RESEARCH ARTICLE

Characterization and Antifungal Resistance Patterns of Fungi Associated with Canine and Feline Mycotic Dermatitis

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Abstract

Mycotic dermatitis is a common clinical condition in companion animals characterized by alopecia, pruritus, and malodor. Due to the diverse etiology involving dermatophytes and non-dermatophytic yeasts and moulds, accurate identification and susceptibility profiling are essential for therapeutic success. The present study was conducted to isolate and identify fungal pathogens associated with mycotic dermatitis in dogs and cats and to determine their *in vitro* sensitivity patterns against commonly used antifungal agents. Clinical samples (n=20), including skin scrapings, hair plucks, tape impressions, and crusts, were collected from symptomatic canine and feline subjects. Samples were processed for fungal culture using Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM). Isolates were identified based on morphological and cultural characteristics. Fungal pathogens were recovered from 100% of the clinical samples. The isolates comprised non-dermatophytic moulds (55%), yeasts (25%), and dermatophytes (20%). Antifungal susceptibility testing (AFST) revealed significant variation in drug efficacy; notably, clotrimazole demonstrated the highest sensitivity across all isolates, whereas fluconazole showed complete resistance (100%). These findings emphasize the predominance of opportunistic fungi and highlight the importance of antifungal susceptibility testing for effective management of mycotic dermatitis.

Key words: Antifungal resistance, Dermatophytes, Moulds, Mycotic dermatitis, Yeasts.

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Introduction

Mycotic dermatitis is an important dermatological disorder in companion animals caused by dermatophytes, yeasts and opportunistic fungi affecting keratinized tissues such as skin and hair. Clinically, mycotic dermatitis is presented as pruritus, alopecia, erythema, scaling, crusting and hyperpigmentation, often mimicking bacterial or allergic skin diseases, thereby complicating diagnosis (Katiraee *et al.,* 2021; Hassanien *et al.,* 2021). Dogs and cats are frequently exposed to environmental fungal spores and factors such as dense hair coat, humidity and compromised skin barriers predispose them to infection. In addition, close contact between pets and humans raises concerns regarding zoonotic transmission of certain fungal pathogens (Bond *et al.,* 2020). In recent years, the emergence of antifungal resistance has become a growing concern in veterinary dermatology, largely attributed to indiscriminate and prolonged use of antifungal agents. This has resulted in reduced therapeutic efficacy and recurrent infections (Arendrup and Patterson, 2017). Although advanced diagnostic tools are available, culture and microscopic identification remain the gold standard for confirming fungal infections. Furthermore, antifungal susceptibility testing (AFST) is increasingly recognized as essential for guiding appropriate therapy (CLSI, 2017; Barua *et al.,* 2022). The present study was therefore undertaken to isolate and identify fungi associated with mycotic dermatitis in dogs and cats and to evaluate their antifungal sensitivity patterns.

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Materials and Methods

Sample Collection, Fungal Isolation and Identification

The study was carried out at Veterinary Clinical Complex, NTR College of Veterinary Science, SVVU, Gannavaram (AP, India). A total of 20 clinical samples comprise of skin scrapings, hair plucks, tape impression samples and crust materials were collected from dogs and cats exhibiting skin lesions

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suggestive of mycotic dermatitis, including alopecia, pruritus, erythema, scaling, hyperpigmentation and malodour.

Samples were subjected to preliminary microscopic examination using potassium hydroxide (KOH) mount method to detect the presence of fungal elements (Quinn *et al.,* 2016). Irrespective of the findings in direct microscopic examination, all the samples were inoculated onto Sabouraud Dextrose Agar (SDA) to ensure maximum recovery of fungal isolates (Bagra *et al.,* 2022). The inoculated media were incubated at 30°C and observed periodically for growth for a duration ranging from 2 days up to 4 weeks (Begum and Kumar, 2020). Fungal identification was carried out based on colony characteristics including texture, pigmentation and growth pattern observed on both the obverse and reverse sides of the SDA plate (Kidd *et al.,* 2022). Microscopic examination of fungal elements was performed using Lactophenol Cotton Blue (LPCB) staining for moulds and using Gram staining technique for yeasts (Bond *et al.,* 2020). Isolates identified as dermatophytes were subcultured onto Dermatophyte Test Medium (DTM) and incubated at 30°C for up to 4 weeks. Confirmation of dermatophytes was based on incubation time, colony morphology, microscopic examination and characteristic colour change on DTM (Morello *et al.,* 2016; Nehru *et al.,* 2024).

Antifungal Susceptibility Testing

Antifungal susceptibility testing was performed by the agar-based disc diffusion method using Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/mL methylene blue (GMB medium) (Afshari *et al.,* 2016). Seven commercially available antifungal discs (M/s HiMedia) tested included itraconazole (10 µg), ketoconazole (30 µg), fluconazole (10 µg), miconazole (30 µg), nystatin (50 µg), clotrimazole (10 µg), and amphotericin B (20 µg). The inoculum was prepared from fresh fungal cultures (4-7 days old) and adjusted to a turbidity equivalent to 0.5 McFarland standard. The inoculum was evenly swabbed onto the surface of the medium, and the antifungal discs were placed aseptically. The plates were incubated at 30°C and examined for zones of inhibition. The diameter of the inhibition zones was measured in millimetres, and the susceptibility pattern of each isolate was recorded (CLSI, 2017; Barua *et al.,* 2022).

Results and Discussion

A total of 20 clinical samples from dogs and cats with suspected mycotic dermatitis were cultured, and all samples yielded fungal growth on Sabouraud Dextrose Agar, indicating a 100% isolation rate and confirming the

Table 1: Distribution of fungal isolates and inhibition zone diameters against antifungal agents used in the study

S. No	Isolates & their number (*n*)	Inhibitory zone diameter (IZD) (mm)						
		IT	KT	FLC	MIC	NS	CC	AP
1.	*T. rubrum* (*n*=1)	29	36	13	27	10	32	15
2.	*T. mentagrophytes* complex (*n*=1)	26	33	12	25	10	32	19
3.	*E. floccosum* (*n=2)*	8	24	0	21	19	20	16
4.		9	26	12	31	19	26	22
5.	*Malassezia* spp.(*n*=3)	0	11	0	14	0	12	0
6.		9	14	12	11	9	14	0
7.		0	16	11	9	11	12	0
8.	*Candida* spp. (*n*=2)	37	43	7	35	22	29	16
9.		32	45	13	37	23	30	18
10.	*Penicillium* spp.(*n*=4)	17	16	0	18	13	16	10
11.		12	26	6	24	18	23	17
12.		14	28	0	22	20	25	18
13.		10	24	5	21	17	22	15
14.	*A. niger* (*n*=3)	13	23	8	21	20	14	12
15.		11	20	0	20	16	14	11
16.		13	24	9	25	14	16	0
17.	*A. flavus* (*n*=1)	14	22	7	21	23	16	18
18.	*Fusarium* spp.(*n*=2)	0	9	0	15	17	21	22
19.		0	14	0	11	15	24	25
20.	*Curvularia* spp.(*n*=1)	22	39	12	31	34	19	14

IT- Itraconazole (10 µg), KT- Ketoconazole (30 µg), FLC- Fluconazole (10 µg), MCN- Miconazole (30 µg), NS- Nystatin (50 µg), CC- Clotrimazole (10 µg), AMB- Amphotericin B (20 µg)

involvement of fungal agents in dermatological conditions. The isolates comprised dermatophytes (20%), yeasts (25%) and non-dermatophytic moulds (55%), reflecting a diverse fungal etiology. Similar diversity of fungal pathogens in companion animals has been reported earlier (Hassanien *et al*., 2021; Katiraee *et al*., 2021), emphasizing the increasing role of opportunistic fungi in dermatological infections. Distribution of fungi isolated from mycotic dermatitis cases in dogs and cats is presented in Table 1.

Among dermatophytes, *Trichophyton* spp. (*T. rubrum* and *T. mentagrophytes* complex; *n*=1 each) and *Epidermophyton floccosum* (*n*=2) were isolated. Microscopically, *T. rubrum* showed numerous microconidia with few clavate macroconidia, while *T. mentagrophytes* exhibited characteristic racket-shaped hyphae along with abundant microconidia. *E. floccosum* was characterized by numerous multicellular, club-shaped macroconidia (Fig. 1). However, dermatophytes constituted a smaller proportion compared to other fungal groups, which is in agreement with reports indicating a declining predominance of dermatophytes in favour of opportunistic fungi (Katiraee *et al*., 2021). Among yeasts, *Malassezia* spp. (*n*=3) predominated over *Candida* spp. (*n*=2), suggesting its role as a commensal organism that becomes pathogenic under favourable conditions. Microscopically, *Malassezia* spp. exhibited oval budding yeast cells with a characteristic footprint (or "shoeprint") appearance, while *Candida* spp. showed oval budding yeast cells along with pseudohyphae (Fig. 1). Similar findings have been reported by Bond *et al.* (2020), who described Malassezia spp. as a common cause of dermatitis in companion animals. Non-dermatophytic moulds formed the predominant group, with *Penicillium* spp. (*n*=4) and *A. niger* (*n*=3) being most prevalent, followed by *Fusarium* spp. (*n*=2), *Aspergillus flavus* (*n*=1) and *Curvularia* spp. (*n*=1). Microscopically, *A. niger* showed characteristic radiate conidial heads, whereas *A. flavus* exhibited radiate to columnar conidial heads arising from the vesicle at the tip of the conidiophore. *Penicillium* spp. displayed a typical brush- or broom-like arrangement of conidiophores with metulae and phialides. *Fusarium* spp. showed septate, branched hyphae with clusters of multicellular macroconidia, while *Curvularia* spp. were characterized by numerous multicellular, geniculate macroconidia with a broad, swollen central cell (Fig. 1). This predominance supports the role of environmental fungi as emerging contributors to dermatomycosis (Hassanien *et al*., 2021).

Antifungal susceptibility testing by agar-based disc diffusion method revealed variable sensitivity patterns among the isolates (Table 1, Fig. 1-I). Among dermatophytes, *T. rubrum* and *T. mentagrophytes* complex showed sensitivity to ketoconazole and clotrimazole, while both were resistant to fluconazole and nystatin, which is consistent with earlier findings indicating higher efficacy of imidazole antifungals (Nair *et al*., 2023). *E. floccosum* isolates were resistant to itraconazole and fluconazole, with sensitivity to clotrimazole, further supporting the effectiveness of topical imidazoles. Among yeasts, *Malassezia* spp. exhibited resistance to most antifungal agents, whereas *Candida* spp. showed sensitivity to itraconazole, ketoconazole, miconazole and clotrimazole, but resistance to fluconazole, which is in agreement with previous reports (Begum and Kumar, 2020).

Among moulds, *Penicillium* spp. were largely resistant to fluconazole, with sensitivity mainly to clotrimazole. *Aspergillus niger* and *A. flavus* also demonstrated resistance to fluconazole while remaining sensitive to ketoconazole and clotrimazole, which may be attributed to reduced efficacy of fluconazole against filamentous fungi (Perlin *et al*., 2017). Notably, *Fusarium* spp. exhibited resistance to most antifungal agents and showed limited sensitivity to clotrimazole, reflecting its known intrinsic multidrug resistance. *Curvularia* spp. showed sensitivity to ketoconazole and clotrimazole, while being resistant to fluconazole. Overall, fluconazole exhibited the highest resistance across all isolates, whereas clotrimazole showed the most consistent sensitivity, followed by ketoconazole (Table 2). This trend was in accordance with previous studies (Nair *et al*., 2023) and highlights the importance of antifungal susceptibility testing for guiding effective therapeutic strategies.

Table 2: Percentage sensitivity and resistance patterns of fungal isolates to antifungal agents used in the study

Antifungal agent	Resistance (%)	Sensitivity (%)
Itraconazole	35	65
Ketoconazole	10	90
Fluconazole	80	20
Miconazole	15	85
Nystatin	45	55
Clotrimazole	0	100
Amphotericin B	40	60

Conclusion

The present study demonstrates that mycotic dermatitis in dogs and cats is caused by a diverse range of fungi, with non-dermatophytic moulds predominating over yeasts and dermatophytes. *Penicillium* spp. and *Aspergillus* spp. were the most common isolates, indicating the increasing role of opportunistic fungi. Antifungal susceptibility testing revealed that fluconazole exhibited the highest resistance across isolates, whereas clotrimazole showed the highest overall sensitivity, followed by ketoconazole. Notably, *Fusarium* spp. displayed marked multidrug resistance with limited sensitivity. These findings highlight the importance of routine culture and antifungal susceptibility testing for guiding effective antifungal therapy in mycotic dermatitis.

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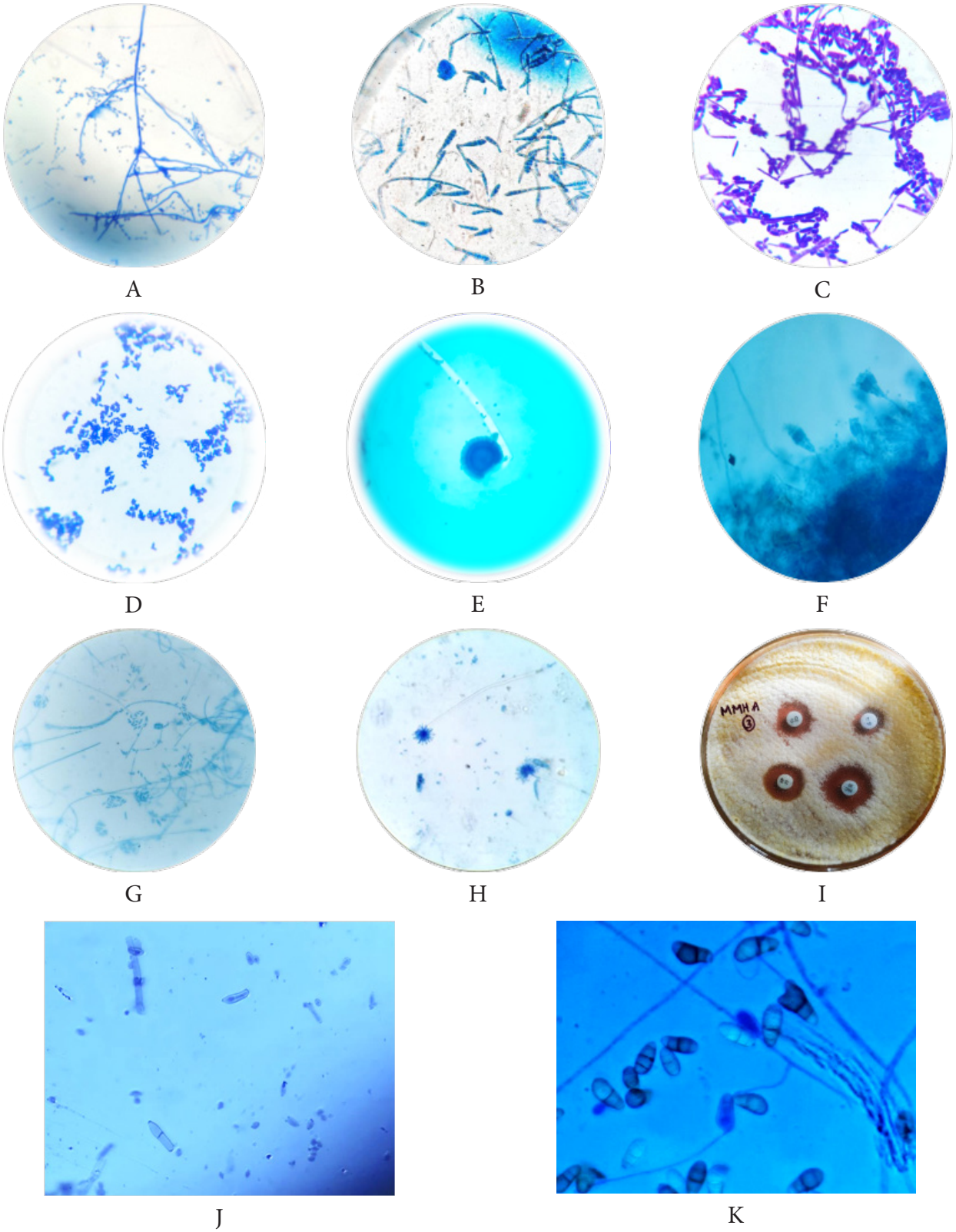


Fig. 1: Microscopic appearance of fungal isolates. (A). *T. mentagrophytes*: racket hyphae and microconidia; (B). *E. floccosum*: Numerous club shaped multicellular macroconidia; (C). *Candida* spp.: oval budding yeast with pseudohyphae; (D). *Malassezia* spp.: oval budding yeast with characteristic foot print like appearance; (E). *A. niger*: Radiate head appearance of conidiophore and conidia; (F). *Penicillium* spp.: Conidiophores with metulae and philalides, cahracteristic brush like appearance; (G). *Fusarium* spp.: Septate branched hyphae with clustered 2 celled macroconidia; (H). *A. flavus*: Radiate to loosely columnar head with phialides covering entire vesicle; (I). Agar based disc diffusion assay; (J). *T. rubrum*: Numerous microconidia and few clavate shaped macroconidia; (K). *Curvularia* spp.: Numerous geniculate multicellular macroconidia with a broad swollen central cell.

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