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Mixed infection of *Microsporum*, *Candida*, and *Malassezia* spp. in a Persian cat: A unique case in Rohilkhand region of Uttar Pradesh, India

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Abstract

Background: Ringworm is one of the most prevalent superficial fungal infections in felines. The natural commensal skin flora in felines includes yeasts and yeast-like fungi, such as *Malassezia* and *Candida*. Mixed fungal infections in cats are uncommon and pose a diagnostic challenge because of overlapping clinical presentations. A breach in the cutaneous barrier associated with dermatophytosis may trigger a flare-up of *Malassezia* and *Candida* species, resulting in a mixed infection. **Case description:** A 2.5-year-old female Persian cat was presented with circular patches of alopecia across the head and ears, coated with thick, crusty lamellar scales. The animal showed no other clinical signs of illness. **Findings/treatment and outcome:** The clinical sample, including hair and skin scrapings, was cultured on Sabouraud's Dextrose Agar (SDA). *Microsporum canis*, *Candida albicans*, and *Malassezia* spp. could be identified from the culture plate using phenotypic and molecular assays. The isolates were further subjected to antifungal susceptibility testing. Based on the disc diffusion assay, miconazole showed the largest zone of inhibition against *M. canis* and *Malassezia* spp., and the second largest against *Candida albicans*. In addition, in the broth microdilution assay, terbinafine had the lowest minimum inhibitory concentration against *M. canis* among the azole drugs. **Conclusion:** This case highlights the significance of mixed fungal infections in cats with persistent dermatological conditions and underscores the need for comprehensive diagnostic approaches in veterinary practice.

Key words: Antifungals, *Candida*, Felines, *Malassezia*, *Microsporum canis*

Introduction

Fungal infections, particularly mycotic skin diseases, are a growing concern worldwide (Elewski *et al.*, 2018; Buil *et al.*, 2020; Gnat *et al.*, 2021). According to a 2017 study, fungal skin diseases in humans account for approximately 10.07% of all skin diseases; however, in 2021, around 1.73 billion cases of mycotic disease were reported (Urban *et al.*, 2021; Li *et al.*, 2025). Although studies on animal mycoses remain limited, superficial mycotic diseases are increasingly reported, especially among companion animals (Silvestre *et al.*, 2021; Chupia *et al.*, 2022; Brathwaite *et al.*, 2025). Furthermore, companion animals can serve as a source of zoonotic infections in humans (Xia, 2022; Capoor *et al.*, 2024). The most common causes of superficial mycoses in dogs

and cats include dermatophytes, *Malassezia*, and *Candida*. Dermatophytes are filamentous, keratinophilic fungal organisms that cause superficial mycoses in humans and animals (Gnat *et al.*, 2020). They are classified into many genera; however, *Microsporum* and *Trichophyton* spp. are commonly reported from animals (Jarjees and Issa, 2022; Lavari *et al.*, 2022). *Microsporum canis* is frequently reported in felines and canines, with felines serving as an important reservoir (Hubka *et al.*, 2018). The infection, commonly known as ringworm, is characterized by hair loss, scaly patches, and crusty deposits in the affected area (Chermette *et al.*, 2008). Although dermatophytosis is usually mild and self-limiting, it is considered a zoonotic disease that significantly affects both human and animal well-being (Gnat *et al.*, 2019; Jartarkar *et al.*, 2021). Proper

diagnosis of dermatophytosis is also important, as some fungal agents can produce similar clinical signs in animals, leading to misdiagnosis (Pin, 2017).

Some yeast and yeast-like fungi are naturally present in the skin flora of cats. However, they can lead to opportunistic infections when the immune system is compromised, resulting in a range of clinical symptoms. *Malassezia pachydermatitis* is a common commensal yeast found in the skin of dogs and cats (Crespo *et al.*, 2002). *M. pachydermatitis* usually does not induce infection in healthy cats but can flare up and produce clinical manifestations during the course of an underlying disease or condition (Ordeix *et al.*, 2007; Juntachai *et al.*, 2009; Hobi *et al.*, 2024). Superficial skin mycoses due to *Candida* spp. are rarely reported in cats, though other manifestations such as urinary tract infections, granulomatous rhinitis and gastrointestinal granulomas have been reported in cats (Fulton and Walker, 1992; Pressler *et al.*, 2003; Lamm *et al.*, 2013; Duchaussoy *et al.*, 2015; Mponda *et al.*, 2025).

In most cases, infections involve a single etiological agent; however, superficial mycoses can involve more than one fungal organism. Mixed mycotic skin infections have been reported in humans, involving multiple fungal organisms (Shama and Kirkpatrick, 1980; Gawaz and Weisel, 2018; Shi *et al.*, 2024). Although combined bacterial and fungal infections in cats have been reported, studies on mixed mycotic skin infections in cats are limited (Soares *et al.*, 2024). In this study, we present a case report on a mixed mycotic skin infection caused by *Mirosporium*, *Malassezia*, and *Candida* species in a Persian cat, and its successful therapeutic management.

Case description

A 2.5-year-old female Persian cat was referred to the Teaching Veterinary Clinical Complex, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, exhibiting circular patches of alopecia across the head and ears, coated with thick, crusty lamellar scales (Fig. 1). The physiological parameters of the cat were within the normal range. The animal exhibited no other clinical signs of illness. Also, the animal had no prior history of antifungal medication and deworming.

Ethical approval

This study did not necessitate official or institutional ethical approval as it did not involve experimental procedures. The examination of the feline and collection of clinical samples were conducted with the owner's consent.

Findings/treatment and outcome

Sample collection, processing, and laboratory investigation

The lesions were cleaned and disinfected with 70% alcohol before collecting samples. The hairs were collected along with the hair follicle using sterile forceps,

and scales were scraped from the edges of the lesions. The samples were collected on clean white paper and brought to the Mycology laboratory. The scrapings and hair samples were treated with 10% potassium hydroxide (KOH), stained with lactophenol-cotton blue, and then examined microscopically. The samples were then inoculated onto Sabouraud dextrose agar (SDA) containing chloramphenicol and incubated at 30°C for 7 days.

The direct microscopic examination of the scrapings and hair samples, using 10% KOH and lactophenol cotton blue staining, revealed a high number of ectothrix arthrospores (Fig. 2).



Fig. 1: A 2.5-year-old female Persian cat presented with clinical signs of alopecia and crusty lesions

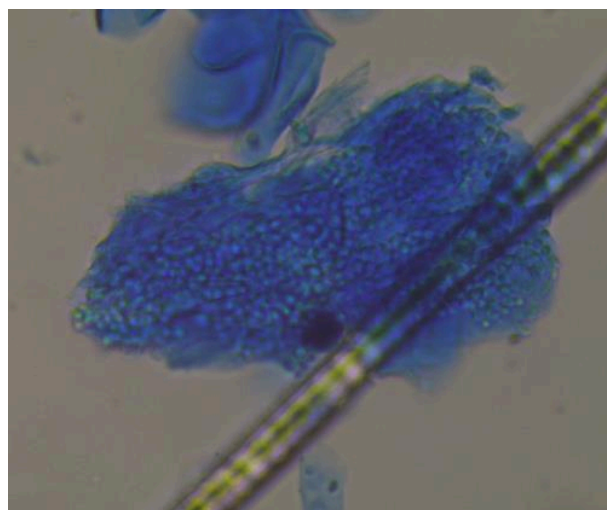


Fig. 2: Direct microscopic examination of the clinical sample using lactophenol cotton blue staining, revealing ectothrix spores

Also, SDA plates inoculated with clinical samples showed colonies indicative of mold and yeast/yeast-like growth. The mold colonies, suspected to be dermatophytes, exhibited submerged growth with a yellowish hue, suggestive of *M. canis*. The yeast/yeast-

like colonies were round, smooth, and cream-colored, with two distinct sizes observed (Fig. 3).

All three colony types were examined microscopically after lactophenol cotton blue staining and/or Gram staining. Spindle-shaped macroconidia characteristics of *M. canis* were observed after lactophenol cotton blue staining of the mold colonies (Fig. 4).



Fig. 3: SDA plate with colonies of *Microsporium* spp., *Malassezia* spp. and *Candida* spp.

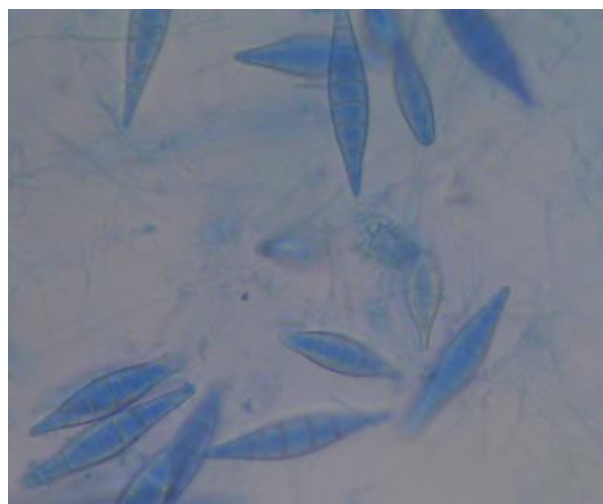


Fig. 4: Microscopic examination of *M. canis* colony after lactophenol cotton blue staining, revealing spindle-shaped macroconidia with hyphae

The yeast/yeast-like colonies were microscopically characterized by observing the shape of the unicellular cells and budding forms (Figs. 5A and B; Figs. 6A and B). One colony type revealed smaller, oblong to cylindrical cells with unipolar, wide-based budding, suggestive of *Malassezia* spp. (Figs. 5A and B). Growth on SDA agar without lipid supplementation strongly indicated *Malassezia pachydermatitis*. The second yeast/yeast-like colony type revealed slightly larger oval-to-spherical cells with budding at the narrow base,

suggestive of *Candida* spp. (Fig. 6A and B).

The suspected *Candida* colonies were subjected to the germ tube test, and tube-like growth from the cells, without any constriction at the base, was observed (Fig. 7B). The presence of true germ tubes indicated a high probability that the colony was *Candida albicans*. This was further supported by the colony's light green colouration on HiCrome Candida differential agar (Fig. 7A) (HiMedia, India).

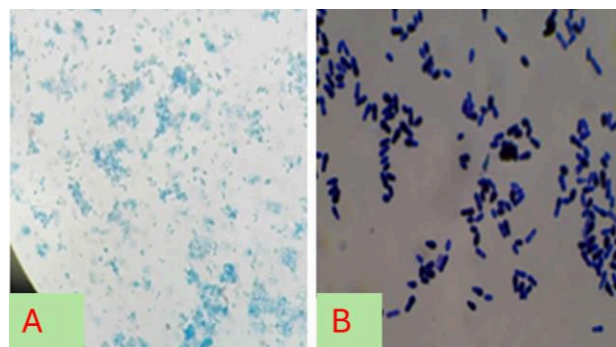


Fig. 5: Microscopic examination of *Malassezia* spp. colony after (A) Lactophenol cotton blue (LCB), and (B) Gram staining

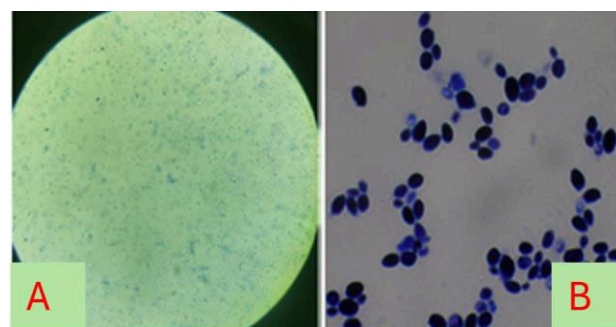


Fig. 6: Microscopic examination of *C. albicans* colony after (A) Lactophenol cotton blue (LCB), and (B) Gram-staining

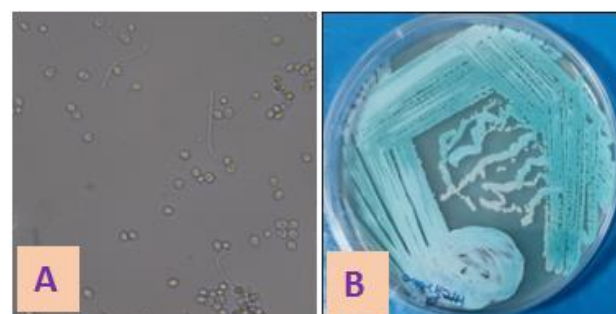


Fig. 7: (A) Production of true germ tube by *C. albicans* isolate, and (B) Light green color of *C. albicans* on Hichrome Candida differential agar

Molecular confirmation

Molecular confirmation of the phenotypically characterized fungal agents was performed using PCR, with *Candida* spp. and *Microsporium* spp. targeted with established primers, and *Malassezia* using in-house standardized PCR (not published). The DNA was extracted using the Zymo: ZR fungal/bacterial Miniprep

Table 1: Primer sequences for the PCR targeting *M. canis* and *C. albicans*

Fungi	Target gene	Primers	Oligonucleotide sequences (5'-3')	Product size	Reference
<i>Microsporium canis</i>	ITS1	Mc-F Mc-R	GTG TGA TGG ACG ACC GTC CCC CCT ATA ATA CAT GGT GCG TTA GGC CAG CCT G	180 bp	Brillowska-Dabrowska <i>et al.</i> (2013)
<i>Candida albicans</i>	26S rDNA	CAL-F CAL-R	TGT TGC TCT CTC GGG GGC GGC CG AGA TCA TTA TGC CAA CAT CCT AGG TTA AA	175 bp	Mannarelli and Kurtzman (1998)

Kit (Zymo Research, USA) (Cat No. 6005). The PCR reaction mixture consisted of 12.5 μ L Dream Taq Green PCR Master Mix (Thermo Fisher Scientific, USA), 1 μ L each of forward and reverse primers (10 pmol each), 3 μ L DNA, and nuclease-free water to bring the volume to 25 μ L. Genomic DNA from *M. canis* (ATCC 36299), *Candida albicans* (ATCC 204305), and in-house-characterized *M. pachydermatitis* were used as positive controls. The mold colony and the suspected *Candida* colony produced the expected amplicons, confirming their identity as *Microsporium canis* and *Candida albicans* (Fig. 8). Molecular confirmation of the *Malassezia* isolate at the genus level was performed using an in-house standardized PCR targeting the ITS-2 and 26S ribosomal regions (PCR methodology not detailed in the present study). Tables 1 and 2 list the primers used and the PCR conditions, respectively.

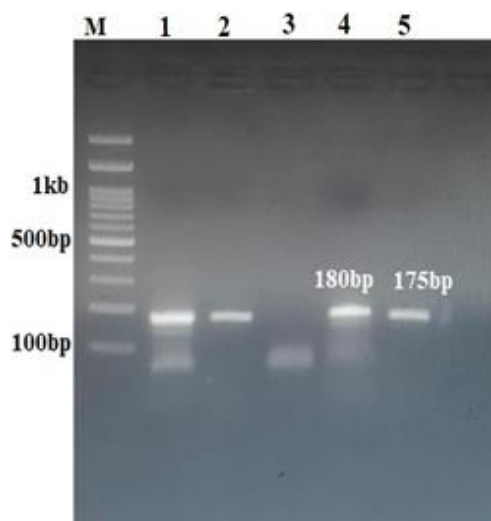


Fig. 8: Agarose gel electrophoresis showing an amplicon of *M. canis* (180 bp), and *C. albicans* (175 bp). M: Marker (1 kb DNA ladder), Lane 1: Positive control (*M. canis*, ATCC 36299), Lane 2: Positive control (*Candida albicans*, ATCC 204305), Lane 3: Negative control, Lane 4: *Microsporium canis* isolate, and Lane 5: *Candida albicans* isolate

Table 2: Cycling conditions for the PCR targeting *M. canis* and *C. albicans*

PCR condition	<i>M. canis</i>	<i>C. albicans</i>
Initial denaturation	94°C for 3 min	94°C for 5 min
Denaturation	94°C for 45 s	94°C for 45 s
Annealing	58°C for 45 s	56.5°C for 1 min
Extension	72°C for 45 s	72°C for 1 min
Final extension	72°C for 10 min	72°C for 10 min
No. of cycles	40	32

Antifungal susceptibility testing (AFST)

The disc diffusion assay was performed for *Microsporium canis*, *Candida albicans*, and *Malassezia* spp. isolates. Six commercially available antifungal discs (HiMedia, India), including miconazole (MIC 30 μ g), fluconazole (FLC 10 μ g), itraconazole (IT 30 μ g), nystatin (NS 50 μ g), clotrimazole (CC 10 μ g), and ketoconazole (KT 30 μ g), were used in the study. For *M. canis*, a clear suspension comprising conidia and hyphae was prepared from culture grown on Potato Dextrose Agar (PDA). The conidia concentration was optimized to 1×10^6 conidia/ml and used as an inoculum (Begum and Kumar, 2021). For *C. albicans* and *M. pachydermatitis*, three to five colonies were picked from a fresh culture grown on an SDA plate and suspended in normal saline. The concentrations were adjusted to 1×10^6 cells/ml and 1×10^7 cells/ml for *C. albicans* and *Malassezia* spp., respectively (CLSI, 2004; Pasquetti *et al.*, 2015). The inoculum was evenly spread on antifungal assay agar (HiMedia, India) and allowed to dry. The antifungal discs were placed on the agar plates at 28°C for *M. canis* and 37°C for *C. albicans* and *Malassezia* spp. isolates till a clear zone of inhibition was visible. Table 3 presents the disc diffusion assay results in the decreasing size of the zone of inhibition for the antifungals tested.

Table 3: Disc diffusion assay for *M. canis*, *C. albicans*, and *Malassezia* using miconazole (MIC 30 μ g), fluconazole (FLC 10 μ g), itraconazole (IT 30 μ g), nystatin (NS 50 μ g), clotrimazole (CC 10 μ g), and ketoconazole (KT 30 μ g)

Isolates	Decreasing order of zone of inhibition
<i>M. canis</i>	MIC>CC>IT>KT>NS>FLC
<i>C. albicans</i>	FLC>MIC>KT>CC>NS>IT
<i>Malassezia</i> spp.	MIC>KT>IT>NS>CC>FLC

To determine the minimum inhibitory concentration (MIC50 and MIC90) of *M. canis* against the most commonly used drugs for dermatophytosis, the broth microdilution assay was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for filamentous fungi, document M38-A2 (CLSI, 2017; Aneke *et al.*, 2020). A conidial suspension of concentration of 1×10^3 to 3×10^3 conidia/ml was used as the inoculum.

We assessed the susceptibility to seven commonly used antifungals acquired from Sigma-Aldrich, USA: fluconazole (FLZ), itraconazole (ITZ), voriconazole (VOR), posaconazole (PSZ), griseofulvin (GRI), terbinafine (TER), and ciclopirox (CPX). The drugs were dissolved in dimethyl sulfoxide to make the stock solution, and the working solution was prepared in

Table 4: Minimum inhibitory concentration (MIC50 and MIC90) values of antifungal drugs against the *M. canis* isolate

Isolates	Ciclopirox		Itraconazole		Terbinafine		Griseofulvin		Fluconazole		Voriconazole		Posaconazole	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
M1-76A	0.0313	-	0.0625	0.0313	0.00782	0.0313	0.0125	-	4	32	0.0313	.0125	-	0.125

RPMI-1640 medium buffered with MOPS (3-N-Morpholinopropanesulfonic acid). 100 µL of the working solution of the drugs was dispensed in a 96-well ("U" shaped) microtiter plate. Subsequently, 100 µL of the inoculum was added into every well so that the final concentrations (in mg/ml) ranged from 0.125 to 64 for fluconazole; 0.03125 to 16 for voriconazole, itraconazole, and posaconazole; and 4 to 0.00781 for ciclopirox, griseofulvin, and terbinafine. Wells containing 100 µL of RPMI-1640 medium and 100 µL of conidial suspension were used as a positive growth control. Wells containing 200 µL of RPMI-1640 medium were included as a sterility control. The microtitre plate was then incubated at 28°C for 4 days before interpretation. The results of the broth microdilution assay are expressed as the concentration of the drug that inhibits 50% and 90% of the growth of the *M. canis* strain (Table 4).

Treatment and outcome

Based on the disc diffusion assay, miconazole showed the largest zone of inhibition against *M. canis* and *Malassezia* spp., and the second-largest against *Candida albicans*. In addition, in the broth microdilution assay, terbinafine had the lowest minimum inhibitory concentration against *M. canis* among the azole drugs. Therapy started on the same day as the clinical and laboratory diagnosis. The regimen included Micodine shampoo (miconazole and chlorhexidine) (applied weekly), Curabless ointment (containing terbinafine hydrochloride, Ofloxacin, Ornidazole, and Clobetasol Propionate) applied topically to the affected areas twice daily for one week, cetirizine tablets 10 mg (1/2 tablet given daily), cephalixin antibiotics (300 mg given as 1/3 tablet daily for seven days), and ZipVit multivitamin syrup (2 ml twice daily, administered orally after meals). The antifungal therapy was consistent with the findings of the disc diffusion and broth microdilution assays. Additionally, multivitamin supplements may have supported the cat's overall health, contributing to the successful outcome. The animal fully recovered without complications within 2-4 weeks.

Discussion

In recent decades, the prevalence of both superficial and systemic fungal infections has gradually risen, posing a significant risk to humans and animals. The rise in pet rearing has further enhanced the transmission of zoonotic pathogens, including fungi, from companion animals to humans. Both opportunistic pathogens and obligate pathogens can cause mycotic infections in dogs and cats. This scientific report presents a unique case of

combined fungal infection involving *M. canis*, an obligate and zoonotic pathogen, and the commensal pathogens *C. albicans* and *Malassezia* spp. in a Persian cat. Dermatophytosis represents a significant clinical condition in small animal practice, especially felines (Moriello and DeBoer, 1995; Moriello *et al.*, 2017). Many researchers have reported infection in cats, especially Persian cats, with *M. canis* (Bond *et al.*, 2001; de Oliveira Nobre *et al.*, 2010; Bagra *et al.*, 2024). *Candida* spp. and *Malassezia* spp. have been isolated from healthy cats without dermatological disease. However, there are fewer reports of *Malassezia* and *Candida* infections in cats than in dogs (Nardoni *et al.*, 2005). *Malassezia*-associated otitis externa and dermatitis have been reported in cats (Hijfte *et al.*, 1997; Crespo *et al.*, 2002, Cafarchia *et al.*, 2005; Nardoni *et al.*, 2005; Shokri *et al.*, 2010). Though *Candida*-associated superficial mycoses are rarely reported in cats, they are mainly associated with urinary tract infection (Pressler *et al.*, 2003; Hernando *et al.*, 2021). Further, combined skin mycotic infections in cats involving *Microsporum*, *Candida*, and *Malassezia* spp. are extremely scarce.

Dermatophytosis in small animals is significant from a public health perspective, as some dermatophytes, including *M. canis*, have zoonotic potential (Cafarchia *et al.*, 2006; Piorunek *et al.*, 2024). In addition, *M. canis* has been associated with atypical tinea presentations in humans (Yang *et al.*, 2021; Hung *et al.*, 2022). However, the zoonotic potential of *C. albicans* and *Malassezia* is not well documented. Persian cats are known to be more susceptible to dermatophytosis than other breeds, possibly because of factors such as their dense, long hair coat and altered skin microenvironment (O'Neill *et al.*, 2019). Further, a particular haplotype of the S100A antimicrobial peptide genes has been associated with severe dermatophytosis in Persian cats (Myers *et al.*, 2022). In this case, the animal may have been infected with *M. canis* spores, likely from the environment or other animals. Dermatophytosis in the animal may have favored overgrowth of the commensal pathogens *Candida* spp. and *Malassezia* spp. Dermatophytes are keratinophilic molds that secrete a wide array of exo- and endo-proteases (Brouta *et al.*, 2002; Monod, 2008; Deng *et al.*, 2023). These proteases can degrade stratum corneum proteins, thereby compromising skin integrity (Dubljanin *et al.*, 2024). Scabs and crusts also create a favorable growth environment for commensal fungal pathogens. Overgrowth of commensal fungal pathogens is mainly associated with immunocompromised conditions. Commensal pathogens, such as *C. albicans*, are more frequently isolated from cats infected with feline immunodeficiency virus (Mancianti *et al.*, 1992). However, *Malassezia* overgrowth has been reported in

otherwise healthy cats with atopic dermatitis (Ordeix *et al.*, 2007). In the present case, although the animal's immune competence was not checked, it showed no signs of systemic disease. Thus, in this case, an initial infection with *M. canis* may supposedly have favored the overgrowth of *C. albicans* and *Malassezia* spp.

Given the increasing trend of antifungal resistance among fungal pathogens, antifungal susceptibility testing, similar to antibacterial testing, is necessary. However, given the time required to grow on artificial media, empirical therapy is often initiated based on clinical findings in cases suspected of superficial mycotic diseases. Disc diffusion has been widely used for *Candida* species; however, it is not routinely recommended for *Malassezia* and dermatophytes (Kustimur *et al.*, 2003). However, because of the ease and minimal reagent requirements, many researchers have used the disc diffusion assay for these two fungal pathogens (Esteban *et al.*, 2005; Nweze *et al.*, 2010; Pasquetti *et al.*, 2015; Khadka *et al.*, 2017; Hsieh *et al.*, 2018; Begum and Kumar, 2021). Although further standardization is needed, the disc diffusion assay provides a general idea of the most suitable drug based on the zone of growth inhibition. In addition, given the significance of *M. canis* as an obligate and zoonotic pathogen and the rising antifungal resistance among the dermatophytes, we have evaluated the inhibitory concentrations of commonly used antifungal drugs against the isolate. The antifungal drugs used in both assays were selected based on the availability of discs and antifungal powder in the laboratory. However, in both the disc diffusion and broth microdilution assays, fluconazole was the least effective, suggesting that the disc diffusion assay may be useful for understanding susceptibility patterns in fungal pathogens, though it requires further protocol standardization.

The present study highlights the importance of dermatophytosis in cats and the secondary yeast/yeast-like infections. Although the lack of long-term follow-up is a limitation of this study, the improvement in clinical signs and the cat's apparent recovery underscore the importance of timely identification and antifungal susceptibility testing of fungal pathogens. The findings of studies further provide insight into complicated clinical manifestations, including mixed mycotic infections. They are indispensable for the effective and appropriate management of mycotic skin diseases and for reducing the probable transmission of zoonotic pathogens to humans.

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Conflict of interest

The authors declare that they have no competing

interests.

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Use of generative AI and AI-assisted technologies statements

No Generative AI tools were used in the preparation of the manuscript. Grammarly software was used to check the grammar and language.

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