

Fungal biota of the domestic animals in a city in Pakistan

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ABSTRACT

Objectives: To determine the prevalence of Keratinophilic fungi from domestic animals in Sukkur City Sindh Pakistan.

Methodology: A total of seven hundred and fifty hair samples from three groups (buffalo, goat and cow) of animals were collected in sterilized polythene bags in Sukkur city. Hair pieces measuring 2cm were placed on the agar plates supplemented with 0.05 g/l chloramphenicol for obtaining the fungal growth on hair. Hairs showing the whitish mycelial growth were transferred to freshly prepared Sabouraud's Dextrose Agar (SDA) for cultural growth and pure culture were maintained at 5°C in refrigerator.

Results: Out of 750 hair samples collected from three domestic animals which were studied in Sukkur city, Sindh, Pakistan, five hundred ninety four (594) isolates belonged to 8 genera and 9 species were isolated. *A. candidus* and *Alternaria alternata* were almost equally prevalent species represented by 14.81% and 14.48% respectively of the total number of isolates. *A. niger* and *Chrysosporium asparatum* were equal in their prevalence and represented 12.63% and 12.46%. *Exophiala sp* comes next constituting 12.29% followed by *Stemphyllium sp.* 10.94%. Other species isolated less frequently included *Cladosporium* 8.08%, *Acrimonium sp.* 7.41% and *Penicillium sp.* 6.90%. The isolation frequency of fungal biota was significantly greater in buffalo from cow and goat (respectively 40.06%, 33.33% and 26.60% $p = 0.02$).

Conclusion: Keratinophilic fungi were isolated from domestic animals and the prevalence of these fungi in domestic animals may have a role in transmission of skin and other infections in human population.

KEY WORDS: Keratinophilic fungi, Keratinase, Dermatophytosis, Domestic animals.

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INTRODUCTION

There are some fungal biota that can colonize, grow, reproduce and degrade hair are known as keratinophilic fungi. These fungi have the ability to digest and utilize the keratin present in skin, nails, hair, hoof and horn that constitute the outer surface of animals.¹⁻⁴ Keratin is highly insoluble protein having fibrous helical structure and numerous disulfide linkages which make it resistant to many proteases but easily digested by enzyme known as keratinase produced by keratinophilic fungi and dermatophytes.⁵ By virtue of keratinase dermatophytes cause dermatophytoses by invading skin, nails and hair.⁶

These keratinophilic fungi including dermatophytes have been reported to cause animal as well as human diseases. In numerous studies these fungi have been isolated from mammals,⁷ from cats and dogs,⁸⁻¹⁰ from cattle in Bangladesh¹¹, from domestic animals in Norway,¹² from hair of cow, goat, rabbit, in Jordan.^{13,14} Due to their pathogenic potential keratinophilic fungi have been receiving considerable attention in recent days and human exposure to these potentially pathogenic fungi pose a serious health risk.

Several non- pathogenic fungi are now being reported as opportunistic pathogen and their prevalence in different environment where they are naturally occurring is not directly investigated. Investigating both environment and fungi where people are exposed to them is of major health concern.¹⁵ *Cladosporium sp.* was isolated from domestic cats with cerebral phaeohyphomycosis caused by these organisms.¹⁶ Infection caused by *Aspergillus sp.* varies from localized pulmonary or paranasal sinus infection to invasive aspergilluosis with intracranial extension. The etiology includes *A. fumigatus*, *A. flavus*, *A. niger*, *A. glaucus*, *A. restrictus*, *A. terreus* and *A. versicolor*.¹⁷ Numerous members of the genus *Exophiala sp.* are potential agents of human and animal mycosis. The majority of these infections are cutaneous and superficial but also fatal systematic infections are also known.^{18,19}

It has been reported that certain fungal diseases could be transmitted from domestic animals to human. Very little work has been done on the isolation and identification of this fungal biota from animal hair in Pakistan. As such this study could be most important step to relate the animal- human pathogenic fungi. The primary objective of this study was to determine what types of fungi are found on the hair of different domestic animals in Sukkur city, Sindh, Pakistan.

METHODOLOGY

Seven hundred and fifty hair samples, two hundred and fifty from each domestic animals buffalo, goat and cow were collected in sterilized polythene bags in Sukkur city. Hair pieces measuring two cm were placed on the agar plates which were prepared by adding 2% agar and supplemented with 0.05 g/l chloramphenicol only, not the other supplements as to recover actual fungi growing on hair and to prevent contamination. Hairs showing the whitish mycelial growth were transferred to freshly prepared Sabouraud's Dextrose Agar (SDA) for cultural growth and pure culture were

maintained at 5°C in refrigerator. The identification of isolates was done as Domesch *et al* (1980) and Dexter (1983)^{20,21}

Statistics: Statistical analysis was done by SPSS version 13 software.

RESULTS

A total number of 594 isolates of fungal biota were isolated from 750 hair samples collected from buffalo, cow and goat in Sukkur city. These belong to 8 genera and nine species. Table-I shows the isolation rate of different keratinophilic fungal biota from various domestic animals. It was also observed that *A. candidus* was dominating 88(14.81%), the other isolates and *Penicillium sp.* was the lowest 41(6.90%) in isolation rate.

Key to Identification of Fungal Species: Colonies grow slowly reaching 1.5-3.0 cm diameter in 14 days on Sabouraud Dextrose agar at 25°C white to yellowish cream with yellowish reverse Conidiophore erect, colorless, large globose vesicles bearing metulae phialides. Conodia globose to sub globose smooth walled hyaline *Aspergillus candidus*

Colonies grow rapidly 2.5-3.0 cm diameter in 10 days on Sabouraud Dextrose agar at 25°C with mycelium white reverse yellow. Conidiophore dark brown to black brown arising from foot cell. Phialides is borne directly on the vesicle. Conidia is mostly globose black in colour..... *Aspergillus niger*.

Colonies slow reaching less than 10 cm in diameter in two weeks on Sabouraud Dextrose agar at 25°C colonies are flat, dry powdery, white olive to brown with white margins and reverse brown. Conidia arise directly on hyphae or on lateral branches. Conidia sub-globose to clavate thick walled hyaline to pale yellow..... *Cryosporium asperatum*. Colonies white are fast growing floccose. The conidia produced in a ball at the mouth of Phialides without the flared collarate..... *Acremonium sp.*

Colonies are olivish black gray to brownish gray. Conidiophores smooth simple straight. Conidia are muriform oblong to pyriform, brown, smooth and they are produced in long chains..... *Alternaria alternata*.

The colonies are mycelial and covered with aerial hyphae. The hyphae are brown, septate, and smooth produced branched or unbranched conidiophore, laterally or on the apex. The conidia are one celled subglobose to cylindrical smooth hyaline *Exophiala sp.*

Colonies rapidly growing dark velvety. Conidiophores brown simple swollen at the apex through

which porospores are produced. Conidia solitary dark brown muriform with smooth or rough walled sub-globose.....*Stemphylium sp.*

The colony is nearby black with velvety texture on Sabourauds Dextrose agar at 25°C spores smooth brown conidia. Conidial chains are produced directly from the side or tip of hyphae or on conidiophores*Cladosporium sp.*

The colony reaches 3.5-4.0 cm in diameter in two weeks at 25°C and becomes blue green as the conidiophores mature. Conidiophores smooth and bear terminal matulae. Each matulae produces several phialides with bear smooth globes to sub globes conidia in chains.....*Penicillium sp.*

DISCUSSION

Keratinophilic fungi could be present in the environment with different distribution patterns. The occurrence of these fungi may be influenced by certain factors such as presence of human and animals. Other factors may be socioeconomic conditions of that particular area where the human and animals under study are living. Sukkur city is highly populated city and the number of domestic animals has increased. The possibility of transmission of infectious agents between human and their domestic animals is a potential risk. This is true not only for dermatophytes, but also for other zoonotic diseases. The fungal species isolated in present study are medically important because numerous studies have been carried out by different scientists in many parts of world, who have investigated the relationship of these keratinophilic fungi with cutaneous and systemic infection caused by these fungi. Philpot and Berry (1984) stated that *Alternaria* and *Cladosporium species* are integral part of the fungal biota of normal dogs but they can cause opportunistic cutaneous infection.²²

In human *Alternaria sp.* has been isolated from immunocompromised organ transplant recipient.^{23,24} Cabanes *et al.* (1986) reported that *Alternaria* and *Cladosporium* were cultured from 80% of dogs.²⁵ Aho (1983) examined dogs with suspected dermatophytosis and recorded *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* were the causative agents in <25% dogs.²⁶ Species of genera like *Chrysosporium* and *Scopulariopsis* were isolated in more than 60% dogs.²⁵ Oyeka (1989) reported the *Chrysosporium sp.* in 9.0% mammals in Anambra state.⁷ In Iran *Chrysosporium keratinophilum* was isolated from the soil of public parks where human and animal activities were increased.²⁷ In some studies many genera of these fungal biota have been reported in combination i.e. *Chrysosporium*, *Alternaria*, *Penicillium*, *Cladosporium* and *Aspergillus* were found in dogs and cats.¹² *Exophiala sp.* are dematiaceous mold which have been infrequently associated with systemic infection recently twenty three cases of fungemia due to these organisms have been reported.¹⁸ The opportunistic fungal infections caused by *Acremonium sp.* have been reported in many parts of the world.²⁸ *Aspergillus* is a fungus found in soil and organic debris from where it can be transferred to animals and human. After getting entrance in animal or human body they can cause various infection including localized pulmonary or paranasal sinus infection and invasive aspergillosis.¹⁷ It is also noteworthy that during this study *Stemphylium sp.* appeared as keratinophilic fungi from all three animals under study and none of dermatophytes was isolated.

To analyze the difference in the isolation frequency of fungal biota among domestic animals the one sample T test was done. A significant difference was observed. The isolation frequency of fungal biota was significantly greater in buffalo from cow and goat (respectively 40.06%, 33.33% and 26.60% $p = 0.02$).

Table-I: Isolation rate of keratinophilic fungal biota from various domestic animal hairs in Sukkur City.

Keratinophilic Fungi	Isolation rate No (%)			Total
	Buffalo(n =250)	Cow(n =250)	Goat(n =250)	
<i>Asprgillus candidus</i>	50(8.41%)	20(3.37%)	18(3.03%)	88(14.81 %)
<i>Alternaria alternata</i>	25(4.20%)	35(5.89%)	26(4.38%)	86(14.48%)
<i>Aspergillis niger</i>	20(3.37%)	30(5.05%)	25(4.21%)	75(12.63%)
<i>Exophiala sp.</i>	30(5.05%)	20(3.37%)	23(3.87%)	73(12.29%)
<i>Chrysosporium asperatum</i>	31(5.21%)	21(3.53%)	22(3.70%)	74 (12.46%)
<i>Stemphylium sp.</i>	40(6.73%)	12(2.02%)	13(2.19%)	65(10.94%)
<i>Cladosporium sp.</i>	20(3.37%)	13(2.19%)	15(2.53%)	48(8.08%)
<i>Acremonium sp.</i>	12(2.02%)	25(4.21%)	07(1.18%)	44(7.41%)
<i>Penicillium sp.</i>	10(1.68%)	22(3.70%)	09(1.52%)	41(6.90 %)
Total	238 (40.06%)	198(33.33)	158(26.60%)	594(100 %)

This work is in the agreement with literature reviewed in this paper from isolation point of view with difference in percentage of prevalence. It is suggested that hair of animals carrying fungal biota shed from the animals may also be colonized and utilized by other soil saprobes as carbon and nitrogen source. *Chrysosporium* species have been frequently reported from human and animal skin lesion.²⁹ Other non-dermatophytic keratinophilic fungi such as *Fusarium*, *Aspergillus*, *Alternaria*, *Exophiala*, *Cladosporium curvularia*, *Onychocola* and *Scopulariopsis* are known to cause cutaneous and other infection in human.²⁹⁻³¹ Masih (1971) and Soomro (1999) reported the pathogenicity of *Chrysosporium asperatum* to cause the skin lesions in rabbits as experimental animals.^{30,32} The results of our study are in the agreement with results of Al Ani *et al.* (2002) who isolated the *A. flavus*, *A. fumigatus*, *Alternaria sp.*, *Fusarium sp.*, *Penicillium sp.* and *Cephalosporium sp.* from cattle (calves).³³

In Pakistan the health facilities are inadequate and the knowledge about occurrence of different diseases in general and about fungal diseases in particular is very limited. Consequently many fungal diseases are prevalent in the area under study; the route of transmission of these diseases may be animal to human. As such it is important that the isolation of pathogenic fungi from domestic animals pose a potential health risk of fungal infection from keratinophilic fungi via domestic animals to the human population.

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Author's Contribution:

Irshad Hussain Soomro was done the conception, design and acquisition of data.
Abdul Hussain Shar was done drafting, critical and intellectual content.
Fateh Mohammad Soomro was done final approval the version for publication.