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Antifungal susceptibility pattern of *Aspergillus* spp. isolated from dogs and cats with mycotic dermatitis

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Abstract

Mycotic dermatitis caused by opportunistic fungal pathogens is increasingly reported in companion animals. The present study was undertaken to isolate *Aspergillus* spp. associated with mycotic dermatitis in dogs and cats and to determine their antifungal susceptibility pattern. Clinical samples comprising skin scrapings and hair materials were subjected to fungal isolation and identification by conventional cultural and microscopic methods. A total of 29 isolates of *Aspergillus* spp. were recovered including *A. niger* ($n=12$), *A. fumigatus* ($n=10$) and *A. flavus* ($n=7$). Antifungal susceptibility testing by agar based disc diffusion method revealed lower zone of inhibition values for fluconazole, whereas ketoconazole, miconazole and amphotericin B demonstrated greater *in vitro* antifungal activity. The study emphasizes the importance of antifungal susceptibility testing in the therapeutic management of *Aspergillus*-associated mycotic dermatitis in companion animals.

Keywords: *Aspergillus* spp., antifungal susceptibility testing, companion animals, agar disc diffusion assay, opportunistic fungi

1. Introduction

Mycotic dermatitis is an important fungal skin disorder affecting dogs and cats and is commonly associated with alopecia, erythema, scaling, crust formation and pruritus. The condition is caused by a variety of fungal pathogens and opportunistic fungi, leading to considerable clinical importance in companion animal practice (Moriello, 2014; Cafarchia *et al.*, 2006) [1, 2]. Recent advances in veterinary mycology have indicated an increasing occurrence of fungal dermatitis associated with opportunistic non-dermatophytic fungi, among which *Aspergillus* spp. are considered predominant due to their frequent isolation from dermatological and superficial lesions in companion animals (Tell, 2005; Segal, 2009) [3, 4]. *Aspergillus* spp. are ubiquitous environmental fungi commonly present in soil, organic debris and air. Species such as *A. niger*, *A. flavus* and *A. fumigatus* have been associated with superficial, cutaneous and systemic infections in animals, particularly in immunocompromised animals or animals with impaired skin barriers (Quinn *et al.*, 2011; Muller *et al.*, 2013) [5, 6]. The identification of *Aspergillus* spp. associated with dermatological lesions is important due to their pathogenic potential and variable response to antifungal therapy. In recent years, emerging antifungal resistance among opportunistic fungal pathogens has become a growing concern in both veterinary and human medicine, thereby necessitating antifungal susceptibility testing for better therapeutic management (Verweij *et al.*, 2016; Chowdhary *et al.*, 2017) [7, 8]. Therefore, the present study was undertaken to isolate and identify *Aspergillus* spp. associated with fungal dermatitis in dogs and cats and to determine their *in vitro* antifungal susceptibility pattern against commonly used antifungal agents.

2. Materials and Methods

2.1 Sample collection

The present study was conducted at NTR College of Veterinary Science, Gannavaram (AP, India) during the period from August 2025 to January 2026. Clinical samples were collected

from dogs and cats presented with dermatological manifestations such as alopecia, scaling, crust formation, erythema, malodour and pruritic skin lesions suggestive of mycotic dermatitis. Samples comprising skin scrapings and hair materials were collected aseptically from the active lesions using sterile scalpel blades after cleansing the affected area with 70% alcohol. The collected samples were transferred into sterile containers, properly labelled and transported to the laboratory for mycological examination.

2.2 Isolation and identification of *Aspergillus* spp.

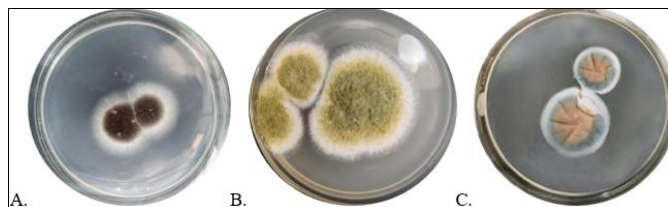
The collected clinical samples were inoculated onto Sabouraud's Dextrose Agar (SDA) supplemented with chloramphenicol and cycloheximide for selective fungal isolation (Larone, 2011) [9]. The inoculated media were incubated aerobically at 28 °C and monitored periodically for fungal growth (Quinn *et al.*, 2011). Fungal colonies exhibiting visible growth within 2 days to a week of incubation were subcultured onto fresh SDA plates to obtain pure isolates. Macroscopic colony characteristics including colony colour, texture, surface topography and reverse pigmentation were recorded following standard mycological procedures (Samson *et al.*, 2014) [10]. Microscopic examination was performed by lactophenol cotton blue (LPCB) staining technique by placing a drop of LPCB stain on a clean glass slide followed by teasing a small portion of fungal material using a sterile needle (Benson, 2001) [11]. The stained fungal preparations were examined microscopically for the presence and arrangement of septate hyphae, conidiophores, vesicles, phialides and conidia for fungal identification (Klich, 2002; Geiser *et al.*, 2007) [12, 13].

2.3 Antifungal Susceptibility Testing

Antifungal susceptibility testing of the fungal isolates was performed by agar based disc diffusion method using modified Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg/ml methylene blue dye (Espinel-Ingroff *et al.*, 2007; CLSI, 2017) [14, 15]. The fungal inoculum was standardized visually by comparison with 0.5 McFarland turbidity standard prior to inoculation. The standardized fungal inoculum was evenly inoculated onto the surface of modified Mueller-Hinton agar plates using sterile cotton swab or sterile L-shaped spreader and antifungal discs were placed aseptically over the media. The antifungal agents used in the study were acquired from HiMedia, India 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 inoculated plates were incubated at 28 °C and examined for zone of inhibition from 2 days up to 1 week of incubation. The antifungal susceptibility pattern of the isolates was determined based on the zone of inhibition around the antifungal discs (Pfaller *et al.*, 2004) [16].

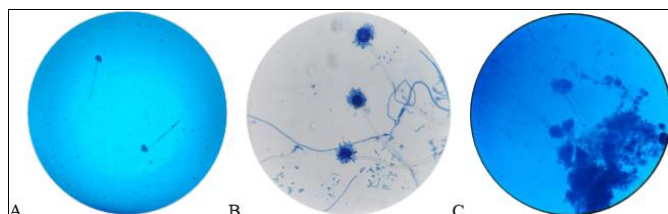
3. Results and Discussion

A total of 29 isolates of *Aspergillus* spp. were recovered from dogs and cats affected with mycotic dermatitis. The fungal isolates exhibited characteristic colony morphology on SDA and microscopic examination revealed septate hyphae, conidiophores, vesicles, phialides and conidia characteristic of *Aspergillus* spp. (Fig. 1 and 2).



(A) *A. niger*: black powdery colonies; (B) *A. flavus*: yellowish-green powdery colonies; (C) *A. fumigatus*: green velvety colonies with white periphery.

Fig 1: Colony morphology of *Aspergillus* spp. on Sabouraud's Dextrose Agar



(A) *A. niger* showing radiate conidial head; (B) *A. flavus* showing rough conidiophore; (C) *A. fumigatus* showing compact conidial head.

Fig 2: Microscopic morphology of *Aspergillus* spp

Based on colony characteristics and microscopic morphology, further species level identification was made revealing 12 isolates of *A. niger*, 10 isolates of *A. fumigatus* and 7 isolates of *A. flavus*. On the obverse side, all isolates initially appeared as white colonies which gradually developed characteristic pigmentation with maturation. Isolates of *A. niger* exhibited black granular colonies, whereas *A. flavus* isolates showed yellowish-green colonies with powdery texture. Isolates of *A. fumigatus* exhibited olive-green colonies with velvety texture and a white periphery. On the reverse side, all isolates of *Aspergillus* spp. exhibited pale to colourless pigmentation. The cultural characteristics observed in the present study were in accordance with the standard morphological descriptions of *Aspergillus* spp. reported by Klich (2002) [12], Quinn *et al.* (2011) [5] and Samson *et al.* (2014) [10]. Similar observations on colony pigmentation, conidial arrangement and microscopic morphology of *Aspergillus* spp. from clinical and environmental sources have also been documented previously by Geiser *et al.* (2007) [13]. Isolation of *A. niger*, *A. fumigatus* and *A. flavus* from dermatological lesions in companion animals observed in the present investigation corroborates earlier reports describing these *Aspergillus* spp as opportunistic fungal pathogens associated with superficial and cutaneous infections in animals (Tell, 2005; Segal, 2009) [3, 4]. Antifungal susceptibility testing of isolates of *Aspergillus* spp. against seven antifungal agents revealed varying zone of inhibition values among the isolates tested (Table 1). Fluconazole exhibited the lowest zone of inhibition in all the isolates of *Aspergillus* spp. tested, indicating comparatively lower *in vitro* activity against the *Aspergillus* isolates recovered in the present study. Similar reduced susceptibility and variable antifungal response among isolates of *Aspergillus* spp. towards azole antifungal agents have been documented previously by Escibano *et al.* (2012) [17], Heo *et al.* (2015) [18], Verweij *et al.* (2016) [7] and Chowdhary *et al.* (2017) [8]. In *A. niger* isolates, ketoconazole and miconazole recorded the highest mean zone of inhibition values, whereas

amphotericin B and ketoconazole exhibited comparatively higher zone diameters against *A. flavus* isolates. Similarly, *A. fumigatus* isolates demonstrated higher zone of inhibition values towards amphotericin B, ketoconazole and miconazole. Comparable findings on the *in vitro* activity of amphotericin B and azole antifungal agents against isolates of *Aspergillus* spp. have also been reported by Herbrecht *et al.* (2002) [19], Gupta *et al.* (2015) [20] and Vahedi-Shahandashti *et al.* (2023) [21]. Variability in antifungal susceptibility patterns observed among isolates of *Aspergillus* spp. in the present investigation may be attributed to species variation and intrinsic differences

in antifungal susceptibility among opportunistic moulds. Similar observations regarding variation in antifungal susceptibility testing methods, antifungal response and interpretive criteria among *Aspergillus* isolates have been discussed previously by Rex *et al.* (2001) [22], CLSI (2013) [23], Berkow *et al.* (2020) [24] and Arendrup *et al.* (2023) [25]. The findings of the present study emphasize the importance of antifungal susceptibility testing for selection of appropriate antifungal agents in the therapeutic management of *Aspergillus*-associated mycotic dermatitis in companion animals.

Table 1: Zone of inhibition (mm) of antifungal discs against isolates of *Aspergillus* spp.

Isolate	IT (10 µg)	KT (30 µg)	FLC (10 µg)	MIC (30 µg)	NS (50 µg)	CC (10 µg)	AP (20 µg)
Isolates of <i>Aspergillus niger</i>							
AN1.	13	23	8	27	20	14	12
AN2.	11	20	0	20	16	14	11
AN3.	13	24	9	25	14	16	0
AN4.	22	21	6	26	20	19	9
AN5.	10	28	14	20	16	18	9
AN6.	18	27	8	26	20	25	16
AN7.	17	26	6	24	17	26	18
AN8.	14	26	9	23	21	16	13
AN9.	15	29	11	25	22	12	14
AN10.	12	24	9	24	21	16	13
AN11.	21	31	11	29	23	26	24
AN12.	15	26	10	22	25	16	14
Mean	15.08	25.42	8.42	24.25	19.58	18.50	12.75
Standard Deviation	3.83	3.13	3.72	2.80	3.20	4.77	6.02
Standard Error	1.11	0.90	1.07	0.81	0.92	1.38	1.74
Isolates of <i>Aspergillus flavus</i>							
Afl 1.	14	22	7	21	23	16	18
Afl 2.	0	0	7	0	0	9	0
Afl 3.	18	20	6	22	12	24	14
Afl 4.	16	22	9	24	18	22	17
Afl 5.	13	16	8	18	10	20	13
Afl 6.	17	19	6	21	12	23	16
Afl 7.	19	22	7	23	14	21	14
Mean	13.86	17.29	7.14	18.43	12.71	19.29	13.14
Standard Deviation	6.69	7.99	1.21	8.13	7.19	5.15	5.84
Standard Error	2.53	3.02	0.46	3.07	2.72	1.95	2.21
Isolates of <i>Aspergillus fumigatus</i>							
Afu1.	17	37	9	29	25	33	0
Afu2.	14	38	0	28	26	31	0
Afu3.	22	20	6	24	13	25	10
Afu4.	20	18	7	22	12	23	17
Afu5.	24	21	6	26	14	27	19
Afu6.	19	17	6	21	11	22	16
Afu7.	21	19	9	23	13	26	15
Afu8.	18	16	11	20	13	21	19
Afu9.	23	21	6	25	14	29	10
Afu10.	17	15	9	19	10	20	15
Mean	19.50	22.20	6.90	23.70	15.10	25.70	12.10
Standard Deviation	3.10	8.31	2.92	3.34	5.51	4.26	7.15
Standard Error	0.98	2.63	0.92	1.06	1.74	1.35	2.26

4. Conclusion

The present study demonstrated the isolation of *Aspergillus* spp., predominantly *A. niger*, *A. fumigatus* and *A. flavus*, from dogs and cats affected with mycotic dermatitis, confirming the involvement of opportunistic moulds in dermatological infections of companion animals. Antifungal susceptibility testing revealed variable *in vitro* responses among the isolates, with comparatively lower susceptibility towards fluconazole, whereas ketoconazole, miconazole and amphotericin B exhibited better antifungal activity. The observed variation in susceptibility patterns emphasizes the

importance of routine antifungal susceptibility testing for appropriate therapeutic management of *Aspergillus*-associated mycotic dermatitis. In recent years, emerging antifungal resistance among opportunistic fungal pathogens has become a growing concern due to indiscriminate antifungal usage and prolonged therapeutic exposure. Development of resistance mechanisms such as target site alteration, efflux pump overexpression and biofilm formation may further complicate antifungal therapy. Therefore, continuous surveillance of antifungal susceptibility profiles and further studies on antifungal resistance mechanisms are essential for effective

disease management and prevention of therapeutic failure in companion animals.

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Conflict of Interest

Not available

Financial Support

Not available

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