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## Research Article

# ALLERGIC AIRWAY DISEASES: ROLE OF MICROBIOTA AND ENVIRONMENTS

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### ABSTRACT

A prospective study on 220 patients were clinically diagnosed with COPD were included in the study. Early morning sputum samples were collected within 24 hours of admission in sterile bottle after an antiseptic oral rinse. Identification and its antibiogram from the sputum samples were done by the standard conventional methods. The quality of the sputum was assessed by Bartlett's grading. The microbiological quality of the patients working environment was assessed by determining the concentrations and composition of bacteria and fungi present in the outdoor air using six stage Anderson's air sampler. Study findings revealed that pathogenic bacterial organisms were found in 50 to 80% of patients with COPD during exacerbations. The major causative organisms are *Staphylococcus aureus*, *Klebsiellapneumoniae*, *Streptococcus pyogenus*, *E.coli* and *Pseudomonas aerogenosa* and the common fungal allergens are *Aspergillusfumigatus*, *Candida albicans*, *Penicillium* and *Rhizobium* are found to be predominant organisms in causing allergic airway diseases. The findings of the present study shows clear evidence for presence of pathogenic microbiota in environment and cause allergic airway disease like COPD among the field working individuals.

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## INTRODUCTION

People are exposed to aeroallergens in various settings, both at home and at work. Fungi are ubiquitous airborne allergens and are important causes of human diseases, especially in the upper and lower respiratory tracts. The concentration of allergens in the environment varies, depending on various factors including climate, vegetation, and air quality.<sup>1</sup> Bacterial infections are the most common observable cause of acute exacerbation and death in COPD. Acute exacerbation is characterized by increase in cough, dyspnoea and alteration in type and amount of sputum production.<sup>2,3</sup> Pathogenic bacterial organisms are found in 50 to 80% of patients with COPD during exacerbations. Respiratory disease caused by micro-organisms, is a major global public health problem that affects millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, with 8-6 million new cases and 1.3 million deaths in 2012.<sup>4,5</sup>

The outdoor allergens are predominantly constituted by pollens and fungal spores. Many of these fungal spores are lesser than 10 microns in diameter and their deposition into lower airways is common. The indoor allergens are represented by allergens

from dust mites, cockroaches and pets.<sup>6,7</sup> Bacteria and fungi from human environment may cause allergic reactions. They are associated with number of allergic diseases in humans including allergic rhinitis, bronchial asthma and allergic bronchopulmonary mycoses resulting from exposure to spores. Recent studies of the respiratory tract micro biota using sputum samples and mixtures of saliva and pharyngeal secretions indicate changes and possible associations with pulmonary infections. Bacterial infections are the most common observable cause of acute exacerbation and death is COPD.<sup>8,9</sup> Isolation and susceptibility testing of organisms make diagnosis to be established and also make drug selection more rational.<sup>10-12</sup> This study was undertaken to determine the role of environment in causing allergic airway disease

## MATERIALS AND METHODS

### Collection of clinical samples

The present study is an institutional based cross sectional study. 220 patients for clinically diagnosed with COPD were included in the study. All the patients were positive allergic skin tests of S.S. Institute of Medical Sciences and Research Centre, Davangere between January 2013 to January 2014. Early

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morning sputum samples were collected from all above patients were instructed to collect deep coughed sputum samples into a sterile container with a screw cap.

These samples were streaked on MHA, MacConkey agar and Blood agar incubated at 37°C for 48 hours. Organisms were identified based on colony morphology and standard biochemical tests with antibiogram. Identification of the organisms and its antibiogram from the sputum samples were done by the standard conventional methods. The quality of the sputum was assessed by Bartlett’s grading. (William M.J. and Firegold S.M. 1982)

**Identification of organisms from environmental study**

Bacteriological examination of above patient’s working environment was done. Anderson’s six stage sampler for sampling of airborne bacteria and fungi in working environment was used. Anderson’s sampler is a multi-stage, multi orifice cascade impacter that automatically separate particles based on size from 10 mm and above to 0.3 mm in diameter. Procedures for sampling was done as per manufacturers’ instruction.

The plates were incubated at room temperature for 24-48 hours for bacterial count and for 5 to 7 days for fungal count. The airborne bacterial and fungal concentrations were calculated and expressed as colony forming units per cubic meter of air (Cfu/m<sup>3</sup>). Isolation, identification of the organisms from patients working environment were done by standard conventional techniques.

**RESULTS**

A total of 220 clinical samples from patients suffering from pulmonary infections among different age groups from both male and female were collected from S.S Institute of Medical Sciences and Research Centre, Davangere.

Among 220 clinical samples, the most common bacterial species isolated from patient’s are *Staphylococcus aureus*.e in 58 samples, *Klebsiellapneumonia* 88 samples, *Streptococcus pyogenus*32 samples, *E.coli*15 samples and *Pseudomonsaerogenosa*22 samples respectively (Table 1).

**Table 1** Bacteria species identified in clinical samples

Bacteria spp. Identified	Clinical samples	
	No.of samples	Percentage
<i>Staphylococcus aureus</i>	58	26.36
<i>Klebsiella pneumonia</i>	88	40.00
<i>Streptococcus pyogenus</i>	32	14.54
<i>E.Coli</i>	15	6.81
<i>Pseudomonas aerogenosa</i>	27	12.27

Among 220 clinical samples, the most common fungi species isolated from patient’s are *Aspergillusfumigatus*120 samples, *Candida albicans*60 samples and *Penicillium species*40 samples respectively (Table 2).

**Table 2** Fungi species identified in clinical samples

Fungi spp. Identified	Clinical samples	
	No.of samples	Percentage
<i>Aspergillusfumigatus</i>	120	54.55
<i>Candida albicans</i>	60	27.27
<i>Penicillium spp.</i>	40	18.18

The most common bacteria species identified from patients working environment are *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pyogenus* and *Pseudomonas aerogenosa* (Table 3).

**Table 3** Bacteria species identified from patients working environment using Anderson’s air sampler

Bacterial organisms	Percentage
<i>Staphylococcus aureus</i>	40.91
<i>Klebsiella pneumonia</i>	31.82
<i>Streptococcus pyogenus</i>	13.64
<i>Pseudomonas aerogenosa</i>	13.64

The common fungi species identified from patients working environment are *Aspergillusfumigatus*, *Rhizobium*, *Candida albicans* and *Penicillium*(Table 4).

**Table 4** Fungi species identified from patients working environment using Anderson’s air sampler

Fungi spp. Identified	Percentage
<i>Aspergillusfumigatus</i>	36.36
<i>Rhizobium</i>	27.27
<i>Candida albicans</i>	22.73
<i>Penicillium spp.</i>	13.64

The organism isolated from both patients’ clinical samples and patients working environment is a particular field were identified and listed as follows (Table 5). In paddy and maize field *Staphylococcus aureus* was identified, in groundnut and maize dust *Aspergillusfumigatus*, in cotton field *Penicillium spp.* in rice field *Aspergillusfumigatus* and *Klebsiella pneumonia* identified.

**Table 5** Organism isolated from both patients clinical samples and patients working environment

Agricultural market Field	% organisms from clinical samples		% organisms from environment samples	
	Organism	%	Organism	%
Paddy	S.aureus	55.0	S. aureus	31.5
	E.Coli	24.2		
Maize	S.aureus	26.4	S. aureus	31.56
	Kl. Pneumonia	66.0		
Groundnut	Aspergillus	55.0	Aspergillus	27.6
		Rhizobium	5.9	
Maize dust	Kl. Pneumonia	48.4	Aspergillus	31.56
	S. Pyogenus	22.0		
Cotton	Penicillium	44.0	Penicillium	8.1
	C.albican	33.0		
Rice	Aspergillus	26.4	Aspergillus	51.2
	Kl. Pneumonia	39.6		

The count of culturable bacteria was observed in the range of 1560 to 17060 cfu/m<sup>3</sup> with an average count of 7120.48 Cfu/m<sup>3</sup> and the fungal load ranged from 220 to 1560 Cfu/m<sup>3</sup> with an average count of 776.19 Cfu/m<sup>3</sup>.

**DISCUSSION**

COPD remains among one of the commonest disease of elderly population with the increase in life expectancy, the prevalence of COPD is increasing. Though a progressive disease, acute exacerbation can account for significant increase in the morbidity and mortality of these patients. The bacteriological profile COPD is constantly changing with advanced diagnostic techniques. In most previous studies airway infection seems to

be the first cause of decompensation and accounts for 80-90% of cases whom triggering factor can be demonstrated<sup>2,3,5</sup>.

Positive cultures of the sputum was seen in 220 patients. 27 (12.27%) of patients were positive for *Pseudomonas aerogenosa* that is consistent with the study by Griffith DE, Muzerek GH *et al*, have also found that traditional pathogens *Klebsiella Pneumoniae* were 88 (40%) are declining its importance, while newer pathogens such as *E.coli* 15 (6.81%) *Streptococcus pyogenus* 32 (14.54%) and *Staphylococcus aureus* 58 (26.36%) are becoming important and *Aspergillus fumigatus* were 120 (54.55%), *Penicillium* were 40 (18.18%), and *Candida albicans* were 60 (27%) respectively, which is consistent with study by Herbert Reynolds who have found these organisms are responsible for acute exacerbation of COPD.<sup>13-15</sup>

The average bacterial count during working days (Monday to Saturday) varied between 6456 to 8410 Cfu/m<sup>3</sup> and weekend count was 3973.33 Cfu/m<sup>3</sup>. The weekend bacterial concentration were significantly higher than counts on the working days<sup>18,20</sup>. A total of 91% gram positive bacteria were found, at agricultural market sites, respectively. It was previously demonstrated that gram positive bacteria have greater resistance and survival ability in atmosphere than gram negative bacteria under strong sunlight. *Bacillus*, *Micrococcus*, *Microbacterium*, *Pseudomonas* and *Staphylococcus aureus* were previously reported as the dominant bacteria in outdoor environment from different parts of the world.<sup>19,20</sup>

The working days count averaged between 760 to 1000 Cfu/m<sup>3</sup> and weekend count was 466.67 Cfu/m<sup>3</sup>. The weekend fungal concentration was significantly lower from working days. The working days counts averaged between 503.33 to 653.33 Cfu/m<sup>3</sup> and weekend count was 806.67 Cfu/m<sup>3</sup>. Many of the fungal species identified in the atmosphere like *Alternaria*, *Aspergillus fumigatus*, *Aspergillus*, *flavus*, *Aspergillus niger*, *Penicillium* and *Rhizobium* are common known to outdoor environment<sup>20,21</sup>. The daily fungal spore concentration is associated with increase in number of emergency visit and hospital admission due to exacerbation is asthma attacks. In most of the cases appropriate antibiotics and antifungal improved the clinical condition of most of the patients.

## CONCLUSION

The role of specific microbial species in the pathogenesis of airway disease like COPD has been much studied over the past two decades in different way in different settings. Despite of ongoing debate, the present study significantly proves there are bacterial and fungal spores are abundant in nature and much evidence is now available to show that bacteria and fungi can cause atopic clinical illnesses. Assessing patient's clinical samples and also their working environment plays a very key role in effective treatment of illness conditions. Aerobiological studies and control of environmental factors can reduce the burden of allergy in human beings and can reduce the patient's hospital stay also. More, such studies from India may help in better understanding of the condition which can lead to proper diagnosis and management of such airway diseases.

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