



Characterization and anti-fungal susceptibility pattern of dermatophytes isolated from dogs, cats and pet owners in and around Kolkata, India

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ABSTRACT

Pet animals mostly suffer from dermatophytic infections and these animals can easily transmit the infection to their handlers or pet owners. Study of 362 clinically suspected cases of dermatophytic infections collected mainly from dogs (n=123), cats (n=202) and few pet owners (n=37) in and around Kolkata, was conducted to detect a total of 285 (78.7%) samples to be positive for significant dermatophytic fungal infections, with cats to be the highest in prevalence (55.4%) followed by dogs (37.9%) and human beings (6.7%) respectively. *Microsporum canis* (60.0%) was the most prevalent pathogen in comparison to *M. gypseum* (22.5%), *Trichophyton mentagrophytes* (15.8%) and *T. rubrum* (1.7%) affecting dogs, cats and human beings. *T. rubrum* was detected only from human cases in this study. Male dogs (58.3%), cats (51.3%) and human patients (78.9%) were mostly infected than the female ones. The anti-fungal susceptibility pattern of these isolates revealed lower MIC₅₀ values of 0.06-0.125 µg/ml for Ketoconazole, Itraconazole, Miconazole, and Amphotericin-B but not for Fluconazole (8-16 µg/ml). The MIC₉₀ values of these antifungal agents were as low as 0.03 µg/ml for all drugs except Fluconazole (32 µg/ml).

Key words: Anti-fungal susceptibility, Cats, Characterization, Dermatophytes, Dogs, Humans.

INTRODUCTION

Dermatophytosis, a specific mycotic disease of epidermal tissues of skin, is prevalent in both sporadic and epidemic forms almost all over the world including India. The dermatophytes are the causative agents of these skin infections leading to infections of the epidermal layer of skin and are quite prevalent in all domestic pets like dogs, cats and other animals. The infection is quite important as these are also communicable to the pet owners or other human beings also. This may also be termed as an occupational mycozoonoses of particularly for the livestock farmers, pet owners, veterinarians and animal handlers (Ruben, 2010). The prevalence of fungal or dermatophytic infections caused by the zoophilic dermatophytes are governed by the geographic region, climatic conditions and animal husbandry practices of that area. In the tropical country, like India with hot and humid weather, dense populations and with lack of knowledge among common people, these infections are very much prevalent mainly in cities or metro-cities (Ngwogu and Otokunefor, 2007). More than 50% cases of mycotic human infections were mainly from the pet animals recorded in India (Day *et al.*, 2012; Moretti *et al.*, 2013). These infections are quite prevalent in young, sick and debilitated pet and stray animals (Sparkes *et al.*, 1993), caused mostly by

Microsporum sp., *Trichophyton sp.* and *Epidermophyton sp.* among which first 2 are mostly prevalent in pet animals. *Microsporum canis* and *Trichophyton mentagrophytes* are reported to be two major zoonotic pathogens of the pet animals causing human skin infections @20-40% (Gangil *et al.*, 2012). Improper management of infected pets can spread the infections among other animals and also in their handlers or owners (Day *et al.*, 2012) with the appearance of specific skin lesions [“Ring worm”]. The infection is not quite fatal but can terminate into dreadful skin infections if not properly countered (Sharma *et al.*, 2009). In densely populated and urbanized cities like Kolkata, the pet animals might be the one of the main sources of human mycotic infections with higher prevalence (Nilce *et al.*, 2008). In this context, this study was aimed to detect the prevalence and distribution of the dermatophytoses in those pet animals and pet owners in and around Kolkata, West Bengal, India followed by their characterization and antifungal sensitivity testing to conclude on their control aspect.

MATERIALS AND METHODS

Collection of samples: A total of 352 animal samples with superficial mycoses (cats = 202, dogs =123) were collected along with few human samples (n=37) mainly from the pet owners from Kolkata area during the period of January to

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August, 2013. The study was also approved by Institutional Bio-safety Committee of this University.

Direct microscopical examination: Collected suspected materials like skin, hair, claw, hoof and nails were considered for direct microscopic examination (Robert and Pihet, 2008) with 10% aqueous solutions of KOH on clean glass slides for demonstration of fungal hyphae, macroconidia, arthrospores etc. Examination of the samples by Calcoflour white staining (Robert and Pihet, 2008) using standard methodology for rapid and accurate diagnosis of dermatophytosis was also done using fluorescence microscope with 330-380 nm excitation filter and an emission filter of >420 nm.

Isolation and characterization: Collected samples were cultured on Sabouraud's dextrose agar with 0.05% Chloramphenicol and Cycloheximide followed by characterization based on their colony characteristics, shape, size, conidial cell structure, presence of septae with number and arrangement of conidial cells around the hyphae (Nilce *et al.*, 2008). Characterizations of the positive samples were done following 'Dermatophyte identification scheme' (Koneman and Roberts, 1985; Seker and Dogan, 2011) by study of physical and morphological details (by lactophenol cotton blue staining) and slide culture technique (Riddell's method). Confirmation of these isolates were performed using rice grain test, urease test, *in-vitro* hair perforation test, temperature tolerance test, growth pattern on trichophyton agar and corn meal agar test (for pigmentation if any) (Day *et al.*, 2012; Mattei *et al.*, 2014).

***In-vitro* antifungal sensitivity testing:** Testing by broth micro-dilution assay using six commonly used antifungal drugs namely, fluconazole, itraconazole, griseofulvin, ketoconazole, miconazole and amphotericin-B (Himedia) which were dissolved using 100% Dimethyl sulfoxide except fluconazole (in sterile water), to the strength of 1mg/ml. Serial twofold dilutions of these antifungals were prepared to achieve the final concentrations ranged from 64.0 to 0.13 µg/ml for fluconazole and 32.0 to 0.06 µg/ml for all the other drugs. The tests were performed using standard technique as per Santos *et al.* (2006). Determination of MIC₉₀ values were performed by visual comparison at 24hrs interval with growth in positive control tube. For amphotericin-B, 100% and for the rests 80% inhibition in growth in comparison to the control tube was considered as the end point.

Statistical analysis: Statistical analysis of the obtained data in this study were performed following the statistical methods with General Linear Model (G.L.M.) of IBM SPSS software package, version 20, as per methods described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

A total of 285 (78.7%) positive isolates of different dermatophytes were detected in this study among which cats were the most infected host (55.5%) followed by dogs (37.9%) and human beings (6.7%) (Table 1).

All the strains of *Microsporum canis* and *M. gypseum* showed woolly aerial mycelium, light to reddish brown colour colonies with well developed macroconidia with 6-12 septa and microconidia. They grew luxuriantly on rice grain medium with reddish to orange pigmentation. Again, *T. mentagrophytes* and *T. rubrum* showed smooth cottony white colonies with mostly microconidia microscopically. Only the *T. mentagrophytes* isolates were positive to urease (in 5-7 days) and hair perforation tests. *T. mentagrophytes* isolates showed slow growth in comparison to no growth of *T. rubrum* at 37°C and luxuriant growth on Trichophyton agar medium with whitish colonies but *T. rubrum* showed huge bright red colonies. Growths on cornmeal dextrose agar of *T. mentagrophytes* (yellowish) and *T. rubrum* (reddish) were also different.

The prevalence of *Microsporum canis* (60.0%) was the highest in comparison to others like *M. gypseum*, *T. mentagrophytes* and *T. rubrum* (Table 1). The isolation rate of this pathogen in different hosts also (42.1- 61.4%) was higher than *M. gypseum* (21-22.8%) and *T. mentagrophytes* (10.5 - 16.7%). *T. rubrum* was isolated only from human samples (26.4%). The incidence of fungal infection was found to be higher in male dogs (58.3%) and cats (51.3%) in comparison to their female counterparts (Table 2) and also in human patients or the male pet owners (78.9%).

In-vitro antifungal susceptibility testing of all isolates revealed that few isolates (12.2%) showed higher MIC₉₀ values of 64 µg/ml for fluconazole and of ketoconazole, it was 2 µg/ml (6.3% isolates) whereas most of the dermatophytes showed lower MIC₅₀ values of 0.06-0.125µg/ml and MIC₉₀ values of 0.03µg/ml for antifungal agents *viz.* ketoconazole, itraconazole, miconazole and amphotericin-B except fluconazole with slightly higher values (8-16µg/ml and 32µg/ml) (Table 3).

Table 1: Incidence rates of different dermatophytes in different hosts

Dermatophytes	Cumulative		Dogs		Cats		Humans	
	No.	%	No.	%	No.	%	No.	%
<i>M. canis</i>	171	60.0	66	61.1	97	61.4	8	42.1
<i>M. gypseum</i>	64	22.5	24	22.2	36	22.8	4	21.0
<i>T. mentagrophytes</i>	45	15.8	18	16.7	25	15.8	2	10.5
<i>T. rubrum</i>	5	1.7	0	0	0	0	5	26.4
Total	285	100.0	108	37.9	158	55.4	19	6.7

Table 2: Sex-wise infection rates of dermatophytes in different hosts

Hosts	Dogs		Cats		Humans	
Positive cases	108		158		19	
Sex	Male	Female	Male	Female	Male	Female
No. of cases	63	45	81	77	15	04
%	58.3	41.7	51.3	48.7	78.9	21.1

Table 3: *In-vitro* antifungal susceptibility patterns of different dermatophytes

Anti-fungal Agents	Effective Range	<i>M. canis</i> (n=171)		<i>M. gypseum</i> (n=64)		<i>T. mentagrophytes</i> (n=45)		<i>T. rubrum</i> (n=5)	
		MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
		(µg/ml)		(µg/ml)		(µg/ml)		(µg/ml)	
Fluconazole	4 - 64	16	32	16	32	16	32	8	32
Ketoconazole	0.06 - 2	0.06	0.125	0.06	0.125	0.125	0.25	0.06	0.125
Itraconazole	0.03 - 0.5	0.06	0.125	0.25	0.5	0.125	0.25	0.06	0.125
Miconazole	0.03 - 1	0.06	0.25	0.06	0.25	0.06	0.125	0.06	0.25
Griseofulvin	0.6 - 1	0.125	0.25	0.125	0.25	0.125	0.25	0.25	0.5
Amphotericin-B	0.03 - 1	0.06	0.125	0.06	0.125	0.03	0.125	0.06	0.125

Prevalence of dermatophytic infections in cats (55.5%) was the highest in this study which was also supported by Nweze (2011) and Esch and Petersen (2013) who reported 58-67% positivity of dermatophytes in cats in their studies. The prevalence of such potential infections in dogs and human beings are in partial to full accordance with the reports of Brilhante *et al.* (2003), Seker and Dogan (2011), Falahati *et al.* (2003) and Stojanov *et al.* (2009) who reported approx 30-45% and 5-12% prevalence rates in dogs and human beings respectively.

The *Microsporum canis* and *M. gypseum* showed typical morphological and growth characteristics during the study (Koneman and Roberts, 1985; Brilhante *et al.*, 2003). *T. mentagrophytes* and *T. rubrum* both showed typical with positive results in different tests which were also reported by Brilhante *et al.* (2003) and Seker and Dogan (2011). Other growth characters of *T. mentagrophytes* and *T. rubrum* were supported by Seker and Dogan (2011) and Falahati *et al.* (2003). All fungal isolates were positive to blue fluorescence in Calcoflour white staining methods also (Robert and Pihet, 2008).

The highest prevalence of *Microsporum canis* followed by other dermatophytes was also reported by Falahati *et al.* (2003), Seker and Dogan (2011) and Mattei *et al.* (2014) before. *M. gypseum* was the 2nd highest prevalent pathogen (Brilhante *et al.*, 2003) followed by *T. mentagrophytes* and *T. rubrum* which were also reported by Falahati *et al.* (2003) and Venkatesan *et al.* (2007).

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The samples from male dogs and cats were found to be more positive in comparison to the female hosts in this study which are also in agreement with the reports of Falahati *et al.* (2003), Seker and Dogan (2011) and Alpun and Ozgur (2009) who also reported more infections in both dogs and cats (19-20%) than bitches and female cats (16-17%). The male human patients were also affected more than the female ones which were also reported by Falahati *et al.* (2003) [65.7% in males and 34.4% in females], Ngwogu and Otokunefor (2007) [29% in males and 1.4% in females].

Antifungal susceptibility testing of the isolates showed higher MIC90 values when tested with fluconazole and ketoconazole for few isolates followed by standard or lower MIC50 values for other antifungal agents. This type of findings indicates the development of a kind of drug resistance in those dermatophytes which were also in agreement with the earlier reports of Jessup *et al.* (2000), Espinel-Ingroff (2001) and Santos *et al.* (2006).

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