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A Study on the Prevalence of Indoor Mycoflora in Air Conditioned Buses

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Authors' contributions

This work was carried out in collaboration between all authors. Author NKUP designed the study and wrote the protocol, author SB wrote the first draft of the manuscript. Authors MRK, SL and KR conducted the sampling and analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To study the presence of indoor mycoflora in A/c Buses to know the commuters risk of exposure to fungal spores.
Place and Duration: Chennai Mofussil Bus Terminus (CMBT), Koyambedu, Chennai, India. Study was conducted from November 2011 to April 2012.
Methodology: Airborne fungi from 50 A/c buses were studied using Reuter Centrifugal Sampler (Biotest, Germany), fungi from the surfaces of air vents through swab sample and bus seats by rubbing sterile petridishes on the seats. Sabourauds Dextrose Agar (SDA) was used for the isolation of fungi from different buses. The collected data were statistically analyzed.
Results: A total of 38 species classified in 21 genera were recorded. Among which, Zygomycetes was represented by 4 species, Ascomycetes and Coelomycetes by single species each and the remaining belongs to Hyphomycetes. The genus, *Aspergillus* was represented by maximum number of species (11 species) followed by *Penicillium* (5 species). A total average of 713 CFU/m^3 of air was recorded within the buses. *Aspergillus*

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niger was the first dominant fungi in the order of dominance followed by *Chrysonilia sitophila*, *Alternaria alternata* and *Aspergillus flavus* in that order. From the surface of bus seats, *Aspergillus niger, A. flavus, Rhizopus stolonifer* and *A. japonicus* were recorded as dominant. However, different mycofloral composition was recorded from air vents. *Cladosporium chlorocephalum* and *Curvularia lunata* dominated the surface of air vents.
Conclusion: The study demonstrates the presence of potential fungal species which pose exposure risk to the immune compromised commuters.

Keywords: A/c buses; bus commuters; exposure risk; mycoflora; reuter centrifugal sampler.

1. INTRODUCTION

Bus is the significant transportation vehicle used as a mass transit system by numerous people worldwide. Buses vary according to the design and nature as high comfort system with Air conditioned (A/c) and Non A/c Buses with lower comfort level. These A/c buses are found to be air tight and no transaction with external air was found as the door was closed. During the journey in A/c buses, the commuters are exposed to the microenvironment throughout their travel time. This includes the presence of microbes within the microclimatic environment and their exposure risk associated with the nature of microbe present within. These microorganisms may be the causative agents or triggering factors of asthma [1] and allergic rhinitis. Among the microbes, fungi are well known for their allergenic, toxigenic and parasitic ability [2]. Many air-borne mycospores have the ability to cause allergies as well as other respiratory diseases in humans, particularly in immune suppressive patients. Mostly, these fungal spores are inhaled by humans during their respiration and these spores affect the respiratory tract as well as lungs. The exposed subjects are at risk to variety of health effects, including infectious, allergic and hypersensitivity reactions [3].

There are only few studies available on the presence of fungi in commuting vehicles. The studies from different transport modes were conducted from different parts of the world which includes Trolley buses in Canada [4], Transit buses in United States [5], Passenger cars and buses in Korea [6], Thailand [7], Hong Kong [8] and Commuter buses in Taiwan [9]. However in India, there was no such report available from the buses so far and hence the present study is conducted. This is to determine the quality and quantity of fungal species present in different A/c buses that ply from Chennai to various parts of Tamil Nadu, India. The commuters in Tamil Nadu, India are taking a minimum travel hour of 3 to the maximum of 14 hours to reach different destined cities within the state. During their journey, they are at risk to variety of health effects, including infectious, allergic and hypersensitivity reactions as they are exposed to air-borne microorganisms. Hence, the knowledge on the presence of indoor mycoflora within buses is utmost important. This also explicits the commuter's risk for fungal species when they travel through A/c buses for long period of time.

2. MATERIALS AND METHODS

2.1 Sampling Information

A total of 50 buses provided with A/c system were selected at random from the Chennai Mofussil Bus Terminus (CMBT), Koyambedu, Chennai, India for the present study. This includes private and government operated buses. They are also termed as luxury buses and are preferred for their comfort provided with different types of seating arrangements like,

tilted seat facility, semi sleeper and with sleeper facility. The windows are sealed and the bus has the facility of single door opening in front of the driver cabinet which serves as entry and exit point. Emergency exit is provided at the back of the bus but not used until emergency occurred. The age of the buses ranges from 0–3 years. The seating capacity ranges from 36 to 45. The seats of the bus possess cushion stuffed with cotton/foam etc., covered with disinfected clothes. These buses ply for the distance of around 320 kilometers at minimum and at the maximum of 700 kilometers a day. The operational speed averages around 60 km/hour. The sampling was carried in Chennai, when the buses are either ready to ply or returned after their journey to destined cities of Tamil Nadu, India.

2.2 Air Sampling [10]

The Reuter Centrifugal Sampler (RCS) (Biotest AG, Landsteinerstr, Dreieich, Germany) was used to collect the air samples. The strips containing Sabouraud's Dextrose Agar (SDA) (Himedia Inc. Mumbai, India) were exposed for a period of 1 minute through RCS sampler with the suction rate of 40 L/minute. Further, our preliminary investigation suggests that operation of the sampler loaded with SDA strips for a time period of 2 minutes results in overcrowd of the fungal colonies or they are found to coalesce. Thus, the sampler was operated for one minute only with the suction rate of 40L per minute. After sampling, the agar strips were incubated at room temperature for 30ºC ± 2ºC for 72-96 hours and the fungal colonies were isolated.

2.3 Surface Sampling [11]

The fungal species present on or within the surface of bus seats were monitored by rubbing the bottom lid of the sterile, plastic petridishes of 9cm diameter and closed immediately using upper lid of the petridish. The sampled petridishes were brought to the laboratory in a sterile polythene bags and processed for isolation of fungal species. Twenty ml. of SDA were added to the petridishes and incubated at 30ºC ± 2ºC for 72-96 hours for the isolation of fungi.

2.4 Air-filter Sampling [12]

The surfaces near A/c vent within the buses were sampled using sterile swab to know the presence of contaminants in air filter system within buses. Swabs were moistened with sterile distilled water and processed for isolation of fungi through swab culture technique using SDA. The growing fungal colonies were isolated and identified after a period of 72-96 hours. The antibiotic, Streptomycin (0.06 g/L) was used to arrest the growth of bacteria.

2.5 Identification

The fungal colonies were isolated and identified after a period of 72-96 hours based on their morphological, i.e. microscopic and macroscopic characters using the manuals [13-15]. The fungi that have been shown to have sexual stages but which are usually observed under their asexual stages in the laboratory were artificially classified under Hyphomycetes.

2.6 Presentation of Data

The developing colonies were studied for their Colony Forming Units (CFU)/ m^3 of air, Frequency occurrence and Percent contribution using the following formulae [16]:

The colony forming units (CFU)/m^3 of air was calculated as follows:

$$X = \frac{Y}{0.04}$$

Where,
0.04 = Volume of air sampled in cubic meter.
X= No. of CFU/m^3.
y= No. of colonies recorded.

Total average CFU of an individual species is calculated as X/n where n = number of samples.

The percent contribution is the ratio of individual fungal species to the total number of CFU of all species isolated which is calculated as,

$$\% \text{ Contribution} = \frac{\text{CFU of an individual fungal species}}{\text{Total No. of CFU of all species}} \times 100$$

The percent frequency occurrence is calculated formula,

$$\% \text{ frequency occurrence} = \frac{N}{n} \times 100$$

Where,

N – No. of samples in which the species occurred, n – Total no. of samples

2.7 Statistical Analysis of Data Obtained

The statistical analysis on the proportion test was conducted and significance was noted as p value for fungal colonies isolated from air samples, air-filter samples and surface sampling using the MINITAB™ (version 16) software. The significant fungal species were denoted as * in the Table 1, 2 and 3.

3. RESULTS

3.1 Air Sampling Results

A total of 31 species of fungi belonging to 19 genera were isolated from the air within the buses. Among which, the class Coelomycetes was represented by single species, Zygomycetes by 3 species and the remaining species belongs to Hyphomycetes. The genus, *Aspergillus* was represented by maximum number of species (10 species) followed by *Penicillium* (3 species) and *Fusarium* (2 species). All other genera were represented by single species each. Among the species, *Aspergillus niger* was recorded as first dominant with an average of 230 CFU/m^3 of air with the maximum contribution of 32.25% to the total. The second and third dominant fungi were *Chrysonilia sitophila* and *Alternaria alternata* with percent contribution of 10.65% and 8.62% respectively. The list of fungal species isolated, their average CFU/m^3 of air and percent contribution is presented in Table 1.

Table 1. Average CFU/m^3 of air and percentage contribution of fungal species isolated from the air sample within Buses (n=50)

Species	Total avg. CFU/m^3 of air	Percent contribution (%)	p-value
Zygomycetes			
Lichtheimia corymbifera	3.5	0.49	0.02*
Rhizopus stolonifer	25.5	3.57	0.10
Syncephalastrum racemosum	2	0.28	0.02*
Hyphomycetes			
Acremonium sp.	9	1.26	0.02*
Alternaria alternate	61.5	8.62	0.44
Aspergillus niger	230	32.25	0.80
A. flavipes	3	0.42	0.02*
A. flavus	50.5	7.08	0.40
A. fumigatus	16	2.24	0.14
A. japonicus	1.5	0.21	0.02*
A. nidulans	6.5	0.91	0.12
A. ochraceus	0.5	0.07	0.02*
A. tamari	5	0.70	0.06
A. terreus	1	0.14	0.04*
A. versicolor	6	0.84	0.08
Aureobasidium pullulans	4.5	0.63	0.08
Candida albicans	9	1.26	0.02*
Chrysonilia sitophila	76	10.65	0.28
Chrysosporium pannorum	2.5	0.35	0.04*
Cladosporium cladosporioides	4.5	0.63	0.06
Curvularia lunata	48	6.73	0.46
Drechslera australiensis	3	0.42	0.02*
Fusarium moniliformis	2	0.28	0.02*
F. oxysporum	8	1.12	0.18
Geotrichum candidum	0.5	0.07	0.02*
Paecilomyces variotii	19.5	2.73	0.06
Penicillium citrinum	13	1.82	0.18
P. funiculosum	1.5	0.21	0.02*
P. oxalicum	3	0.42	0.06
Trichoderma viride	23	3.22	0.10
Coelomycetes			
Phoma sp.	7.5	1.05	0.08
Yeast	16	2.24	0.12
Non sporulating colonies	50	7.01	0.38

3.2 Surface Sampling

In surfaces of bus seats, 27 different fungal species belonging to 17 genera were isolated. Among which the class, Ascomycetes was represented by single species, Zygomycetes by 4 species and the remaining species belongs to Hyphomycetes. The genus, *Aspergillus* was represented by maximum number of species (8 species) followed by *Penicillium* (3 species) and *Paecilomyces* (2 species). All other genera were represented by single species each. Among the species, *Aspergillus niger* was the dominant with 42.51% contribution to the total. This was followed by *Aspergillus flavus* (26.33%), *Rhizopus stolonifer* (5.82%) and

Aspergillus tamarii (2.66%) in the order of dominance. The list of fungal species isolated from the surface of bus seats, their average CFU and percent contribution is presented in Table 2.

Table 2. Frequency occurrence and percent contribution of fungal species isolated from Bus seats (n=50)

Species	Total no. of colonies isolated	Frequency Occurrence (%)	Percent contribution (%)	p-value
Ascomycetes				
Chaetomium globosum	1	2	0.04	0.02*
Zygomycetes				
Lichtheimia corymbifera	17	10	0.77	0.10
Mucor racemosum	7	6	0.31	0.06
Rhizopus stolonifer	128	38	5.82	0.38
Syncephalastrum racemosum	12	14	0.54	0.1
Hyphomycetes				
Acremonium sp.	4	2	0.18	0.02*
Alternaria alternate	45	18	2.04	0.18
Aspergillus flavus	579	86	26.33	0.86
A. fumigatus	44	14	2	0.14
A. japonicas	113	34	5.13	0.34
A. nidulans	6	4	0.27	0.04*
A. niger	935	96	42.51	0.96
A. Tamari	59	24	2.68	0.24
A. terreus	17	26	0.77	0.26
A. ustus	1	2	0.04	0.02*
Aureobasidium pullulans	1	2	0.04	0.02*
Chrysonilia sitophila	46	42	2.09	0.42
Cladosporium oxysporum	25	10	1.13	0.10
Curvularia lunata	49	32	2.22	0.32
Drechslera australiensis	3	6	0.13	0.06
Fusarium oxysporum	5	6	0.22	0.06
Paecilomyces carneus	1	2	0.04	0.02*
P. variotii	6	12	0.27	0.12
Penicillium citrinum	4	4	0.18	0.04*
P. frequentans	4	6	0.18	0.06
P. oxalicum	3	4	0.13	0.04*
Trichoderma viride	9	14	0.4	0.14
Non sporulating colonies	75	32	3.41	0.32

3.3 Air-filter Sampling

In surface sampling, 29 different fungal species belonging to 16 genera were observed. Among which the class Ascomycetes and Coelomycetes were represented by single species, Zygomycetes by 2 species and the remaining species belongs to Hyphomycetes. Unlike air and bus seat surface, filter samples from the air vent produced a different kind of results. In this *Cladosporium chlorocephalum* belonging to Hyphomycetes, showed its dominance in both percent contribution and percent frequency occurrence than any other

fungi recorded. The species, *Cladosporium chlorocephalum* contributed around 25 % followed by *Curvularia lunata* (17.74 %), *Aspergillus niger* (17.18 %) and *Aspergillus flavus* (14.71 %). Table 3 presents the list of fungal species isolated, their percent occurrence and percent contribution from the surfaces of the air vent within buses.

Table 3. Frequency occurrence and percent contribution of fungal species isolated from air filters of Bus (n=50)

Species	Total no. of colonies isolated	Frequency occurrence (%)	Percent contribution (%)	p-value
Ascomycetes				
Chaetomium globosum	2	4	0.15	0.04*
Zygomycetes				
Mucor racemosum	3	6	0.23	0.06
Rhizopus stolonifer	25	10	1.98	0.10
Hyphomycetes				
Acremonium sp.	9	6	0.71	0.06
Alternaria alternate	3	6	0.23	0.06
Aspergillus flavus	183	32	14.71	0.32
A. fumigatus	28	30	2.22	0.30
A. japonicus	3	2	0.23	0.02*
A. nidulans	2	4	0.15	0.04*
A. niger	208	70	17.18	0.70
A. tamarii	2	4	0.15	0.04*
A. terreus	3	6	0.23	0.06
A. ustus	2	2	0.15	0.02*
Chrysonilia sitophila	7	10	0.55	0.10
Chrysosporium pannorum	8	6	0.63	0.06
Cladosporium cladosporioides	3	2	0.23	0.02*
C.chlorocephalum	316	6	25.13	0.06
Curvularia lunata	223	26	17.74	0.26
Drechslera australiensis	12	10	0.95	0.10
Fusarium oxysporum	7	14	0.55	0.14
Paecilomyces carneus	1	2	0.07	0.02*
P. variotii	7	8	0.55	0.08
Penicillium chrysogenum	1	2	0.07	0.02*
P. citrinum	4	6	0.31	0.06
P. frequentans	12	18	0.95	0.18
P. funiculosum	3	6	0.23	0.06
P. oxalicum	11	10	0.87	0.10
Trichoderma viride	5	4	0.39	0.04*
Coelomycetes				
Phoma sp.	4	6	0.31	0.06
Yeast colonies	1	2	0.07	0.02*
Non sporulating colonies	149	76	11.85	0.76

4. DISCUSSION

Microbes are found to cause disease in humans by three ways namely, pathogenic, allergic and toxic. When the required humidity, temperature and water activity are available to the

microorganism, they use to survive and propagate. The microenvironment in A/c bus is one such example where those factors are rightly available for the microbes. These are found to become airborne within the buses during commuting. Airborne microorganisms are widely known to cause diseases among immune compromised individuals. Especially, air-borne fungi are often associated with many diseases in humans such as asthma, allergy and lung diseases [1]. The study conducted provides a data on airborne fungi within the A/c buses in which human exposure to microorganisms are clearly evident. The study proves that the commuters are exposed to different species of fungi in the micro environment of bus. However, the exposure risk of the commuters to airborne fungi within A/c bus depends upon their immune system.

A total of 38 species classified in 21 genera were recorded. Among which, Zygomycetes was represented by 4 species, Ascomycetes and Coelomycetes by single species each and the remaining belongs to Hyphomycetes. The genus, *Aspergillus* was represented by maximum number of species (11 species) followed by *Penicillium* (5 species). *Aspergillus niger* was recorded as dominant fungi in air sample within buses followed by *Chrysonilia sitophila*, *Alternaria alternata* and *Aspergillus flavus* in that order. From the surface of bus seats, *Aspergillus niger, A. flavus, Rhizopus stolonifer* and *A. japonicus* were recorded as dominant. However, different mycofloral composition was recorded from air vents. *Cladosporium chlorocephalum* and *Curvularia lunata* dominated the surface of air vents. Isolation of large number of species (20 species) in common in all three samples (air sample, surface sample of the seat and air vents) within bus proves that they are interrelated. The entry of the species through air vent, their settling on bus seats and proliferation within buses and becoming airborne. However, few species showed their difference in mycofloral composition, especially in air vents which is due to the external air being filtered. The presence of the species, *Cladosporium chlorocephalum* and *Curvularia lunata* in high amount is due to their presence outdoor. Similar result was recorded with Air Handling Units in Singapore [11].

The Aspergilli, belonging to class hyphomycetes, are the most predominant causative agent of allergies in humans when exposed. The exposure to fungal spores is through inhalation of conidia or through contact [17]. The inhalation of fungal spores form clumps of mycetoma within the cavity of lungs, called Aspergilloma. In the present study, the genus *Aspergillus* is most predominant in nearly all the samples taken in A/c buses. Thus, the probability of getting allergies and infections due to the genus, *Aspergillus* is high when immune compromised people are travelling through A/c buses.

Airborne fungal concentrations in the A/c buses in Chennai showed 713 CFU/m^3 of air whereas the recommended limit of airborne fungi within work environment is 300 CFU/m^3 of air [18]. The Healthy Building International (HBI) recommended concentration of less than 750 CFU/m^3 [19] and National Instititute of Occupational Safety and Health (NIOSH) have recommended upto 1000 CFU/m^3 of air [20]. Brazilian health ministry as 750 CFU/m^3 of air [21] and which did not exceed the recommended standard (1000 CFU/m^3) in Taiwan [9]. However, these limits were brought using the outdoor concentration of molds as reference. If similar nature is taken into account, the average cfu/m^3 of air recorded from the outdoor environment of Chennai was 514 CFU/m^3 of air [22]. The total average count recorded from the bus (713 CFU/m^3 of air) exceeds the published outdoor level of fungi. This is due to the entry of the species through air vents, their dissemination in air and settling of the fungal spores in the seats of the buses. Further, the growth of the deposited mycospores are favored by the eatables and water spilled by the commuters within bus. This poses a high

risk for the commuters as the level of fungi within the bus exceeds that of outdoor in Chennai.

Apart from these, many harmful substances like, volatile organic chemicals [23] and particulate matters [24-26] also affect the commuters of A/c buses and this microenvironment poses high risk for the commuters. The symptoms of watery and itchy eyes, rhinorrhea and headaches were reported due to the fungal invasion in Canadian buses [4]. Santilli [27], reported about a suffering of patient due to exposure in her transport vehicle. So, it is recommended that the safety measures must be taken by the commuters while travelling in A/c buses to prevent themselves from health hazard. Reduction of microbial level within buses can be reached through providing exhaust ventilation fans [28]. The treatment of fungi infected buses with UV radiation from Florida, US was reported [5]. These measures can be implemented in A/c buses if warranted.

5. CONCLUSION

The study demonstrates the presence of many fungal species within buses and the exposure risk of immune compromised commuters when they travel through A/c bus. The air sample within buses, surfaces of bus seat and air vents were analyzed for the presence of fungal flora. The genus, *Aspergillus* was represented by maximum number of species (11 species) followed by *Penicillium* (5 species). The study is first of its kind in providing data on A/c Buses in India. The current study serves as a reference report in case infection of mould incidence rises when the age of the bus progress.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

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