-like crusty lesions covering most of the ear pinna (C), on the head (D)
- Ringworm in a cat: circular patches of hair loss with crusts or dandruff on the fur and skin (E)
- Calf ringworm lesions: circumscribed, grayish-white, thick, crusty, and raised lesions on the neck region (F)

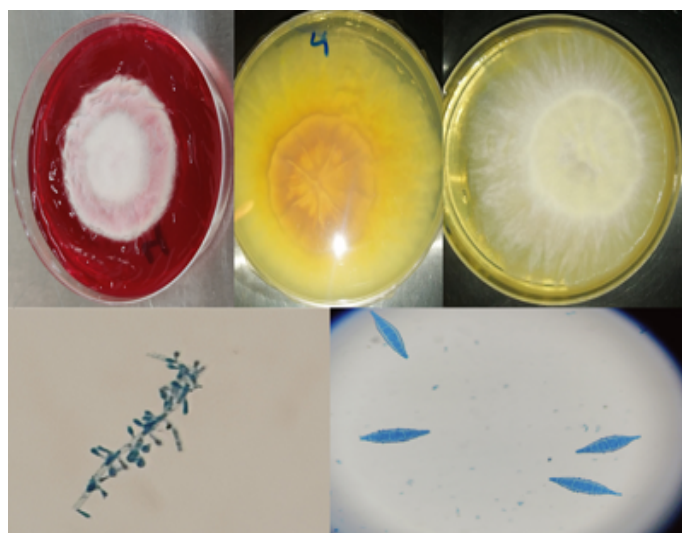


Fig. 3. Macroscopic and microscopic appearances of colonies

isolates (14.91%) matched *A. otae*. However, while ITS sequencing is widely accepted for accurate species-level identification, it has limited discriminatory power in identifying strain level variation among *M. canis* isolates.

According to the results of PCR amplifications in the present study, the SUB2 and SUB3 genes were successfully amplified, yielding amplicons of 173 bp and 181 bp, respectively, (Fig. 4). Similarly, amplification of the MEP1 and MEP3 genes produced fragments of 153bp and 133bp, respectively (Fig. 5). Whereas the SUB1 and MEP4 genes generated larger amplicons of 1540bp and 1200bp, respectively (Fig. 6). The successful amplification of target genes confirms the specificity and reliability of primers used for molecular detection in this study. Highly significant differences were observed between the distribution of the two form across different hosts (Table 2). However, all clinical isolates of *M. canis* and its teleomorph *A. otae* carried SUB-related genes (SUB1-3) and MEP genes (MEP1, MEP3, MEP4), with no statistically significant differences observed between the two forms ($p \geq 0.05$) (Table 3). All animal and human isolates investigated in the current study demonstrated, 100% positivity for SUB2, SUB3, MEP1, and MEP3. While SUB1 and MEP4 were detected in 37.5% and 62.5% of

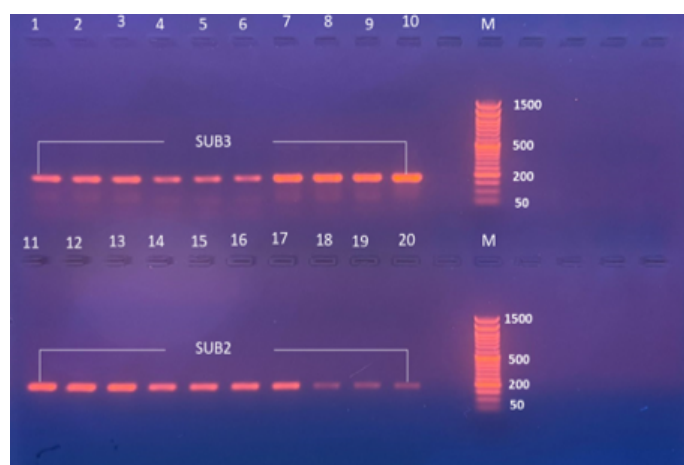


Fig. 4. Agarose gel electrophoresis showing amplification of SUB3 (181 bp) and SUB2 (173 bp). Lanes 1–10 represent positive amplification of SUB3, while lanes 11–20 correspond to SUB2 amplification. M: DNA molecular weight marker (50–1500 bp)

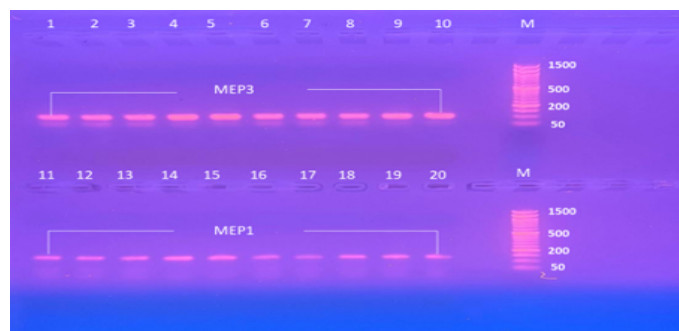


Fig. 5. Agarose gel electrophoresis showing amplification of MEP3 (133 bp) and MEP1 (153 bp). Lanes 1–10 represent positive amplification of MEP1, while lanes 11–20 correspond to MEP3 amplification. M: DNA molecular weight marker (50–1500 bp)

M. canis isolates, and 35.29% and 58.82% of *A. otae* isolates, respectively. In contrast, neither MEP2 nor MEP5 was detected in any of the *M. canis* or *A. otae* isolates. The evaluation of the prevalence of virulence-associated genes among human ($n = 25$) and animal ($n = 32$) isolates revealed that SUB2, SUB3, MEP1, and MEP3 genes were detected in 100% of isolates in both groups, suggesting complete conservation across all samples. The SUB1 gene was detected in 24.0% of human isolates and 46.9% of animal isolates. Statistical analysis using Fisher's exact test showed no significant difference between groups ($p = 0.08$).

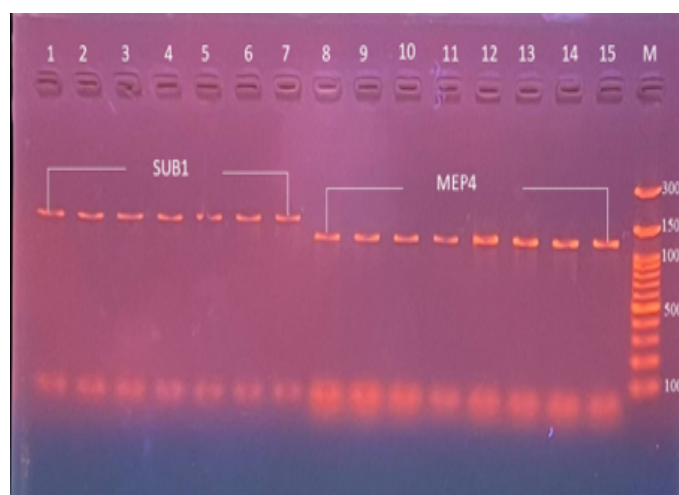


Fig. 6. Agarose gel electrophoresis showing amplification of SUB1 (1540 bp) and MEP4 (1200 bp). Lanes 1–7 represent positive amplification of SUB1, while lanes 8–15 correspond to MEP4 amplification. M: DNA molecular weight marker (100–3000 bp)

The odds ratio ($OR = 0.36$; 95% CI: 0.11–1.15) suggests a lower likelihood of SUB1 occurrence in human isolates compared to animal isolates. Similarly, MEP4 gene was identified in 68.0% of human isolates and 56.3% of animal isolates, with statistically no significant difference between two groups ($p = 0.41$), and an odds ratio ($OR = 1.65$; 95% CI: 0.55–4.91), indicating comparable gene distribution between host groups (Table 4). The universal presence of SUB2, SUB3, MEP1, and MEP3 genes in the isolates suggests their potential involvement in fungal infection and could be associated with highly conserved nature irrespective of host origin. The comparable distribution patterns of virulence-associated genes between *M. canis* and *A. otae* isolates, suggest a high degree of genetic and functional similarity between the two forms and the absence of statistically significant differences ($p = 0.999$) supports the concept that *A. otae* represents the teleomorphic

Table 2. The percentage prevalence of *M. canis* and *A. otae* in the present study

Isolates	<i>M. canis</i>		<i>A. otae</i>		Total	
	No.	(%)	No.	(%)	No.	(%)
Human	20	50.0	5	29.4	25	43.9
Cats	6	15.0	8	47.1	14	24.6
Dogs	0	0.0	4	23.5	4	7.0
Sheep	8	20.0	0	0.0	8	14.0
Cattles	6	15.0	0	0.0	6	10.5
Total	40	35.1	17	14.9	57	50.0

CalX² = 86.4, TabX² = 15.51, DF = 8, p-value < 0.01
 CalX²: Calculated Chi-square; TabX²: Tabulated Chi-square; DF: Degrees of Freedom

(perfect) state of *M. canis*, despite the loss of sexual reproduction in most strains and these findings are compatible with a previous study (Makimura et al. 2001). Furthermore de Hoog et al. (2017) reported that *M. canis* was previously used to only refer to the anamorphic form, while the teleomorph was called *Arthroderma otae* or *Nannizzia otae*. Currently, *M. canis* refers to both forms. However, the comparable detection of protease-encoding genes in isolates from both human and animal sources may indicate the presence of a conserved pathogenic strategy that may facilitate zoonotic transmission, persistent infection, and cross-species transmission. While the observed variability in the distribution of SUB1 and MEP4 genes may be attributed to adaptive mechanisms during the infection.

Table 3. Number and percentage (%) of *M. canis* and *A. otae* isolates positive for subtilisin (SUB) and fungalyisin (MEP) genes

Genes	<i>M. canis</i> (n = 40)		<i>A. otae</i> (n = 17)	
	No.	(%)	No.	(%)
SUB1	15	37.50	6	35.29
SUB2	40	100.0	17	100.0
SUB3	40	100.0	17	100.0
MEP1	40	100.0	17	100.0
MEP3	40	100.0	17	100.0
MEP4	25	62.50	10	58.82

CalX² = 0.250; TabX² = 11.07; DF = 5; p-value = 0.999

The results of the current study revealed that a high prevalence of the SUB2, SUB3, MEP1, and MEP3 genes, suggesting that the proteins encoded by these genes may contribute to the infection process and are necessary for adherence to keratinized tissues and establishment of infection. These findings are consistent with previous studies who reported that Subtilisin 3 (SUB3), produced by *M. canis*, may plays a critical role during the early stages of infection and serves as an indicator of metabolically active fungal cells (Baldo et al. 2010; Grumbt et al. 2013). However, functional expression studies are required to confirm the biological role of these genes in pathogenicity. The absence of SUB1 and MEP4 genes in some isolates in the present study suggests potential alternative gene repertoires involved in the degradation of keratin and is in agreement with a previous study (Kaplan et al. 2020). Furthermore, the level of gene expression and proteolytic activity of SUB genes and their coded proteases may be associated more closely with the severity of the infection rather than the mere ability to cause infection (Basiri et al. 2022). However, Robati et al. (2018) reported that

the absence of SUB1 genes in *M. canis* isolates from severe infections indicates that this gene is not essential for dermatophyte invasion but the proteases encoded by SUB genes are thought to play a potential role during the initial stage of contact with the host, only facilitating the adherence of fungi to host cells. However, there is a possibility that gene family sequences are subjected to genetic or regulatory changes during infection development, enabling the fungus to express and secrete other enzymes that sustain fungal pathogenesis despite the absence of specific genes such as SUB and MEP.

Table 4. Gene prevalence according to host source

Gene	Human (No.)	Human (%)	Animal (No.)	Animal (%)	Odds Ratio	95% CI	Fisher p-value
SUB1	6	24.0	15	46.9	0.36	0.11-1.15	0.08
SUB2	25	100	32	100	-	-	-
SUB3	25	100	32	100	-	-	-
MEP1	25	100	32	100	-	-	-
MEP3	25	100	32	100	-	-	-
MEP4	17	68.0	18	56.3	1.65	0.55-4.91	0.41

The absence of MEP2 and MEP5 genes in all isolates examined in the present study suggested that not all keratin-induced proteases are actively involved in the infection process. These results are consistent with Datt et al. (2021) and Burmester et al. (2011), who demonstrated that only a subset of keratin-induced proteases is strongly expressed during fungal-keratinocyte interactions. However, variations in the distribution of virulence-associated genes among isolates may be influenced by several factors such as host species, environmental conditions, and genetic variability among fungal strains. Furthermore, to confirm the genetic relationship between the two studied forms and reference strains, a phylogenetic tree was constructed using the neighbor-joining method implemented in the MEGA software (version 11). Nine local Iraqi isolates were analyzed and compared with reference sequences available in the Genbank database using the Basic Local Alignment Search Tool (BLASTN). Sequencing analysis revealed that six isolates were identified as *M. canis*, and three isolates as *A. otae*. The six *M. canis* isolates were distributed across several sub-clades but remained closely related to global reference strains. They showed high sequence similarity to a reference Iraqi and Iranian strain with accession number OR230140.1 and JN134109.1, respectively. Five isolates displayed 100% nucleotide identity and one isolate revealed 98.6% nucleotide identity (Table 5). The Iraqi-related strain OR230140.1 clustered with Indian and Thai strains, supported by high bootstrap values (94–99), suggesting a strong evolutionary relationship and potential regional dissemination or shared ancestry (Fig. 7). These results are in agreement with a recent molecular study (Nair et al. 2025) which reported that *M. canis* isolates from human and animal origins across different geographic regions tend to cluster closely, reflecting limited genetic divergence and supporting zoonotic transmission. Similarly, two *A. otae* isolates showed 100% nucleotide identity with an Iranian reference strain with accession number KP789402.1 and an Estonian reference strain with accession number KC833523.1, while one isolate showed 100% identity with a Belgian and a Chinese reference strains with accession numbers KP132445.1 and KT003284.1, respectively, (Table 6). Furthermore, multiple sequence alignment of ITS region sequences showed a high degree of sequence conservation among *M. canis* and *A. otae* isolates and their corresponding reference strains, regardless of their geographic source. The identity profiles

Table 5. Percentage identity of ITS region sequences between local *M. canis* isolates and the reference strain retrieved from GenBank

GeneBank accession No.	Sequence identity (%) with local isolates						Reference country
	Isolate 10	Isolate 35	Isolate 37	Isolate 38	Isolate 68	Isolate 69	
OR230140.1	100	100	98.60	100	100	100	Iraq
JN134109.1	100	100	98.60	100	100	100	Iran
PP446672.1	99.86	100	98.60	99.86	100	99.85	Iran
OW984899.1	99.86	99.84	98.60	99.86	99.85	99.85	Belgium
PV350118.1	99.85	100	98.60	99.85	99.85	99.85	China
MW759863.1	99.40	99.51	98.0	99.40	99.52	99.37	Poland
MT921620.1	97.02	97.22	95.39	97.02	97.30	97.17	India
KT155712.1	96.36	96.73	94.99	96.36	96.62	96.36	Netherlands
MW358008.1	95.68	95.76	94.00	95.68	96.05	95.95	Thailand
LC535028.1	95.12	94.95	92.81	95.12	95.30	95.23	India
OR230140.1	100	100	98.60	100	100	100	Iraq

displayed only a limited number of polymorphic sites, which indicated a minimal nucleotide variation, as illustrated in Fig. 8.

This close phylogenetic relationship may provide a reasonable explanation for the comparable distribution patterns of SUB and MEP virulence-associated genes detected in both forms. These findings are consistent with a previous study using mitochondrial DNA markers (Kawasaki et al. 1995), which revealed close clustering among *Microsporium* and *Arthroderma*. Also, molecular and conventional taxonomic studies of the *M. canis* complex (Graüser et al. 2000) and *A. otae* complex (Hasan 2018) indicated that, despite minor morphological differences, there is a high degree of genetic similarity among isolates. However, minor clustering differences observed among some isolates of the present study may reflect strain-level diversity or geographic adaptation rather than species-level differentiation.

Table 6. Percentage identity of ITS region sequences between local *A. otae* isolates and the reference strain retrieved from GenBank

GeneBank accession No.	Sequence identity (%) with local isolates			Reference country
	Isolate 10	Isolate 35	Isolate 37	
KP789402.1	100	100	99.85	Iran
KP132445.1	99.86	100	99.85	Belgium
KC833523.1	99.72	100	100	Estonia
KT003284.1	99.26	100	99.38	China
JQ922442.1	98.65	98.74	98.51	Tunisia
AJ252339.1	98.30	98.93	98.68	Germany

Antifungal susceptibility testing was conducted on *M. canis* isolates, as this species represented the predominant dermatophyte identified in the present study. Focusing on the most prevalent species enables a more consistent and representative evaluation of antifungal activity within the studied population. Although, *A. otae* isolates (n = 17) were also identified and confirmed molecularly, they were included descriptively to reflect the diversity of dermatophytes in the study setting and were not subjected to antifungal susceptibility testing. Furthermore, *A. otae* represents the teleomorphic state of the same species complex, further supports this approach. Furthermore, molecular analysis of virulence-associated genes revealed a higher frequency of genes in *M. canis* isolates compared to *A. otae*, may indicate a potentially pathogenic association and justifying prioritization of *M. canis* in antifungal susceptibility evaluation. Results of microdilution plates were read spectrophotometrically and revealed

variable growth inhibition among the tested agents. Based on optical density measurements at 630 nm, the results demonstrated a statistically significant difference in the antifungal efficacy among the tested agents against *M. canis* isolates, as indicated by the Kruskal-Wallis (H = 14.24, p = 0.0066). Among the tested antifungal agents, terbinafine exhibited the highest antifungal activity, as evidenced by the lowest mean MIC value (0.275 µg/mL), along with low MIC₅₀ and MIC₉₀ values (Table 7). This high efficacy can be attributed to its mechanism of action, which involves inhibition of the enzyme squalene epoxidase, leading to toxic accumulation of squalene and disruption of ergosterol biosynthesis in fungal cells. These findings are in agreement with previous studies that have consistently reported terbinafine as one of the most effective antimicrobial agents against dermatophytes (Appelt et al. 2021), and it was further reported that, although the resistance of dermatophyte species towards terbinafine is growing, it remains the preferred treatment choice due to its consistent clinical efficacy and lower recurrence rates (Mahmood et al. 2024).

In contrast, fluconazole demonstrated the highest mean MIC value (0.625 µg/mL) with a broader MIC range, indicating the lowest antifungal activity and suggesting reduced susceptibility among the isolates. This reduced efficiency may be explained by the emergence of resistance mechanisms such as increasing drug efflux and modifications in target enzymes. Dunn post-hoc analysis (Table 8) supported these findings, displaying significant differences between fluconazole and other agents. Increasing resistance to fluconazole among dermatophyte species have been reported earlier as well (Channoum 2016). Itraconazole, griseofulvin, and minocycline, revealed moderate to high antifungal activity in this study, with no significant differences observed in most pairwise comparisons. This suggests a relatively comparable activity among these drugs. Itraconazole showed identical

Table 7. MIC values of antifungal agents against *M. canis*

Antifungal agent	Mean ± SD (µg/mL)	MIC ₅₀	MIC ₉₀	MIC range (µg/mL)
Terbinafine	0.275 ± 0.137	0.25	0.5	0.125–0.5
Itraconazole	0.375 ± 0.176	0.5	0.5	0.125–0.5
Fluconazole	0.625 ± 0.353	0.5	1	0.125–1
Griseofulvin	0.362 ± 0.176	0.25	0.5	0.0625–0.5
Minocycline	0.312 ± 0.176	0.25	0.5	0.0625–0.5

MIC: Minimum inhibitory concentration; SD: Standard deviation

Table 8. Dunn post-hoc comparison of antifungal agents against <i>M. canis</i>		
Comparison	p-value	Significance
Terbinafine vs Itraconazole	0.42	Not significant
Terbinafine vs Fluconazole	0.004	Significant
Terbinafine vs Griseofulvin	0.08	Not significant
Terbinafine vs Minocycline	0.11	Not significant
Itraconazole vs Fluconazole	0.01	Significant
Itraconazole vs Griseofulvin	0.36	Not significant
Itraconazole vs Minocycline	0.29	Not significant
Fluconazole vs Griseofulvin	0.03	Significant
Fluconazole vs Minocycline	0.02	Significant
Griseofulvin vs Minocycline	0.75	Not significant

MIC₅₀ and MIC₉₀ values (0.5 µg/mL), indicating a narrow MIC distribution and uniform susceptibility pattern among isolates. Interestingly, minocycline, although primarily classified as an antibacterial drug, showed an inhibitory effect, which was not significantly different from griseofulvin but significantly higher than fluconazole. This observation suggests its potential as an adjunct or alternative therapeutic option. However, further studies are required to clarify its exact mode of action. However, several studies have reported that, in addition to its antibacterial activity, minocycline displays pleiotropic biological effects, including antioxidant, anti-inflammatory, anti-apoptotic, and immunomodulatory properties (Dunston et al. 2011; Desjarlais et al. 2014). It has been shown to inhibit matrix metalloproteinases (MMPs), which are involved in tissue degradation, by inhibiting the enzymatic activity of gelatin-degrading proteases (Machado et al. 2006). These characteristics suggest that minocycline may affect fungal growth and host–pathogen interactions. Furthermore, emerging evidence suggests that certain non-antifungal agents may improve the effectiveness of conventional antifungal drugs through

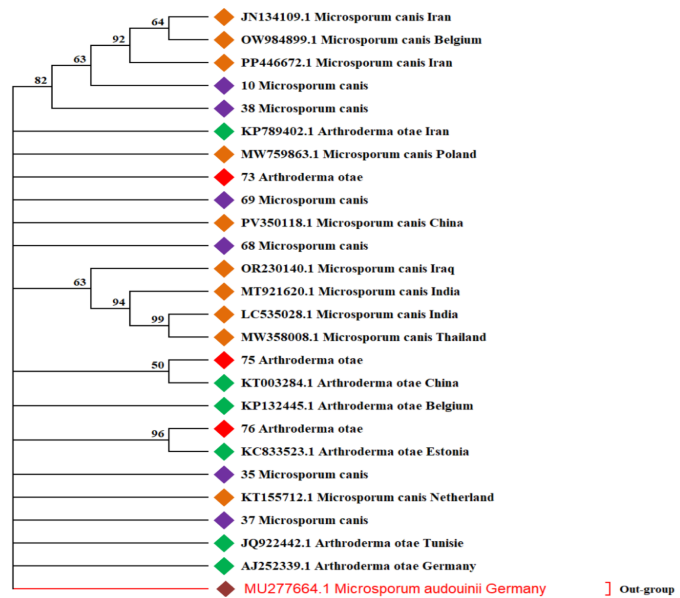


Fig. 7. Phylogenetic tree showing the evolutionary relationships among local *M. canis* and *A. otae* isolates and reference sequences retrieved from GenBank. Bootstrap values (>50%) are indicated at the nodes. *Microsporium audouinii* was used as an out-group

synergistic mechanisms (Brescini et al. 2021), supporting the evaluation of minocycline as a potential adjuvant in antifungal susceptibility testing.

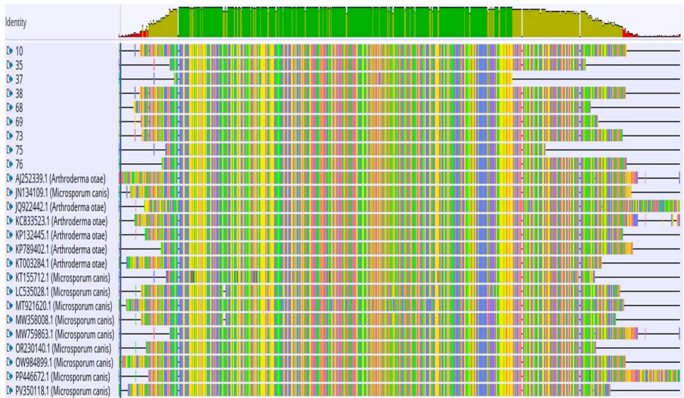


Fig. 8. Multiple sequence alignment of ITS region sequences of *M. canis* and *A. otae* compared with the reference sequences from GenBank

The ranking of antifungal effectiveness observed in this study (terbinafine > minocycline > griseofulvin > itraconazole > fluconazole) provides a clear pattern of drug efficacy and may have important clinical consequences for selecting appropriate treatment strategies, particularly in the context of emerging antifungal resistance. These findings highlight the importance of routine antifungal susceptibility testing to select appropriate therapy and improve clinical outcomes. Overall, the current study supports the use of terbinafine as a first-line treatment for dermatophytosis infection, while caution is advised when considering fluconazole due to its relatively lesser effectiveness. Furthermore, the potential antifungal role of non-conventional agents such as minocycline warrants further investigation. The inclusion of antifungal susceptibility testing provides a valuable phenotypic perspective along with the molecular findings. Although the detection of virulence-associated genes does not directly confirm their pathogenic function, combining phenotypic antifungal response patterns with molecular detection offers a more comprehensive understanding of dermatophyte epidemiology and therapeutic behavior. In the present study, a higher frequency of virulence-associated genes was detected, accompanied by increased antifungal resistance among the isolates. In contrast, Hashoosh and Al-Aaraji (2024) reported the presence of only a single virulence gene and observed that *M. canis* and *A. otae* isolates were completely sensitive to antifungal agents, with no detectable growth. Notably, these authors also reported a direct correlation between the number of virulence genes, particularly metalloprotease genes (MEP1–5), and the degree of antifungal resistance, suggesting that isolates harboring a higher number of these genes showed higher resistance. Though this observation is in agreement with the findings of the present study, where isolates with a higher number of virulence genes may be associated with increased resistance to antifungal agents, the potential role of virulence-associated genes in modulating antifungal susceptibility requires further research to establish causality.

4. Conclusions

The present study provides molecular evidence supporting the close evolutionary relationship between *Microsporium canis* and *Arthroderma otae*, as demonstrated by their phylogenetic proximity and the comparable distribution of SUB and MEP virulence-associated genes among human and animal-derived isolates, suggesting that

evolutionary relatedness contributes to the conservation of key pathogenic protease-encoding genes. Moreover, the primers designed in the present study may serve as useful molecular tools for future diagnostic and therapeutic applications. However, the direct role of virulence-associated genes in antifungal resistance remains unclear. Therefore, further studies with larger sample sizes, broader geographic representation, and functional analyses are required to clarify the precise role of these genes during the infection process. Antifungal susceptibility testing revealed terbinafine as the most effective antifungal agent with relatively low susceptibility to fluconazole. However, regular surveillance of antifungal susceptibility patterns is also recommended to guide appropriate therapeutic strategies to prevent the unnecessary use of potentially toxic drugs and minimize treatment failures.

Declarations

Funding: The authors did not receive any financial support from any organization for the conducting this study

Conflict of interest: The authors declare that they have no conflicts of interest

Acknowledgements: None

Ethics approval: All procedures performed in the present study were in accordance with the ethical standards of the scientific and ethics committee at the College of Medicine/University of Basrah Ref. No. 1 on February 11/2024. Before collecting the specimens, the patients were given a thorough description of the study and asked for their agreement. For animal samples, permission was obtained from the owners before the specimen collection. All clinical specimens were collected and handled following institutional biosafety and ethical guidelines; also, all applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Data availability: Data will be available upon reasonable request from the corresponding author

References

- Aneke CI, Rhimi W, Hubka V, Otranto D, Cafarchia C. (2021). Virulence and Antifungal Susceptibility of *Microsporium canis* Strains from Animals and Humans. *Antibiotics* 10(3): 296. <https://doi.org/10.3390/antibiotics10030296>
- Appelt L, Nenoff P, Uhrlass S, Kruger C, Kuhn P, Eichhorn K, Buder S, Beissert S, Abraham S, Aschoff R, Bauer A. (2021). Terbinafine-resistant dermatophytes and onychomycosis due to *Trichophyton rubrum*. *Hautarzt* 72(10): 868–877. <https://doi.org/10.1007/s00105-021-04879-1>
- Baldo A, Mathy A, Tabart J, Camponova P, Vermout S, Massart L, Maréchal F, Galleni M, Mignon B. (2010). Secreted subtilisin SUB3 from *Microsporium canis* is required for adherence to but not for invasion of the epidermis. *British Journal of Dermatology* 162(5): 990–997. <https://doi.org/10.1111/j.1365-2133.2009.09608.x>
- Basiri S, Hazaveh SJH, Ghazvini RD, Khodavaisy S, Hematzadeh M, Forooshani AR, Yaraghi AAS, Kord M. (2022). Investigation of Subtilisin Genes in Dermatophytes Isolated from Human Dermatophytosis in Ardabil, Northwest of Iran. *Journal of Experimental Zoology India* 25(1): 141–146. <https://connectjournals.com/03895.2022.25.141>
- Brescini L, Fioriti S, Morroni G, Barchiesi F. (2021). Antifungal Combinations in Dermatophytes. *Journal of Fungi* 7(9): 727. <https://doi.org/10.3390/jof7090727>
- Brouta F, Descamps F, Monod M, Vermout S, Losson B, Mignon B. (2002). Secreted metalloprotease gene family of *Microsporium canis*. *Infection and Immunity* 70(10): 5676–5683. <https://doi.org/10.1128/IAI70.10.5676-5683.2002>
- Burchacka E, Pięta P, Lupicka-Słowik A. (2022). Recent advances in fungal serine protease inhibitors. *Biomedicine and Pharmacotherapy* 146: 112523. <https://doi.org/10.1016/j.biopha.2021.112523>
- Burmester A, Shelest E, Glockner G, Heddergott C, Schindler S, Staib P, Heide A, Felder M, Petzold A, Szafranski K, Feuermann M, Pedruzzi I, Priebe S, Groth M, Winkler R, Li W, Kniemeyer O, Schroeckh V, Hertweck C, Hube B, White TC, Platzer M, Guthke R, Heitman J, Wostemeyer J, Zipfel PF, Monod M, Brakhage AA. (2011). Comparative and functional genomics provide insights into the pathogenicity of dermatophytic fungi. *Genome Biology* 12: R7. <https://doi.org/10.1186/gb-2011-12-1-r7>
- Cafarchia C, Figueredo LA, Coccioli C, Camarda A, Otranto D. (2012). Enzymatic activity of *Microsporium canis* and *Trichophyton mentagrophytes* from breeding rabbits with and without skin lesions. *Mycoses* 55(1): 45–49. <https://doi.org/10.1111/j.1439-0507.2010.01997.x>
- Clinical and Laboratory Standards Institute (CLSI). (2017). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard-third edition (M38). Clinical and Laboratory Standards Institute, Wayne, PA, USA.
- Datt S, Das S, Ansari MA, Sharma R, Datt T, Bhattacharya SN. (2021). Gene expression profiling of protease and non-protease genes in *Trichophyton mentagrophytes* isolates from dermatophytosis patients by qRT-PCR analysis. *Scientific Reports* 11: 403. <https://doi.org/10.1038/s41598-020-79839-1>
- de Hoog GS, Dukik K, Monod M, Packeu A, Stubbe D, Hendrickx M, Kupsch C, Stielow J, Freeke J, Goker M, Rezaei-Matehkolaei A, Mirhendi H, Graser Y. (2017). Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. *Mycopathologia* 182 (1): 5–31. <https://doi.org/10.1007/s11046-016-0073-9>
- Desjarlais M, Pratt J, Lounis A, Mounier C, Haidara K, Annabi B. (2014). Tetracycline Derivative Minocycline Inhibits Autophagy and Inflammation in Concanavalin-A-Activated Human Hepatoma Cells. *Gene Regulation and Systems Biology* 4(8): 63–73. <https://doi.org/10.4137/GRSB.S13946>
- Dubljanin E, Zunic J, Vujcic I, Calovski IC, Grujcic SS, Mijatovic S, Dzamic A. (2024). Host-Pathogen Interaction and Resistance Mechanisms in Dermatophytes. *Pathogens* 13(8): 657. <https://doi.org/10.3390/pathogens13080657>
- Dunston CR, Griffiths HR, Lambert PA, Staddon S, Vernallis AB. (2011). Proteomic analysis of the anti-inflammatory action of minocycline. *Proteomics* 11(1): 42–51. <https://doi.org/10.1002/pmic.201000273>
- Ghannoum M. (2016). Azole Resistance in Dermatophytes: Prevalence and Mechanism of Action. *Journal of the American Podiatric Medical Association* 106(1): 79–86. <https://doi.org/10.7547/14-109>
- Graüser Y, Kuijpers AFA, El Fari M, Presber W, De Hoog GS. (2000). Molecular and conventional taxonomy of the *Microsporium canis* complex. *Medical Mycology* 38(2): 143–153. <https://doi.org/10.1080/mmy.38.2.143.153>
- Grumbt M, Monod M, Yamada T, Hertweck C, Kunert J, Staib P. (2013). Keratin Degradation by Dermatophytes Relies on Cysteine Dioxygenase and a Sulfite Efflux Pump. *Journal of Investigative Dermatology* 133(6): 1550–1555. <https://doi.org/10.1038/jid.2013.41>
- Hasan KMA. (2018). Evaluation of The Phenotypic and Genotypic Variations for Clinical Isolates of *Arthroderma otae* Complex. *Journal of University of Babylon for Pure and Applied Sciences* 26(5): 1–15. <https://www.journalofbabylon.com/index.php/JUBPAS/article/view/854>
- Hashoosh QH, Al-Aaraji AMY. (2024). Determination of Metalloprotease Gene in a Few Dermatophyte Species and its Relationship with Antifungal Resistance. *Journal of Communicable Diseases* 56(1): 127–134. <https://doi.org/10.24321/0019.5138.202417>
- Kaplan E, Gonca S, Kandemir H, Doğ'en A, Hilmiog'lu-Polat S, Ilkit M, Tanaka R, Yaguchi T, Uhrlab S, Nenoff P. (2020). Genes Encoding Proteolytic Enzymes Fungalsin and Subtilisin in Dermatophytes of Human and Animal Origin: A Comparative Study. *Mycopathologia* 185: 137–144. <https://doi.org/10.1007/s11046-019-00367-2>
- Katirae F, Kosari YK, Soltani M, Shokri H, Minoieanhighi MH. (2021). Molecular identification and antifungal susceptibility patterns of dermatophytes isolated from companion animals with clinical symptoms of dermatophytosis. *Journal of Veterinary Research* 65: 175–182. <https://doi.org/10.1007/s11046-019-00367-2>

doi.org/10.2478/jvetres-2021-0020

- Kawasaki M, Aoki M, Ishizaki H. (1995). Phylogenetic relationships of some *Microsporium* and *Arthroderma* species inferred from mitochondrial DNA analysis. *Mycopathologia* 130(1): 11-21. <https://doi.org/10.1007/BF01104344>
- Lemsaddek A, Chambel L, Tenreiro R. (2010). Incidence of fungalsin and subtilisin virulence genes in dermatophytes. In: Mendéz-Vilas A, editor, Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Microbiology Book Series 2. Formatex Research Center, Badajoz, Spain. Pp. 658-665.
- Machado L, Kozak A, Ergul A, Hess D, Borlongan C, Fagan S. (2006). Delayed minocycline inhibits ischemia-activated matrix metalloproteinases 2 and 9 after experimental stroke. *BMC Neuroscience* 7: 56. <https://doi.org/10.1186/1471-2202-7-56>
- Mahmood HR, Shams-Ghahfarokhi M, Salehi Z, Razzaghi-Abyaneh M. (2024). Epidemiological trends, antifungal drug susceptibility and SQLE point mutations in etiologic species of human dermatophytosis in Al-Diwaneyah, Iraq. *Scientific Reports* 14(1): 12669. <https://doi.org/10.1038/s41598-024-63425-w>
- Makimura K, Tamura Y, Murakami A, Kano R, Nakamura Y, Hasegawa A, Uchida K, Yamaguchi H. (2001). Cluster Analysis of Human and Animal Pathogenic *Microsporium* Species and Their Teleomorphic States, *Arthroderma* Species, Based on the DNA Sequences of Nuclear Ribosomal Internal Transcribed Spacer 1. *Microbiology and Immunology* 45(3): 209-216. <https://doi.org/10.1111/j.1348-0421.2001.tb02609.x>
- Martinez JPO. (2023). Understanding enzyme-mediated keratin hydrolysis and evaluation of new keratin binding peptides. PhD. thesis. School of Biology and Environmental Science, Faculty of Science, Queensland University of Technology, Australia.
- Midgley G, Clayton Y, Hay R. (1997). *Diagnosis in color: Medical mycology*. 1st edition. Mosby-Wolfe, London, UK. Pp. 155.
- Mohamed MF, Naser El-din A, Abu El-hamd M. (2016). Isolation, Identification, and In Vitro Antifungal Susceptibility Testing of Dermatophytes from Clinical Samples at Sohag University Hospital in Egypt. *Electronic Physician* 8(6): 2557-2567. <https://doi.org/10.19082/2557>
- Moriello K. (2014). Feline dermatophytosis: Aspects pertinent to disease management in single and multiple cat situations. *Journal of Feline Medicine and Surgery* 16 (5): 419-431. <https://doi.org/10.1177/1098612X14530215>
- Moskaluk AE, VandeWoude S. (2022). Current Topics in Dermatophyte Classification and Clinical Diagnosis. *Pathogens* 11(9): 957. <https://doi.org/10.3390/pathogens11090957>
- Naeimipour F, Hashemi SJ, Rezaie S, Bayat M. (2021). Subtilisin Gene Activity in Dermatophytes: A study on the Presence of the Subtilisin Gene in *Trichophyton verrucosum* and *Microsporium gypseum* in Clinical and Nonclinical Samples in Tehran, Iran. *Archives of Razi Institute* 76(2): 253-259. <https://doi.org/10.22092/ari.2019.126417.1343>
- Nair SS, Thomas P, Abdel-Glil MY, Prajapati SK, Athira V, Reddi L, Kumar B, Saikumar G, Dandapat P, Rudramurthy SM. (2025). Whole genome sequence analysis *Microsporium canis*: A study based on animal strains isolated from India. *The Microbe* 7: 100329. <https://doi.org/10.1016/j.microb.2025.100329>
- Robati A K, Khalili M, Hazaveh SJH, Bayat M. (2018). Assessment of the subtilisin genes in *Trichophyton rubrum* and *Microsporium canis* from dermatophytosis. *Comparative Clinical Pathology* 27: 1343-1347. <https://doi.org/10.1007/s00580-018-2745-y>
- Seyedmousavi S, Bosco S, de Hoog S, Ebel F, Elad D, Gomes R, Jacobsen I, Jensen H, Martel A, Mignon B, Pasmans F, Piecková E, Rodrigues A, Singh K, Vicente V, Wubbelt G, Wiederhold N, Guillot J. (2018). Fungal infections in animals: A patchwork of different situations. *Medical Mycology* 56(1): 165-187. <https://doi.org/10.1093/mmy/myx104>
- Vite-Garín T, Estrada-Cruz NA, Hernández-Castro R, FuentesVenado CE, Zarate-Segura PB, Frías-De-León MG, MartínezCastillo M, Martínez-Herrera E, Pinto-Almazán R. (2024). Remarkable Phenotypic Virulence Factors of *Microsporium canis* and Their Associated Genes: A Systematic Review. *International Journal of Molecular Sciences* 25(5): 2533. <https://doi.org/10.3390/ijms25052533>
- Zafar MA, Naqvi FZ, Tahir AH, Pasha RH, Khan MA, Rahim MF. (2023). Dermatophytosis in One-Health Perspective. *Zoonosis* 4: 481-489. <https://doi.org/10.47278/book.zoon/2023.171>

Citation

Hadi NS, Jasim HA, Alhamdi DK. (2026). Molecular detection of subtilisin and fungalsin genes and antifungal susceptibility of *Microsporium canis* from human and animal dermatophytosis. *Letters in Animal Biology* 06(2): 13 – 21. <https://doi.org/10.62310/liab.v6i2.344>