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ISOLATION AND IDENTIFICATION OF AIR BORNE POLLENS AND PATHOGENS CAUSING PULMONARY INFECTIONS IN SOME POLLUTED AREAS OF BANGALORE CITY

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ABSTRACT

Pulmonary infections are common which are mostly lung infections and are caused by a wide range of microorganisms.In the field of medicine, especially clinical point of view, knowing the details about the occurrence of the pollen and spore load in the atmosphere is most important. Based on previous investigation on airborne pollen and fungal spores are an epidemiological study which is done as an essential tool to diagnose allergy.The polluted atmosphere of the Bangalore city was surveyed by using plate exposure method and Vaseline coated slides followed by isolation and identification of air micro-flora. In this present study most dominant pollens and fungal spores that were found in the polluted areas of Bangalore city are *Argemone M, Bauhinia, Carica, Casuarina, Cedrus, Cocus, Cannabis, Cassia, Parthenium, Basidomycetes, Cladosporioides,* *Aspergillus*and*Fusarium*and some specific bacteria such as *Bacillus, Streptococcus, pseudomonas, Klebsiella, Escherichia, Staphylococcus, Salmonella, Enterobacter and Proteus spp*..So,the present study is one of the approaches that, to collect air samples, isolating and identifying airborne pathogens in order to maintain proper records about aeroallergens causing respiratory infections and implementing therapeutic aid to the allergic patients and thereby controlling air pollution.

KEYWORDS: Pulmonary infections, Aeroallergens, pollen, fungal spores, air micro-flora.

INTRODUCTION

Pollution of air is the main notable risk factor for a number of health issues that includes infections in respiratory tract, heart and lung diseases, and COPD (chronic obstructive pulmonary disorder), and stroke. Disorders that occur due to air pollution may involve breathing difficulties, coughing, asthma, wheezing, rhinitis, bronchitis and cardiac conditions might get worse. Allergic reactions such as, rhinitis, asthma, eczema, atopic dermatitis etc., are caused by fungi and pollens. Respiratory allergic reactions caused by fungi in patients were reported in a study carried out by a researcher where he noted that fungi are the one and only source of outdoor polluted environment.[1] In another survey, it was reported that inside human sera antibodies to moulds were separated and characterized and the importance of human precipitins to typical antigens of fungi in allergic response.[2] Then later during an investigation was confirmed during some other survey.[3]

It is estimated that 30-40% of the whole world inhabitants are suffering from allergic diseases.Airborne pollens, bacteria and fungi are the main agents for cause of respiratory disorders. In one of the previous study it was conducted that, both indoor and outdoor environments fungal spores are very common and due to this reason nearly 10 % of people worldwide have fungal allergies.[4] And in some other research it was confirmed that the most viable source of airborne bacteria is the presence of human.[5] One of the famous researcher detected that pollen is the only aeroallergen which causes hay fever (allergic rhinitis).[6] Later, in some other survey it was established that the most specific cause of hay fever are grasses in U.K.[7] After more than 40 years in USA, a investigation was carried out for field evaluation and aerial examinations to record aeroallergens/airborne particles from the polluted atmosphere.[8] Earliest known allergens are pollen grains and “hay fever” is a major cause of allergies historically known that was confirmed during one survey about allergies.[9][10] Around 20-30% of world population suffers from various types of allergies and 10-15% of the world citizens suffer from pollinosis alone. In Finland, a survey was conducted that showed a widespread allergic rhinitis which was 14% and 2.5% of asthma.Among Greek population, Asthma is indicated as high as 9% and 27% of children suffering from wheeze in Australia. An aerobiological study was conducted by some researchers whoinitiated in UK at Cardiff, and was later spread in other stations of Great Britain.[11]

In a recent investigation was undertaken, i.e. “All India Coordinated Project on Aeroallergens and Human Health” in 18 different centers of the country to research about the quantitative

and qualitative concentration of Bioaerosols.[12] In a current survey, a re-evaluation on importance of aerobiology in asthma diagnosis, allergy and management was conducted and established.[13] Around the same time, large investigations on aerobiological study were startedby some researchers in urban environment of Bangalore.[14] During one survey in Bangalore, 34% of allergic rhinitis patients *Partheniumhysterophorus*pollen extracts showedallergenicity and patientssuffering from bronchial asthmawere recorded atleast 12%.[15] Maximum skin reactions in patients were recorded from Bangalore city bysome investigatorswhich are caused by*Casuarinaequisetifolia*.[16] Some fungal allergens such as *Aspergillus, Cladosporium, Penicillium* and *Rhizopus*were mostly detected in urban areas of Bangalore city and *Staphylococcus, Streptococcus* and *Bacillus* are detected which are responsible for respiratory disorders.Sampling devices such as Gravimetric, filtration and impaction devices are essential for monitoring airborne allergens. In 1925, aerobiological survey was initiated by one scientist in USA.[17] In 1946, a sampler was adopted by American Academy of Allergy which was devised by Durham. It is a Gravimetric sampler which was used to recognize airborne components. So in this present investigation, proper utilization of air sampling techniques was understood andwas drawn to study airborne pollen analysis and aeroallergens were enumerated in some specific sampling sites/polluted areas followed by isolationand identification of micro-flora.

MATERIALS AND METHODS

Collection of samples from sampling sites

Air sampling was done in some selected sampling sites. Samples were collected from four different polluted air sources such as from traffic areas (Majestic, Vijayanagar, Sunkadakatte, Jayanagar and K.R.market traffic signal areas), hospital areas (jayadeva hospital east stand, Rajiv Gandhi hospital jayanagar, K.C.general hospital malleshwaram, D.G. hospital banashankari) and industrial areas (Peenya, Vijayanagar, Jayanagar and Yeshwantpur industrial areas) and poultry shop areas (Chikkabanavara, Sunkadakatte, Jayanagar and Jalahalli poultry areas) of the Bangalore city. Sterile Petri-plates for both bacteria and fungi containing 15 ml of sterilized Nutrient agar and Sabouraud Dextrose Agar were exposed in the polluted areas for a period of 1 hour and were kept for incubation. Likewise for pollen, Biolin (petroleum gel) was used in preparing Vaseline coated slides and those slides were exposed for one hour in polluted areas for further identification.

Treatment of samples and isolation of air micro-flora: Enumeration of total viable colonies and colony morphology of both bacteria and fungi were recorded according to CFU (colony forming units) per plate. And after exposure of Vaseline coated slides those were aseptically transferred to the laboratory and were stored in refrigerator for further pollen identification. Characteristic colonies based on their shape, size and color were identified. Well isolated unique colonies were picked using a sterile loop and cultured on nutrient agar (NA) (Hi-media M001) and Sabouraud Dextrose agar (SDA) (Hi-media MH 063) slants.

Sub-culturing process: For identification of microorganisms, the isolates were sub-cultured in tryptone broth (Hi-media tryptone broth) (M463) for 18-24h and log phase cultures were used for all the identification tests.

Identification of air micro-flora and airborne pollens

Air micro-flora was identified by gram staining [(Hi-media kit: Gram's Crystal Violet (SO12), Gram's Iodine (S013), Gram's Decolourizer (SO32), Safranin, 0.5% w/v (SO27)], Biochemical tests and Direct Microscopic Technique. The identification of both pollen and fungi was done by using Direct Microscopy technique. The pollens were identified based on their characteristics such as shape, size and other morphological features by keeping exposed Vaseline coated slides directly under the microscope and were recorded. Using the inoculating needles, a small or little portion of the growth on the culture plate was transferred, teased to separate the spores properly. Then fungi were identified clearly based on their characteristics such as shape, size and other morphological features.

By using conventional techniques pure bacterial cultures that were sub-cultured were further processed for identification. Motility testing was performed by preparing a wet amount on a glass slide and was observed under microscope. Later, it was followed by Gram staining procedures to determine both Gram-negative and Gram-positive bacteria. After staining techniques, various biochemical tests were conducted to get closer to bacterial identification. And those tests include Lactose fermentation test (Hi-media: MacConkey Agar (MH081) was used, Catalase test (3% H_2O_2 (hydrogen peroxide) was used, Citrate utilization test (Simmons Citrate Agar (M099) was used, Triple sugar iron test (Triple Sugar Iron Agar (MM021) was used, Urease test (Urea Agar Base (M112S) was used, Oxidase test (1% tetramethyl-p-phenylenediamine dihydrochloride) reagent was used, Carbohydrate fermentation tests (Phenol red fermentation broth (0.5%-1.0% of carbohydrate, nutrient broth and pH indicator phenol red) was used to carry out for sucrose, mannitol, arabinose and inositol test.

RESULTS AND DISCUSSION

The study carried to identify aeroallergens in some specific polluted areas in the Bangalore city. There are many reports about airborne microorganisms responsible for causing respiratory or pulmonary infections. In this study it was found that:

Table I: Total viable count of bacteria collected from the polluted areas.

Source	Sample	Total Viable Count (Cfu/Plate)
TRAFFIC AREA	Sunkadakatte traffic area(T1)	443
	Vijayanagar traffic area (T2)	200
	K.R. market traffic area (T3)	355
	Jayanagar traffic area (T4)	209
	Majestic traffic area (T5)	239
INDUSTRIAL AREA	Peenya industrial area (I1)	80
	Vijayanagar industrial area (I2)	165
	Jayanagar industrial area (I3)	95
	Yeshwantpur industrial area (I4)	120
HOSPITAL AREA	Jayadeva hospital east stand (H1)	125
	Rajiv Gandhi hospital jayanagar (H2)	120
	K.C.generalhospital malleshwaram (H3)	140
	D.G. hospital banashankari (H4)	130
POULTRY SHOP AREA	Chikkabanavara poultry shop area (P1)	180
	Sunkadakatte poultry shop area (P2)	165
	Jayanagar poultry area (P3)	160
	Jalahalli poultry shop area (P4)	182

Table II: Total viable count of fungi collected from the polluted areas.

Source	Sample	Total Viable Count (Cfu/Plate)
TRAFFIC AREA	Sunkadakatte traffic area (T1)	150
	Vijayanagar traffic area (T2)	200
	K. R. market traffic area (T3)	189
	Jayanagar traffic area (T4)	212
	Majestic traffic area (T5)	167
INDUSTRIAL AREA	Peenya industrial area (I1)	70
	Vijayanagar industrial area (I2)	109
	Jayanagar industrial area (I3)	65
	Yeshwantpur industrial area (I4)	98
HOSPITAL AREA	Jayadeva hospital east stand (H1)	118
	Rajiv gandhi hospital jayanagar (H2)	85
	K.C.generalhospital malleshwaram (H3)	98
	D.G. hospital banashankari (H4)	94
POULTRY SHOP AREA	Chikkabanavara poultry shop area (P1)	89
	Sunkadakatte poultry shop area (P2)	72
	Jayanagar poultry shop area (P3)	155
	Jalahalli poultry shop area (P4)	180

Above table number I and II explains that the enumeration of micro-flora present in the air by using CFU/plate. Total viable count of bacterial and fungal colonies was done which were collected from four different polluted areas e.g. traffic; industry, hospital and poultry shop areas. Likewise, gram staining was also carried out for bacterial identification. The selected bacterial isolates that were stored were gram stained to determine which one is gram negative and gram positive bacteria. So in this survey it was observed that in polluted areas of hospital 5 Gram-positive bacilli, 2 Gram positive cocci and 4 Gram negative bacilli were found. Likewise in polluted Industrial areas, 4 Gram positive bacilli and 4 Gram negative bacilli were found. In polluted traffic areas, 2 Gram positive bacilli were found and in polluted ares of poultry 1 Gram positive cocci and 1 Gram negative bacilli was found.

Table III: Identified pollens from selected sampling sites/polluted areas.

Sampling sites	**Identified pollens**
Traffic areas	*Parthenium, Prosopis, Cassia, Coccus, Casuarina, Argemone, Bauhinia.*
Hospital areas	*Bauhinia, Carica, Casuarina, Cedrus, Cannabis, Eucalyptus, Frestuca.*
Industrial areas	*Prosopis, Parthenium, Cannabis, Cedrus, Carica, Argemone.*
Poultry shop areas	*Carica, Casuarina, Cedrus, Cannabis, Cassia.*

This above table shows identified pollens that were collected by using Vaseline coated slide method.

Table IV: Common fungi that were identified from selected sampling sites or polluted areas.

Sampling sites	**Common identified fungi**
Traffic areas	*Aspergillus, Cladosporioides, Basidiomycetes,Periconia.*
Hospital areas	*Cladosporioides*and *Basidiomycetes.*
Industrial areas	*Fusarium*and*Aspergillus.*
Poultry shop areas	*Periconia, Aspergillus, Cladosporioides, Basidiomycetes.*

The above table shows identified fungi that were found in the polluted areas of Bangalore city via plate exposure method.

Table V: Identified bacteria via conducting various biochemical tests.

Isolate no.	Gram reaction	type	Motility Test	Lactose Fermentatio-n test	Citrate Utilization test	H_2S	Urease test	Catalase test	Oxidase test	Sugar utilization test				Identification
										Sucr-ose	Man-nitol	Arabinose	Inos-itol	
1	+	Bacilli	-	-	-	-	-	+	-	-	-	-	-	*Bacillus*
2	+	Bacilli	-	-	-	-	-	+	-	-	-	-	-	*Bacillus*
3	+	Bacilli	-	-	-	-	-	+	-	-	-	-	-	*Bacillus*
4	+	Cocci	-	-	+	-	-	-	-	-	-	-	-	*Streptococcus*
6	-	Bacilli	+	-	+	-	-	+	+	+	+	+	+	*Pseudomonas*
7	-	Bacilli	-	+	+	-	-	+	-	+	+	+	+	*Klebsiella*
8	-	Bacilli	-	+	-	-	-	-	-	-	+	+	-	*Escherichia*
9	-	Bacilli	-	+	-	-	-	-	-	-	+	+	-	*Escherichia*
10	+	Cocci	-	+	-	-	-	-	-	-	-	-	-	*Streptococcus*
11	+	Bacilli	-	-	-	-	-	+	-	-	-	-	-	*Bacillus*
12	+	Bacilli	-	-	-	-	-	+	-	-	-	-	-	*Bacillus*
13	+	Bacilli	-	-	-	-	-	+	-	-	-	-	-	*Bacillus*
14	-	Bacilli	-	+	-	-	-	-	-	-	+	+	-	*Escherichia*
15	-	Bacilli	+	+	+	-	-	-	-	+	+	+	+	*Enterobacter*
16	-	Bacilli	-	-	-	-	-	+	-	+	+	+	-	*Yersinia*
17	-	Bacilli	+	-	+	-	-	-	-	-	+	-	-	*Salmonella*
18	+	Bacilli	-	-	-	+	-	+	-	-	-	-	-	*Bacillus*
19	+	Bacilli	-	-	+	+	-	+	-	-	-	-	-	*Bacillus*
20	+	Bacilli	-	-	+	+	-	+	-	-	-	-	-	*Bacillus*
21	-	Bacilli	+	-	-	+	-	+	-	+	+	-	-	*Proteus spp*
22	-	Bacilli	+	-	+	+	-	+	-	+	+	+	-	*Citrobacter*
23	+	Cocci	-	+	+	+	-	+	-	-	-	-	-	*Staphylococcus*
24	-	Bacilli	-	+	+	-	-	+	-	+	+	+	+	*Klebsiella*

This above table shows various genuses of bacteria that were identified through various biochemical tests.

So in this survey, isolation and identification of aeroallergens were done and recorded. Common airborne pollens observed were *Parthenium, Casuarina, Cedrus, Cassia, Eucalyptus., Cannabis, Prosopis, Carica, Argemone, Bauhinia, Frestuca*. Common airborne fungal sporesrecoreded were *Aspergillus, Cladosporioides, Basidiomycetes, Periconia, Fusarium, and Alternaria*. Bacteria such as *Bacillus, Streptococcus, pseudomonas, Klebsiella, Escherichia, Staphylococcus, Salmonella, Enterobacter and Proteus spp.* were identified and recorded.In the previous investigation done by some investigators with the similar work reported that fungi such as *Fusarium, Cladosporium, Aspergillus* and pollens such as *Parthenium, Cannabis, Casuarina* were the most dominant aeroallergens in the air traffic polluted areas of the Bangalore city. Differences between these findings may be due to different sampling methods,different sampling seasons, different geographical conditions and different culture media. So by comparing the epidemiological study of aeroallergensin both previous and present study it was found that proper remedial measures are not taken to cure allergic patients and concentration of spore load, airborne pollen has not decreased much. Sampling of air for airborne fungal spores, pollens and pathogens must be done by industrial and occupational hygienists to determine the presence of airborne spores and pollens, their composition and concentrations in situations where occupants complain of ill health. This information could be used in accessing the possibility of hidden spore growth, pathogens and human exposure. For example, in hospital environments monitoring must be done where mostly presence of *Aspergillusfumigatus, Aspergillusniger* and *A. flavus* were detected that causes Aspergillosis in immuno-compromised patients. Records and reports of aeroallergens prevailing in the polluted atmosphere of every city must be maintained. Polluting of air must be controlled. Continuous monitoring of air borne allergens is recommended. There are various sampling devices used to monitor qualitative and quantitative prevalence of allergens such as Gravimetric sampler, Impaction samplers, Suction samplers and Filtration samplers. In India more than 30% of the people are suffering from various types of pulmonary infections every year. To reduce these infections therapeutic aid and remedial measures must be taken and a proper survey must be conducted. Lastly, standardizing analytical methods are urgently needed for quantifying airborne spores and pathogens so that from different laboratories results could be easily compared.

CONCLUSION

Air sampling was done in some selected sampling sites of urban environment of Bangalore city such as in traffic areas, hospital areas, poultry shop areas and industrial areas to record current status of the aeroallergens present in the atmosphere of this city which are responsible for respiratory disorders.Air sampling was then later followed by isolation, identification and characterization of aeroallergens. Different types of pollens, fungi and bacteria were identified which are specifically present in those polluted areas. It was found that pollens such as *Cannabis sativa l., Parthenium, Cassia, Prosopis and Argemone*are the most common pollens in the polluted areas of Bangalore city. Common fungal spores were predicted such as *Aspergillus, Periconia, Fusarium, BasidomycetesCladiospores*and *Alternaria.* And common bacteria found were*Bacillus, Streptococcus, pseudomonas, Klebsiella, Escherichia, Staphylococcus, Salmonella, Enterobacter and Proteus spp* . So the survey carried out before and now indicates that acute laryngitis, sinusitis, pharyngitis and common cold cases are caused by bacteria, most commonly *Streptococcus* and *Bacillus*. Important fungi such as *Aspergillus, Cladosporium,Basidiomycetes* are responsible for causing bronchitis, rhinitis, allergic bronchopulmonarymucoses and hypersensitivity pneumonitis. Pollens such as *Parthenium*cause asthma and bronchitis, *Casuarinas, Cedrus* and *Bauhinia, Eucalyptus*. causes sneezing, rhinitis and skin reactions. These observed aeroallergens are sufficient to cause various pulmonary or lung infections which becomes a threat to human health. Thus, overall experiment shows that important measures should be taken to control aeroallergens and sampling of air must be carried out during every season to check and enumerate the rate of aeroallergens in the atmosphere. The detailed information about aeroallergens is of paramount importance and very useful in diagnosis and management of allergic patients in the country.

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