ORIGINAL ARTICLE

Isolation of potentially pathogenic fungi from selected pigeons' feeding sites in Karachi: A new dimension to health hazard

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Abstract

Objective: To determine the presence of pathogenic fungal strains in areas where pigeons are present in a large number.

Methods: This study was conducted at the Federal Urdu University of Arts, Science and Technology, Karachi, from February 2015 to March2016, and comprised samples of soil contaminated with pigeons' excreta. The samples were collected from 20 different pigeon-feeding places in the city. These samples were processed for the isolation and identification of fungi by using standard conventional methods. The fungal strains isolated were also tested for their susceptibility to commonly used antifungal agents by disc diffusion technique.

Results: There were 105 samples. A wide variety of fungal strains belonging to different genera of Aspergillus, Rhizopus, Penicillium, Fusarium and Candida were isolated and identified by using conventional methods. The antifungal resistance pattern of these strains also depicts emergence of resistance against commonly used antifungal agents such as amphotericin B and fluconazole.

Conclusion: The soil and air of places densely populated with pigeons were found to be loaded with fungal spores and many of them were potential pathogens.

Keywords: Feral pigeons, Fungal infections, Antifungal resistance. (JPMA 67: 901; 2017)

Introduction

Feral pigeons (*Columbia livia*), also known as city pigeons or street pigeons, are considered as descendants of the domestic pigeons. They have adapted to urban life, and are abundant in towns and cities throughout much of the world.[1] In Karachi, the population of this bird is drastically increasing with time. These pigeons are in abundance particularly in areas where certain places are reserved for feeding them. Pigeons' feeding has importance in many religions therefore certain places, particularly near mosques, temples and churches, have been reserved for feeding these birds. Provision of food, fresh water and shelter to these birds results in aggregation and formation of large roosts in such places and facilitate them to reproduce and increase their population rapidly. Because of their abundance in specific places, the faecal material they produce also accumulates in the environment and contributes in the facilitation of microbial growth in such soil.[2] Therefore, the congregation of these birds in public places can be considered as environmental pests as they are responsible for the transmission of many diseases, including bacterial, viral, fungal and parasitic diseases, in humans and animals. This has been observed in 77 epidemiological studies that feral pigeons harbour 60 different human pathogens, including nine species of bacteria, five viruses, one protozoa and 45 species of fungi.[3]

Pigeons and fungal infections in human have long recognised relationship which might be due to the ecological background of fungi. Most of the fungi being saprophytes prefer to grow in soil rich in organic substances. The presence of growth-promoting factors in birds' excrement in the soil of places where birds are found in a large number serves as an excellent niche for these fungi, particularly human pathogenic fungi like *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Aspergillus* (A.), *Candida* species (spp.), etc. Because of the fact that most of the fungal infections in humans are caused by aerosolised transmission of fungi, therefore in places where potential pathogens are in abundance there is a bright chance for their invasion, colonisation and infection in humans.[3,4]

Cryptococcus neoformans is considered as the most prevalent zoonotic pathogen in feral pigeon population. Because of high body temperature, i.e. around 40°C, pigeons do not serve as amplifying host of this fungi. But their dried excreta contains certain low molecular weight nitrogenous compounds which promote growth of *Cryptococcus spp*. as well as other fungi. *Cryptococcus neoformans* has been reported to cause severe forms of illness in both immunocompetent as well as

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immunocompromised individuals.[5] However, the risk of Cryptococcasisis is 1,000-fold high in immunocompromised patients such as acquired immune deficiency syndrome (AIDS) patients as compared to immunocompetent individuals.[6]

Histoplasma capsulatum, causative agent of Histoplasmosis, also prefers to grow in places which are rich in dried excreta of birds like pigeons, bats, starlings, etc. This infection is acquired by inhalation of fungal spores and its manifestations may vary from mild flu like illness to severe systemic infection.[7] Besides, many other fungal pathogens like *Aspergillus spp.*, *Candida spp.*, *Rhizopus spp.*, Trichosporon, Dermatophytes, etc. are inhabitant of soil and may reach human body through inhalation or traumatic implantation and cause infections.[8-10]

The current study was designed to evaluate the prevalence of fungi in places where pigeons are present in a large number.

Materials and Methods

This study was conducted at the Department of Microbiology, Federal Urdu University of Arts, Science and Technology (FUUAST), Gulshan-e-Iqbal campus, Karachi, from February 2015 to March 2016, and comprised samples of soil contaminated with pigeons' excreta. The samples were collected from 20 different places of Karachi reserved for pigeons' feeding. These places were randomly selected on the basis of draw method.[11] Multiple samples (average 5) from each selected site were collected at an interval of 2-3 months so as to observe maximum season-dependent variation in isolation of fungal strains.

The soil samples were then processed for the isolation of fungi by transferring one gram of soil in ten millilitres of sterile distilled water and were vortexed to make homogenised suspension. The serial dilutions of homogenised suspension were made and surface plated on Sabouraud's dextrose agar (Oxoid) and incubated at 25°C for at least 5 days. The colonies appeared were counted and reported as colony forming units per gram of dry weight (CFU/g dwt).[12]

The recovered fungal strains were purified by re-culturing on appropriate media and then identified by macroscopic and microscopic studies. The macroscopic or colonial characteristics of isolated fungal strains such as rate of growth, colour of colonies, texture of colonies, pigment production and colour of reverse of colony were recorded. Microscopic characteristics of isolated fungal strains were observed in 10X and 40X of microscope by employing wet mount technique.[13]

Antifungal susceptibility of isolated fungal strains was determined by using disc diffusion method. For this purpose, fungal inoculum was prepared by inoculating 24-hour-old culture in 5ml of sterile saline. The suspension was vortexed and the turbidity was adjusted to yield 1 x10^6 - 5x10^6 cells/ml(i.e. 0.5 Macfarland standard). With the help of sterile swab, the fungal suspension was gently and uniformly spread on Mueller-Hinton agar (Oxoid). The plates were allowed to dry for 5-15 minutes and then the antifungal discs (6mm in diameter) were placed on fungal lawn with the help of sterile forceps. The plates were then incubated at 25°C for 48 hours. After the incubation period, the zone of inhibition was measured (in millimetres) around each antifungal disc and recorded.[14,15]

Results

A total of105 samples of soil contaminated with pigeons` excreta were collected. A variety of fungi was found from almost all samples. However, no season-dependent variation in isolation was observed during the study and the fungal strains were isolated more or less on the same pattern throughout the year. The soil samples collected from Saddar, Bacha Khan Chowk, New Town, Numaish Chowrangi and Paposh Nagar exhibited the highest

Table-1: Distribution of fungal growth with respect to pigeons `feeding places.

Location of Pigeons' feeding places	No. of samples collected	Average Number of colonies observed per sample	Average types of colonies observed per sample
Bacha Khan Chowk	5	9	5
Abdullah College, North Nazimabad	5	6	4
Board office, Nazimabad	4	4	4
Hyderi, North Nazimabad	5	6	4
Liaqatabad Market	4	5	4
Urdu University	6	6	4
Hassan Square	7	7	5
IslamiaCollege	6	6	5
New Town	7	9	5
NumaishChowrangi	8	10	5
Gurumandir	5	7	4
Mosamiyat	7	8	6
Saddar	5	9	5
Paposhnagar	5	9	5
Karimabad	4	6	5
Shahrah-e-Faisal	4	5	4
NipaChowrangi	3	2	2
Garden	5	4	4
Clifton area	5	4	4
Site area	5	5	4

Table-2: Isolation of fungal strains and their antifungal susceptibility profile.

Name of fungi isolated	Strains Isolated		Susceptibility to antifungal drugs (Average zone of inhibition in mm)					
	No.	%	Amphotericin B	Fluconazole	Itraconazole	Griseofulvin	Terbinafine	Nystatin
Aspergillus flavus	14	13.5	12	16	20	24	28	13
Aspergillus niger	10	9.6	12	25	23	23	30	20
Aspergillus fumigatus	10	9.6	15	13	28	15	32	10
Aspergillus terreus	10	9.6	15	24	18	22	35	10
Aspergillus nidulans	02	1.9	15	9	26	20	32	9
Aspergillus versicolor	10	9.6	22	30	27	30	32	30
Aspergillus glaucus	03	2.9	23	26	24	24	34	24
Rhizomucor spp.	04	3.8	22	0	20	0	32	0
Rhizopus sp.	04	3.8	23	11	23	14	34	10
Mucor spp.	03	2.9	21	13	24	12	36	15
Penicillium spp.	05	4.8	25	20	24	21	35	23
Monilia sitophila	02	1.9	22	21	22	20	34	22
Epicocum nigrum	02	1.9	15	0	0	0	34	15
Chrysosporium sp.	01	0.9	15	15	20	25	35	15
Trichophyton terrestre	01	0.9	16	16	21	26	32	17
Trichophyton rubrum	01	0.9	20	19	21	27	36	18
Microsporium canis	01	0.9	22	20	22	26	39	18
Syncephalastrum racemosum	01	0.9	NT	NT	NT	NT	NT	NT
Cryptococcus neoformans	02	1.9	16	17	21	18	37	18
Candida albican	10	9.6	0	16	20	15	34	18
Non albican Candida spp.	08	7.7	12	17	26	19	37	13
Total	104							

Spp/sp: Species.

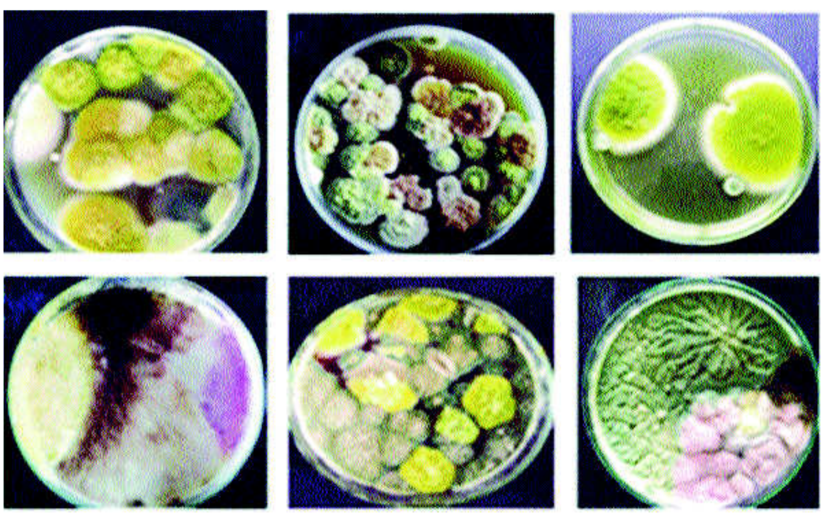

Figure-1: Isolation of Fungal strains from soil samples collected from different Pigeons' feeding sites in Karachi.

number of colony forming units of fungi per gram. Moreover, the samples from these places also showed more variety of fungal strains (Table-1).

Of the 104(99.04%) different fungal strains isolated, most belonged to genera Aspergillus, Rhizopus, Mucor, Candida, Penicillium, etc. Among *Aspergillus* species, *Aspergillus flavus* 14(13.5%), *A.niger* 10(9.6%), *A. fumigates* 10(9.6%), *A. versicolor* 10(9.6%) and *A. terreus* 10(9.6%) were isolated in a large number from most of the samples (Figure 1).Other fungi such as *Candida albicans* 10(9.6%), *non-albicans Candida* (NAC) species 8(7.7%), *Penicillium spp.* 5(4.8%) also isolated from these soil samples.

The antifungal susceptibility profile of these isolated fungal strains also showed emerging trends of resistance against commonly used antifungal agent. Amphotericin B, the most commonly used antifungal in systemic infections, was found less effective against *C. albicans* and *non-albicans Candida* species. More or less similar pattern was observed in case of Aspergillus species where *A.niger*, *A. flavus* and *A. fumigatus* showed much resistance against this drug. Members of Zygomycetes and *Penicillium* species were found susceptible to this antifungal agent (Table-2).

Another antifungal Fluconazole was found effective against *Aspergillus* species; however, resistance against

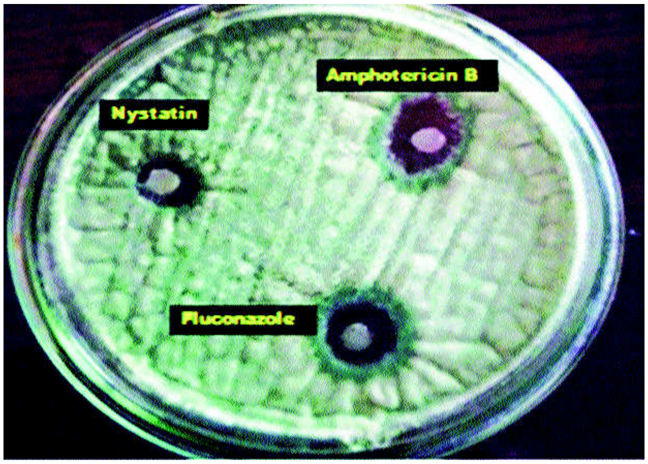

Figure-2: Antifungal susceptibility pattern of *A. fumigatus*.

this drug was observed from members of Zygomycetes such as *Rhizopmucor*, *Mucor* and *Rhizopus spp.* Itraconazole also showed good results against almost all the fungal strains tested. Similarly, all the strains were found susceptible to terbinafine. Griseofulvin was found effective against *Aspergillus*, *Trichophyton*, *Microsporum* species, while strains of Zygomycetes and *Candida spp.* showed resistance against this drug. In case of another polyene, Nystatin, most of the *Aspergillus spp.* and Zygomycetes showed remarkable resistance against this antifungal drug (Figure-2).

Discussion

This study was the first of its kind in assessing the risk factors associated with pigeons' population in Karachi with particular reference to fungal infections. The study was aimed to determine the presence of fungal species in the environment opulent of pigeons and their faeces. This study also depicted the overwhelming increase in the population of pigeons in Karachi, which might be due to increase in feeding places of pigeons which were reserved for this purpose in many areas of city. Although pigeons feeding sites are also common in many Western countries for a long time, proper cleaning system and the average low temperature of most of these countries do not facilitate growth of many microorganisms in such feeding sites. In Karachi, however, poor sanitation and average warm temperature might help flourish most of the potentially pathogenic microorganisms in these sites. Therefore, these feeding sites pose a threat to the human population of Karachi because of infections associated with pigeons and their excreta. To evaluate the fungal presence in these pigeons feeding sites, we selected 20 places reserved for feeding pigeons from different parts of city, such as Bacha Khan Chowk, Paposh Nagar Chowk, Hyderi Market, Nipa, Clifton, etc. The presence of a large number of such sites in different parts of city indicated that this custom is found in every part of society irrespective of their socio-economic group (Table-1). However, these places are not safe for human health because the environment of such places was found laden with different microorganisms.[3] The present study revealed high load of fungal species from all the soil samples collected from such places. These fungi comprised a variety of potential and opportunistic species of fungi which were also in accordance with previous literature[3] (Table-2).

Feral pigeons' population has been assessed very rarely as a hazard to human health. Even the epidemiological researches have been focused on health status of pigeons and other birds while the risk factors associated with avian population are still rarely addressed and very limited research has been done in this regard. Feral pigeon was a rare species just few decades back in Karachi. But now these birds have been predominating over other species. The drastic increase in population of these birds might be due to the increasing trend of pigeons' feeding places in urban areas. Pigeons have been found living in close association with man and they are characteristically found in flocks and form large roosts. In pigeons' feeding places, these birds also forms flocks, feeds together and produce immense amount of excreta in these places.[1]

Fungus, being a saprophytic organism, has been found as the most prevalent organism not only in the soil contaminated with pigeons' droppings, but also the air of such places is heavily contaminated with fungal elements and spores. As most of the fungal infections are transmitted by inhalation of dust and aerosols, these environments pose high risk to acquire fungal infections.[16] Among fungi, *Aspergillus* species, which were isolated in a significant number from samples collected in our study, indicated this hazard. Aspergillus is a large genera of fast-growing fungi, found mainly as saprophyte on decaying organic substances and in the faeces of wild birds.[17] But these species are also recognised as opportunistic mould having ability to cause both allergic and severe invasive infections, particularly in immunocompromised individuals. Among these species, *Aspergillus fumigatus* has been already reported as the most pathogenic specie responsible for causing 90% of all aspergillosis in humans and animals.[17] Other species, such as *A. niger*, *A. flavus* and *A. terreus*, were also isolated in a remarkable number from pigeons feeding places in this study. These strains have been documented as frequently isolated species after *A. fumigatus* from cases of invasive aspergillosis and reported to cause lethal infections.[18]

Moreover, the antifungal susceptibility pattern of isolated *Aspergillus* species in the current study depicted increasing resistance against commonly used antifungal drugs such as amphotericin B and azoles, which are in accordance with a previous relevant study conducted on *A. fumigatus* isolated from domestic geese. In that study, 70.6% to 90.6% of the total isolates of *A. fumigates* also showed resistance against these two groups of antifungal drugs.[19]

Likewise, genera of zygomycetes such as rhizopus, mucor, etc. were also found associated with pigeons. This was also in accordance with the previous literature. This family is recognised as opportunistic pathogen having ability to cause infections in humans ranging from mild or acute infections to fulminant infections in host.[20] These species were normally found susceptible to amphotericin B and were resistant to triazoles and Echinocandins,[21] which also supported our findings where isolated strains of *Mucor spp.*, *Rhizopus spp.* and *Rhizomucor spp.* showed similar pattern towards antifungal drugs. Additionally, these isolates also showed resistance against Griseofulvin.

Candida species are also recognised as opportunistic pathogen which are normally found as micro-flora of human and animals. The isolation of this genera was also reported in this study which was also in accordance with previous findings.[3] In recent years, the serious manifestations of candidiasis have been increasing and their management is problematic because of a growing prevalence of non-albicans *Candida* species which are resistant to amphotericin B as well as newer azole antifungal drugs.[22] In our study, however, both *Candida* albicans and non-albicans *Candida* species depicted resistance towards most of antifungals (Table-2), which is in contrast with previous literature which reported *C. albicans* as susceptible specie to most of the anti-fungals while NAC spp. were reported as more resistant species.[4]

Cryptococcus neoformans is a recognised pathogen isolated from pigeons faeces and reported to cause severe and lethal infections in human,[23] but in our study it was not isolated in high rate. Similarly, *Histoplasma capsulatum*, which is another fungi usually associated with birds' excreta,[24] was also not isolated in this study. This might be due to limited geographic distribution of this fungi. Besides, other fungal strains observed in the present study, such as *Trichophyton*, *Microsporum*, *Chryosporum* and *Penicillium* species, were also reported in previous literature as potential pathogen.[25]

Conclusion

The soil and air of places densely populated with pigeons were found to be heavily loaded with fungal spores and many of them were potential pathogens. Therefore, such places can be a risk for human health. There is a need to control this overwhelming population of pigeons. Moreover, the people, particularly immunocompromised ones, should be aware about hazards associated with pigeons and must be advised to limit their exposure to pigeons' feeding places.

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