

Assessment of airborne fungi from animal establishments in and around Kolkata, West Bengal

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ABSTRACT

Background: Airborne transmission of pathogenic fungi from animals to humans is very common and causes various diseases such as Aspergillosis, allergic sinusitis, and chronic lung disease. **Materials and Methods:** The present study was conducted to analyze the fungi present in and around the environment of local meat market, fish market, and farms. This was determined through air quality sampling using Sabouraud's dextrose agar-agar plate after incubation using Lactophenol cotton blue mount. **Results:** The highest number of fungi was isolated from farms (37%) followed by chicken shop (24%), mutton shop (23%), and fish shops (16%). *Aspergillus flavus* (21%) was prominent in chicken shop, while *Aspergillus fumigatus* (19%) was prominent in mutton shop. *Fusarium* (19%) and *Rhizopus* spp. (18%) were prominent in farm air. **Conclusion:** The study is significant from one's health point of view and stresses the importance of proper ventilation, sanitation, and importance of sunlight to minimize load of fungi in air.

Key words: Fungi, Air, Animal establishments

Air is essential for life; without it, we can survive only a few minutes. It constitutes the immediate physical environment of living organisms. Airborne transmission of pathogenic microorganisms to humans from the environment, animals, or other humans can result in various diseases. Inhalation is an important route of exposure as the lung is more susceptible to infection than the gastrointestinal tract [1]. The extent to which airborne transmission plays a role in the spread of diseases between animal handler, farm and the relationship between microorganisms and dust remain unclear. The common airborne disease which can occur to humans are aspergillosis, aspergilloma (fungus ball), allergic sinusitis, and allergic bronchopulmonary disease, flu-like illness, chronic lung disease, influenza, pneumonia, acute respiratory distress, Legionnaires' disease and Pontiac fever, tuberculosis, acute febrile disease with an exanthematous rash, dry cough, sore throat, headache [2]. Airborne fungus is very common and nearly 10% individuals globally have some sort of fungal allergy. Fungal aerosols which are normally present around animals' establishment can be very harmful to caretakers and farmers. They can transmit the infection to the visitors and the chain continues. Many studies have been carried out over airborne fungi concentrated in the vicinity of animals' house and hospitals, and the most common species they found were *Aspergillus* spp., *Cladosporium* spp., *Curvularia* spp., *Penicillium* spp., *Alternaria* spp., *Fusarium* spp., *Helminthosporium* spp., and *Trichoderma* species [3]. Fungi are ubiquitous in nature and are a serious threat to public health in indoor environments as well [4]. Many fungi that

are reported to cause allergy belong to *Ascomycota*, *Basidiomycota* or anamorphic fungi. There are many reports on fungi isolated from indoor environments [5]. Fungi are able to grow on almost all natural and synthetic materials, especially if they are hygroscopic or wet. Inorganic materials get frequently colonized as they absorb dust and serve as good growth substrates for *Aspergillus fumigatus* and *Aspergillus versicolor* [6]. Keeping all these things in mind, we attempted to assess and study the airborne pathogenic fungi in ambient air of various animal facilities.

MATERIALS AND METHODS

Ours was a Laboratory-based observational study that was carried out in the Department of Microbiology, of a public health institute in Kolkata. Air samples were studied from local market of and farms in and around Kolkata region for fungi. One hundred samples from air of different cow shed or farms (organized or unorganized), poultry shed, commercial shop of poultry, mutton shop, fish market, and in and around, area of Kolkata district were taken.

In this study, air samples were collected in Petridish containing Sabouraud's dextrose agar (SDA) from different areas of Kolkata like GD block Market, Saltlake, New Town NKDA Market, IA block Market, Saltlake, IB Market, Saltlake, CK block Market, Saltlake, ICMarket, Saltlake, KB-KC Market Saltlake, Beliaghata, Singur (Hooghly district), Atharatala (Rajarhat), Hathiar, Paikpara, Nayapatty, SukanataNaNagain Sector-4, Saltlake.

Methodology

Two SDA plates of 90 mm diameter were labeled and placed open in the center of any animal establishment 3 feet above the ground level and 3 feet away from the walls, for 3 h and then closed and transported aseptically to the laboratory. As soon the sample reached the laboratory, plates were incubated in incubator at 37°C and 25°C. The two plates of SDA were incubated at different temperatures one at 25°C and other at 37°C, for minimum 3 weeks before ruling out no fungal growth. The fungal colonies grown at both 25°C and 37°C were processed for identification by lactophenol cotton blue

mounting and then observed under microscope for morphology. For confirming *A. fumigatus*, thermotolerance was observed at 45°C by subculturing on SDA plate which was kept at 45°C overnight.

RESULTS

A variety of fungi were identified from all the animal establishments. Overall, the most common fungi encountered were *Aspergillus flavus*, *A. fumigatus*, and *Rhizomucor pusillus*. The findings are highlighted below in Tables 1 and 2, and Figures 1-6.

Table 1: Species distribution of the fungi

Sr. No.	Fungi	37°C (number with percentage in that temperature)	25°C (number with percentage in that temperature)
1	<i>Aspergillus flavus</i>	18 (14.17%)	12 (12.24%)
2	<i>Aspergillus fumigatus</i>	14 (11.02%)	10 (7.87%)
3	<i>Aspergillus versicolor</i>	6 (4.72%)	4 (4.08%)
4	<i>Aspergillus nidulans</i>	-	4 (4.08%)
5	<i>Aspergillus niger</i>	10 (7.87%)	8 (8.16%)
6	<i>Aspergillus candidus</i>	2 (1.57%)	-
7	<i>Rhizopusarrhizus</i>	12 (9.44%)	16 (16.32%)
8	<i>Rhizomucor pusillus</i>	16 (12.59%)	12 (12.22%)
9	<i>Acremonium spp.</i>	8 (6.29%)	-
10	<i>Exophiala dermatitidis</i>	8 (6.29%)	-
11	<i>Scedosporium spp.</i>	4 (3.14%)	4 (4.08%)
12	<i>Conidiobolus incongruus</i>	-	2 (2.04%)
13	<i>Fusarium spp.</i>	14 (11.02%)	16 (12.59%)
14	<i>Alternaria alternata</i>	8 (6.29%)	2 (2.04%)
15	<i>Penicillium</i>	6 (4.72%)	8 (8.16%)
16	<i>Fungus under Phylum Glomeromycota</i>	1 (0.78%)	-
	Total	127	98

Table 2: Distribution of fungi according to market area and farms

Sr. No	Fungi	Chicken Shop	Mutton Shop	Fish Shop	Farms
1	<i>Aspergillus flavus</i>	11	6	3	10
2	<i>Aspergillus fumigatus</i>	5	10	2	7
3	<i>Aspergillus versicolor</i>	2	4	4	
4	<i>Aspergillus nidulans</i>	3		1	
5	<i>Aspergillus niger</i>	6	5	3	4
6	<i>Aspergillus candidus</i>		1	1	
7	<i>Rhizopusarrhizus</i>	4	5	3	16
8	<i>Rhizomucor pusillus</i>	4	6	3	15
9	<i>Acremonium spp.</i>	2	2	2	2
10	<i>Exophialadermatitis</i>		3	4	
11	<i>Scedosporium spp.</i>	3	2	1	2
12	<i>Conidiobolus incong</i>	0	0	0	2
13	<i>Fusarium spp.</i>	2	3	8	16
14	<i>Alternaria alternata</i>	3	2		5
15	<i>Penicillium spp.</i>	7	3	0	4
16	<i>Fungus under Glomeromycota</i>	1	0	0	0

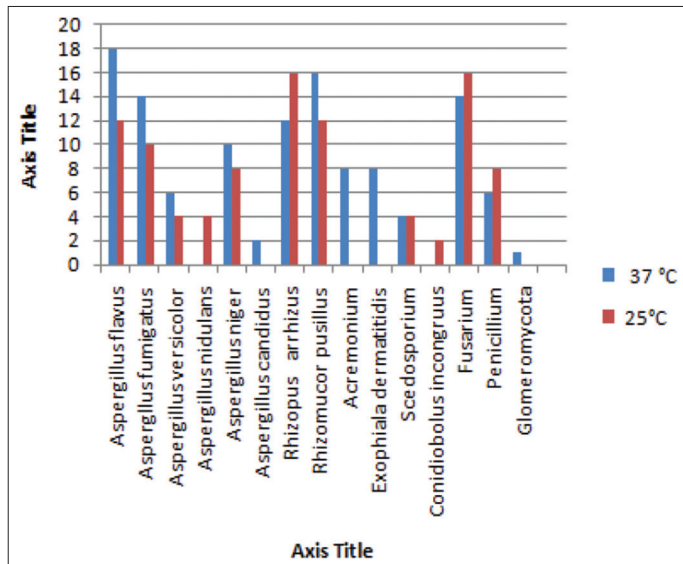


Figure 1: Bar diagram showing isolation of fungi at different temperatures

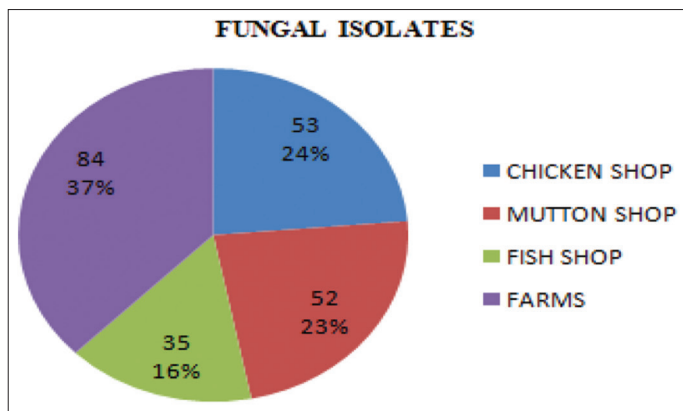


Figure 2: Pie chart showing percentage of fungi isolated from market and farms

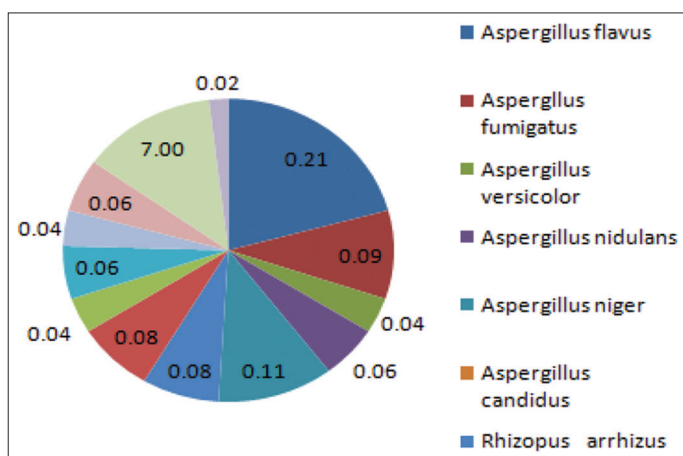


Figure 3: Pie chart showing fungal isolation from chicken shop

The highest number of fungi have been isolated from farms sample (37%) followed by chicken shop (24%), mutton shop (23%), and fish shop (16%).

A. flavus (21%) were prominent in air of chicken shop, while *A. fumigatus* (19%) were prominent in mutton shop.

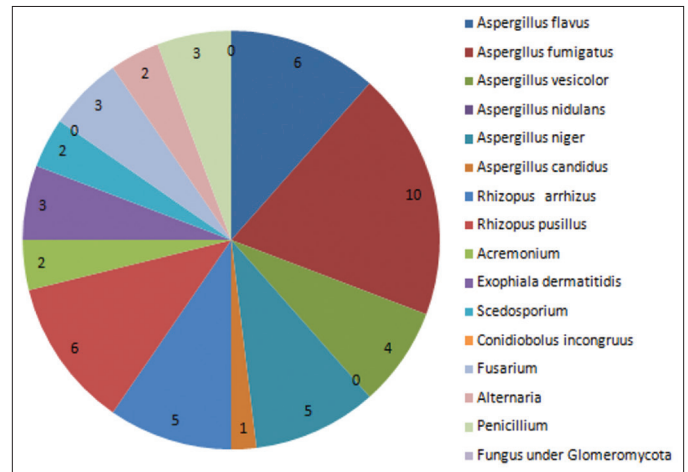


Figure 4: Pie chart showing fungal isolation from mutton shop

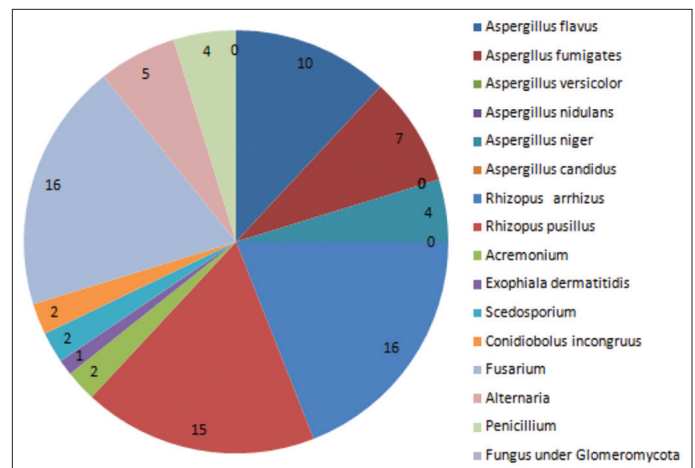


Figure 5: Pie chart showing fungal isolation from farms more number of fungi were isolated at 37°C than at 25°C

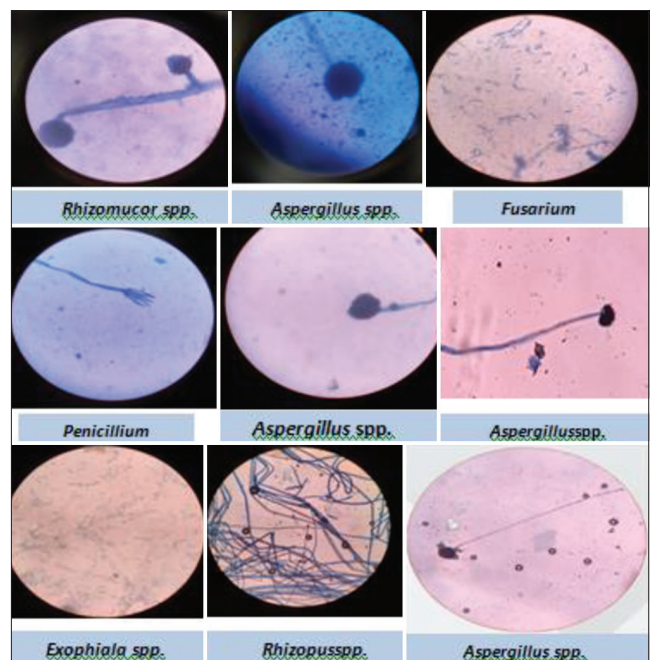


Figure 6: Microscopic morphology of different fungi isolated

Fusarium (19%) and *Rhizopus* spp. 18% were prominent in farms isolates. *Fusarium* was also found in greater proportion in air of

fish shops. A wide variety of fungi were found in ambient air of chicken shops and mutton shops, while the maximum numbers of fungi were recovered from farms isolates.

Microscopic views are highlighted in Figure 6:

DISCUSSION

Kolkata (22°33'40"N, 88°22'1"E) is one of the largest and populated metropolitan cities (population density: 24.760/2 km). Ratio of population: of male to female is 1000: 956. The local market usually present in the dense crowded areas. These areas are affected by high level of road traffic and gaseous and particulate emissions from motor vehicles.

This study revealed that a great variety of fungi are present both at farms and at local market shops selling chicken, mutton and fish. Maximum number fungi are isolated from farms than shops. This reveals the greater chances of transmission of fungi in these environments to both man and animals. A study done by Pavan and Manjunathin cowshed at Hesaraghatta Village, Bangalore found *Acremonium* sp., *Alternaria alternata*, *Ascomycetes* sp., *Aspergillus* sp., *A. flavus*, *A. niger*, *A. oryzae*, *A. ochraceus*, *A. fumigatus*, *A. terreus*, *Curvularia* sp., *C. lunata*, *Cladosporium* sp., *Cladosporioides*, *C. herbarum*, *C. acremonium*, *Fusarium* sp., *Mucor* sp., *Rhizopus* sp., *Penicillium* sp., *P. versicolor*, *P. citrinum*, *P. nigricans*, *Scopulariopsis* sp., and *Trichoderma* sp., were found and predominant airborne fungi were *Cladosporium* sp., *Aspergillus* sp., *Aspergillus niger*, and *A. alternata* [3]. Similar fungi have also been found in our study. Fungi in indoor and outdoor air pose great health risks of allergic and invasive infections such as allergic bronchopulmonary aspergillosis [7]. *A. flavus* (21%) were prominent in ambient air of the chicken shops in our study, while *Aspergillus fumigates* (19%) were prominent in Mutton shop. Overall, *Aspergillus* sp. and *Rhizopus* spp. were common. Factors such as building dampness, indoor temperature, relative humidity, and hygiene in indoors and in the surrounding environment favor the growth and proliferation of fungi including the pathogenic species [8]. There is adequate clinical evidence that exposure to molds and other dampness-related microbial agents increase the risk of rare conditions such as hypersensitivity, pneumonitis, allergic alveolitis, chronic rhinosinusitis, and allergic fungal sinusitis [9]. Ventilation is one of the key factors which affect particle deposition rates in indoor air [10-12]. In another study done by Yassin *et al.*, The indoor and outdoor median viabilities of fungi were 55% and 25 %, respectively, which indicates that an indoor environment provides more favorable conditions for the survival of aerosolized fungi [13]. The air is abundant of fungal spores, although it is not a good medium for growth unlike the soil, water, surfaces of living organisms, and nonliving materials. In India, about 70% of the population is non-vegetarian, and their percentage is also increasing. Air around the animals includes varieties of microorganisms which not only affects the animals, but also the people who come in contact with them. Maximum farms in India are unorganized, most of which are concentrated in rural areas. Many a time's slaughters are done in

open areas which are not legalized [13-15]. This study focuses on the prevalence of different fungi which harbor in air of local market and farms located in and around Kolkata to get the idea of pathogenic fungi and their potential adverse effect on animal and human health. According to a study carried out by Quintana *et al.*, the microorganisms present in the different areas of a farm have an influence on its environmental quality, using the air as a vehicle of dissemination [16,17]. This study demonstrates that proper ventilation reduces the microbial load of the aerial environment of dairy farms, enhancing the quality of the air and, therefore, the wellbeing of the animals and their handlers. The present study also shows the importance of overall hygiene and ventilation to reduce the microbial load in the air. Bulk of animal meat is available in local shops in market that do not follow any regulations and guidelines, hence can act as chain for zoonosis. Most people in Kolkata, West Bengal buy meat from local market available in their area. Various Microorganisms exist in the environment which may be pathogenic to animals as well as Humans. If the person is immune compromised, then chances of getting affected from commensals are greatly increased. Therefore, proper hygiene and cleanliness around the animals and local shops are very essential. Awareness about the different microorganism and their possible transmission were discussed with farmers and handlers to prevent infections, an interactive session was done with them to make them understand better about the severity of infection they can get. Proper waste disposal and importance of sunlight was told to farmers and caretaker to minimize the moisture to prevent the growth of Microorganism in that area. There should be adequate public health awareness about this because these things are often neglected and form an important component of one health. Hence, our study attempts to answer these questions. As far as we now, such studies have still not been done from this part of the country. One limitation of our study is that it is not a quantitative estimation of the airborne fungi. Furthermore, if time would have permitted, more samples could have been tested. More such studies are hence urgently needed in these interesting aspects of public health.

CONCLUSION

A variety of zoonotic fungae present in ambient air which can act as potential source of allergic or invasive infection for both animal and human in animal establishments.

This study points out the importance of one's health as the unknown cause of air-borne illness which not only reduces the productivity of animal but also leads to diseases in humans. Animal handlers are the one who directly comes in contact of animal which can act as chain for zoonosis to others human and can lead to unknown outbreak and allergic and invasive fungal diseases. It has a definite implication from the public health point of view. The findings indicate the substantial presence of various airborne fungi in the areas which needs to be evaluated at regular interval and proper hygienic precautions should be taken in market area. Hence, it is recommended that regular check of airborne fungi should be done in animal facilities and appropriate remedial measures taken at once.

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