

# Study of respiratory viruses and their coinfection with bacterial and fungal pathogens in acute exacerbation of chronic obstructive pulmonary diseases

Rahat Jahan<sup>1</sup>, Baijayantimala Mishra<sup>1</sup>, Bijayini Behera<sup>1</sup>, Prasanta Raghav Mohapatra<sup>2</sup>, Ashok Kumar Praharaj<sup>1</sup>

<sup>1</sup>Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India, <sup>2</sup>Department of Pulmonary Medicine, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

## ABSTRACT

**Background:** Patients with chronic obstructive pulmonary disease (COPD) develop acute exacerbations (AE), with varying natural history. The exacerbation is triggered by infection, leading to increased morbidity and mortality. The study on infectious aetiology of AECOPD is largely restricted to only viral or only bacterial aetiology. There are no studies from India that have investigated multiple viral, bacterial, and fungal associations from the same group of patients. This prospective study was conducted over 2 years to estimate the incidence and profile of viral infections in AECOPD patients, their coinfection with other bacterial and fungal agents, and association of the type and pattern of infective agent with the clinical severity. **Materials and Methods:** Seventy-four AECOPD cases were included in the study. Multiplex polymerase chain reaction was performed from nasopharyngeal swab using Fast Track Diagnostics Respiratory Pathogens 21 Plus Kit. Ziehl–Neelsen (ZN) stain, Modified ZN, and potassium hydroxide (KOH) mount were performed for *Mycobacteria*, *Nocardia*, and fungal elements. Bacterial cultures and fungal cultures were done as per the standard techniques. Serum samples were tested for *Mycoplasma* and *Chlamydia pneumoniae* immunoglobulin M enzyme-linked immunosorbent assay. **Results:** The number of AECOPD events involving only viral infection, only bacterial infection, bacterial–viral coinfection, and no infection were 43 (58.1%), 32 (43.2%), 20 (27%), and 19 (25.7%), respectively. Influenza A virus was the most common virus (22/43, 51%) identified. In 26 patients, monoviral infections were found, and in 17 patients, polyviral infections were identified, the most common pattern being influenza A and B virus, followed by human rhinovirus and human parainfluenza. The most common bacteria isolated were *Pseudomonas aeruginosa* (9/32, 28%) followed by *Acinetobacter baumannii* and *Klebsiella pneumoniae* (7/32, 21%). Among the viral–bacterial coinfection, human coronavirus NL63 infection was always associated with a bacterial infection. **Conclusion:** This information on the various viral and bacterial etiologies of respiratory infections in AECOPD in this part of India will improve the understanding of the management of AECOPD using a timely institution of antivirals and reduce the overuse of antibiotics and the implementation of routine influenza vaccination.

**KEY WORDS:** Acute exacerbations of chronic obstructive pulmonary disease, virus, influenza, bacteria, coinfections

**Address for correspondence:** Dr. Baijayantimala Mishra, Department of Microbiology, All India Institute of Medical Sciences, Bhubaneswar - 751 019, Odisha, India. E-mail: micro\_baijayantimala@aiimsbhubaneswar.edu.in

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## INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is presently the third most common cause of death worldwide and second in India.<sup>[1]</sup> It is one of the important causes of morbidity and mortality throughout the world, affecting more than 400 million people worldwide. The prevalence, morbidity, and mortality vary across countries.<sup>[1]</sup> The reported prevalence of COPD is highly variable ranging from 0.2% in Japan to 37% in the United States.<sup>[1]</sup> The estimated burden of COPD in India is about 15 million cases based on questionnaire-based prevalence rates and hence may underestimate the true spirometry-based prevalence of COPD.<sup>[1]</sup> An acute exacerbation of COPD (AECOPD) is defined as an acute event which is characterized by a sustained worsening of any of the patient's respiratory symptoms (cough, sputum quantity and/or character, and dyspnea) that is beyond the normal day-to-day variation and leads to a change in medication.<sup>[1]</sup> Exacerbation in COPD cases often leads to hospitalization and associated with increased mortality and is thought to be caused by complex interactions between the host, microbes, and environmental pollution.<sup>[2,3]</sup> However, respiratory infections have been implemented as the major cause of acute exacerbation.<sup>[2,3]</sup>

Previously, it was thought that bacteria are the main cause of exacerbation in COPD. However, with the advent of newer techniques for detection of viruses, the high prevalence of respiratory viral infection in AECOPD cases was revealed.<sup>[4]</sup> Bacterial infections are mostly secondary to primary viral infection. Influenza virus and rhinoviruses are the most prevalent viruses detected in most of the studies.<sup>[4]</sup> The profile of viral agents and their epidemiology is different in different geographical regions. Knowledge regarding this can reduce the overuse of antibiotics and help in preventing exacerbation with timely vaccination.<sup>[2]</sup>

Mixed bacterial and viral infections have been linked to more severe exacerbations of COPD.<sup>[5]</sup> The burden of viral or bacterial infections in AECOPD has been independently assessed in several studies, but there is a paucity of information on viral, bacterial, and fungal coinfections in AECOPD events. The profile of respiratory viruses in the etiology of AECOPD patients and coinfection pattern vary in different geographical regions. The present study aims to find the prevalence of various respiratory viruses as well as bacterial and fungal pathogens in AECOPD patients.

## MATERIALS AND METHODS

### Study design and subjects

The present prospective study was carried out for a period of 2 years from July 2017 to June 2019. Adult patients previously diagnosed as COPD (as per the GOLD criteria<sup>[6]</sup>) presenting to the Department of Pulmonary Medicine, AIIMS, Bhubaneswar, with COPD exacerbations were included in the study within 24 h of their presentation,

after obtaining informed written consent. The assessment of COPD severity was done according to the GOLD criteria.<sup>[6]</sup> The study was commenced after obtaining the approval of the institute ethics committee.

### Inclusion and exclusion criteria

Patients were recruited from the outpatient department, inpatient department, and intensive care unit. They were categorized into moderate and severe on the basis of the mode of presentation of clinical symptoms. Moderate exacerbation of COPD is defined as worsening of respiratory symptoms beyond normal day to day variations and leading to a change in medication but not requiring hospitalization and severe exacerbation are those of the same clinical presentation but increased in severity along with hypoxia who requires hospitalization. Pregnant women, patients who received systemic antibiotics and/or antifungal agents within the last 72 h, those with confirmed pneumonia (on chest radiograph) within 48 h, and those with underlying pulmonary malignancy and asthma were excluded from the study.

The following demographic and clinical parameters were recorded on admission for each case: age, sex, stage of COPD (according to the GOLD criteria), smoking habits, influenza vaccination, comorbidities (confirmed by medical records), current medication as well as signs and symptoms of respiratory tract infection. All patients underwent routine blood examination and chest radiograph.

### Sample collection and processing

Nasopharyngeal swab, sputum, and blood were collected before administration of antibiotics (if needed) from all the patients clinically diagnosed with AECOPD who were subjected for detection of viral agents and bacterial and fungal culture. Bronchoalveolar lavage and endotracheal tube secretion were collected from selected cases when clinically indicated.

### Viral assay

RNA was isolated from the nasopharyngeal swab samples using a viral RNA isolation kit (Qiagen RNeasy Mini Kit, Qiagen, Germany) as per the manufacturer's instructions. The viral RNA extracts were stored at  $-70^{\circ}\text{C}$  until amplification. The isolated viral RNA was subjected to multiplex real-time polymerase chain reaction (RT-PCR) assay using Fast Track Diagnostics (FTD) Respiratory Pathogens 21 Plus Kit (FTD, Luxembourg). In brief, the kit consists of six separate primers and probes, which at specific wavelength are specific for detection of respiratory viruses (influenza A virus [IAV] [H3N2] and influenza A [H1N1] virus [swine lineage]); influenza B virus; human coronaviruses NL63, 229E, OC43, and HKU1; human parainfluenza viruses 1, 2, 3, and 4; human metapneumovirus (hMPV) A and B; human rhinovirus (HRV); human respiratory syncytial viruses (RSVs) A and B, human adenovirus; Enterovirus; human parechovirus; human bocavirus; *Mycoplasma*

*pneumoniae*; *Chlamydia pneumoniae*; *Streptococcus pneumoniae*; *Staphylococcus aureus*; and *Haemophilus influenzae* from the nasopharyngeal swab by RT-PCR.

### Sputum microscopy and culture

Gram's stain of sputum was examined for polymorphonuclear leukocytes and epithelial cells. Samples containing <10 epithelial cells and >25 leukocytes per low power field were further subjected to bacterial culture.<sup>17</sup> A sputum sample was also subjected to Ziehl-Neelsen (ZN) staining and modified acid-fast staining for the detection of acid-fast bacilli and *Nocardia*, respectively. The sputum sample was subjected to KOH mount and fungal culture and incubated upto 4 weeks at 25°C and 37°C following standard methods.

### Detection of IgM antibody for atypical bacterial pathogens

All sera were subjected to detection of immunoglobulin M (IgM) enzyme-linked immunosorbent assay (Calbiotech, USA) of *M. pneumoniae* and *C. pneumoniae* species, according to the manufacturer's instruction.

### Statistical analysis

Continuous data were expressed as mean ± standard deviation. Categorical data were expressed as proportions. *P* < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows version 22.0 (IBM Co., Armonk, NY, USA).

## RESULTS

### Patient characteristics

A total of 74 patients with AECOPD were enrolled who fulfilled the inclusion criteria and from whom all the three required samples (nasopharyngeal swab and sputum) could be collected. Among these 74 patients, 65 were male. The participants of this study were of age group between 40 and 86 years, and the mean age was 65.49 ± 10.40 years. During the course of their exacerbation, 61 (82.5%) of patients were hospitalized and 17.6% were treated as outpatients and of the admitted patients, 40 (40/61, 65%) were admitted to the intensive care unit. Overall, 42 (42/74; 56.8%) patients had associated comorbid conditions. Hypertension (14/42; 18.9%) was the most common comorbidity followed by ischemic heart disease in 13 (13/42; 17.6%) patients.

### Infections in acute exacerbations of chronic obstructive pulmonary disease cases

Of the 74 AECOPD patients, 58 (58/74; 78.30%) cases were found to have a respiratory infection with either viral, bacterial, tubercular, atypical bacterial, or fungal agents. Only viral infection was found in 23 (23/74; 31%) patients, only bacterial infections in 12 (12/74; 16%), bacterial-viral coinfection in 20 patients (20/74; 27%), 3 had a fungal infection, and no infective agents could be detected in 16 patients (16/74; 22.70%). This amounted to viral infection in 43 patients (43/74, 58%), followed by a bacterial infection in 32 (32/74, 43.2%) patients.

### Pattern of viral infections in patients with acute exacerbations of chronic obstructive pulmonary disease

Of the 43 patients with a viral infection, monoviral infection was found in 26 patients and polyviral infection was identified in 17 patients. Of the later, >2 viral infection was found in 9 (9/17, 52.9%), >3 in 6 (6/17; 35%), and >4 in 2 (2/17; 11%) events. The monoviral and polyviral infection pattern of various respiratory viruses is depicted in Table 1. The spectrum of detected respiratory viruses is shown in Figure 1. Among the polyviral infection, coinfection with influenza A and B (IAV + IBV) was found to be the most common combination followed by HRV and human parainfluenza 3 (HRV + hPIV3) coinfection. The details of the pattern of polyviral infections are shown in Figure 2.

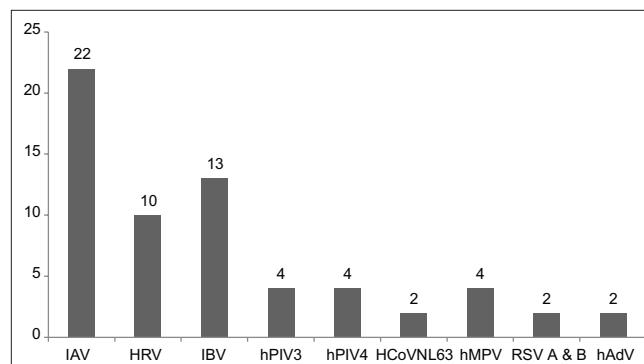
### Bacterial infections in the patient of acute exacerbations of chronic obstructive pulmonary disease

Of the 74 sputum samples tested, the bacterial culture was positive for 32 samples (32/74; 43%). The most common bacteria isolated were *Pseudomonas aeruginosa* in 9 (9/32; 28%) samples, followed by *Acinetobacter baumannii* and *Klebsiella pneumoniae* in 7 each (7/32; 21%), *Escherichia coli* in six samples, *S. aureus* in two samples, and *H. influenzae*, *Chryseobacterium indologenes*, and *M. pneumoniae* in one sample each. One sample was found to be positive for *Mycobacterium tuberculosis* by ZN stain and cartridge-based nucleic acid amplification test (CBNAAT). None of the samples were

**Table 1: Monoviral and polyviral infection pattern of various respiratory viruses in acute exacerbations of chronic obstructive pulmonary disease patients**

	Mono viral	Poly viral	Total
Influenza A virus	11	11	22
HRV	8	2	10
Influenza B	3	10	13
Human parainfluenza virus 3	0	4	4
Human parainfluenza virus 4	1	3	4
Human corona virus NL63	2	0	2
hMPV	4	0	4
HRSVA and B	0	2	2
HAdV	0	2	2

HRV: Human Rhinovirus, hMPV: Human metapneumovirus, HAdV: Human Adenovirus; HRSVA: Human rhinovirus A



**Figure 1: Distribution of respiratory viruses detected in acute exacerbations of chronic obstructive pulmonary disease patients**

positive for *Nocardia* spp (by modified ZN staining). *M. pneumoniae* and *H. influenzae* type B were detected in one each sample, each tested by FTD Respiratory Pathogen 21 Plus Kit. The profile distribution of bacterial pathogens is shown in Figure 3.

**Viral-bacterial coinfection in the patient of acute exacerbations of chronic obstructive pulmonary disease**

Viral-bacterial coinfection was observed in 20 of 74 AECOPD patients (27%). Sixteen of 45 influenza virus isolates (IAV and IBV together) had bacterial coinfection. The details of viral-bacterial co-infection are given in Figure 4.

Among the bacterial agents, most of the bacterial infections were associated with co-infection with viruses except *K. pneumoniae* which was isolated as single agent in (3/7, 42.8%) of cases.

**Association of severe acute exacerbations of chronic obstructive pulmonary disease with infection**

Severe group of AECOPD patients (Stage III and Stage IV) was found to have a significantly higher number of polyviral infections as compared to that of a moderately severe group of patients (82% in the severe group as compared to 69% in the moderate group,  $P < 0.01$ ).

Among the monoviral infections, IAV, rhinovirus, and influenza B virus were found to be significantly associated

with severe AECOPD cases, ( $P = 0.04$ ,  $P = 0.018$ , and  $P = 0.019$ , respectively), whereas hMPV was found to be significantly associated with moderately severe group of AECOPD patient as compared to the severe group of patients.

There was no significant association found between the severity of the AECOPD patient with the presence of coinfection ( $P = 0.101$ ).

**Prevalence of respiratory viral infection in different age groups of acute exacerbations of chronic obstructive pulmonary disease patients**

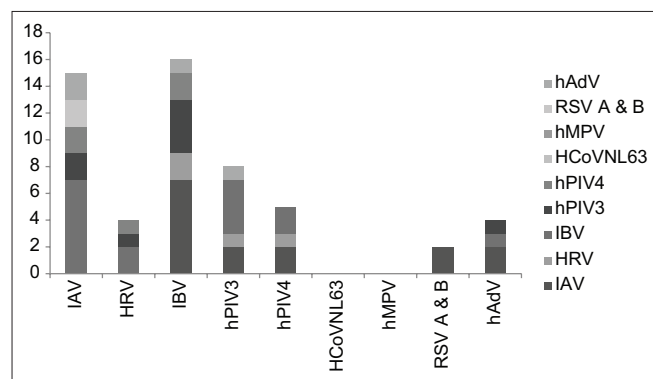
The prevalence of respiratory viral infection was found to be high in patients with  $\geq 60$  years of age (52) as compared to patients of 35–49 years (4) and 50–59 years (14). Of the total 22 IAV isolates, 19 (86%) were found in  $> 60$  years of age group [Figure 5].

**Prevalence of fungal infection in acute exacerbations of chronic obstructive pulmonary disease patients**

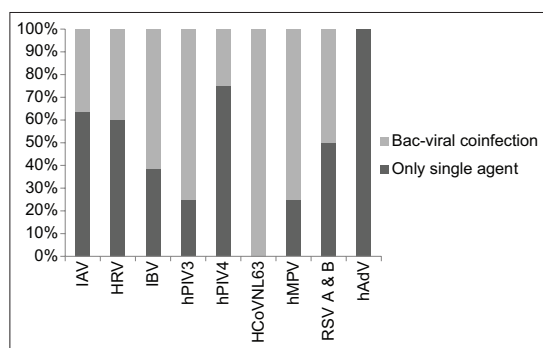
Sputum samples of three patients (3/74.4%) were found to be positive for the growth of *Aspergillus flavus*.

**DISCUSSION**

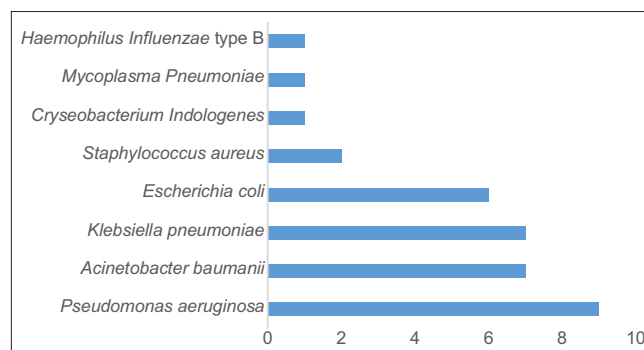
Respiratory viral infections are shown to be the most common etiology (58%) in AECOPD patients in the present



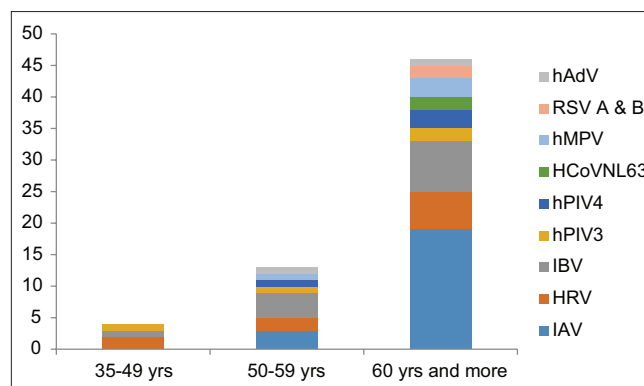
**Figure 2:** Pattern of polyviral infection in acute exacerbations of chronic obstructive pulmonary disease patients



**Figure 4:** Viral bacterial co-identification in acute exacerbations of chronic obstructive pulmonary disease patients



**Figure 3:** Distribution of bacterial agents in acute exacerbations of chronic obstructive pulmonary disease patients



**Figure 5:** Prevalence of respiratory viral infection in different age groups of acute exacerbations of chronic obstructive pulmonary disease patients

study on the patients from Eastern India. Viral infection in AECOPD patients has been reported from 20% to 64% in various studies in recent years.<sup>[8-17]</sup> The difference in the rate of viral infection in different studies could be due to geographic variation. In an Indian study from Kashmir, viral infection has been reported in 20% of hospitalized COPD patients.<sup>[8]</sup> The higher rate of viral infection in our study could be due to differences in the climatic condition in the eastern part of the country as compared to Northern India. Majority of these studies including ours have used molecular assay for the detection panel of respiratory viruses. Use of newer techniques such as multiplex real-time PCR and microarray have revealed the importance of viral infection in COPD patients.<sup>[13,18]</sup> Influenza virus, HRV, and RSV are among the common viruses detected from patients with AECOPD.<sup>[8-11]</sup> The detection of influenza virus as the common viral agents in the present study supports the reports of others and could be due to a high number of influenza unimmunized individuals in our study population.<sup>[19]</sup> HRV is detected as one of the common viral agents (23%) in our AECOPD patients. A similar report has also been published in a systemic review of 19 studies using PCR as the detection method.<sup>[11,20]</sup> It is to be noted that HRV, which was mainly known only as a causative agent of common cold, appears to be one of the important pathogens in patients with COPD with acute exacerbation in majority of studies. Risk of infection has been reported to be higher with age.<sup>[21]</sup> On comparing the rate of viral infections, we found a higher frequency of viral infection in elderly AECOPD patients (>60 years) as compared to the non-elderly AECOPD patients.

Bacterial infections have also been found in a substantial proportion of patients (43%) of AECOPD patients. Several other studies have also reported a similar proportion of bacterial infection (42%–49%).<sup>[22]</sup> Most of the Indian studies have reported Gram-negative pathogens as the common bacterial infection in this group of patients.<sup>[22-24]</sup> Our study is in agreement with other Indian studies by reporting *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii* as the common bacterial agents in AECOPD patients.

Association of severity in AECOPD patients with infection due to any particular type of pathogens such as viral, bacterial, or coinfection has remained controversial.<sup>[10]</sup> Koul *et al.* have shown the association of respiratory viruses in hospitalized AECOPD patients.<sup>[8]</sup> The present study found a significant association of polyviral infection with severe AECOPD patients, whereas the association of bacterial infections was found to be present with a moderately severe group of AECOPD patients. Among the monoviral infections, influenza A, influenza B, and rhinovirus were significantly associated with the severe AECOPD patients. This is in agreement with Koul *et al.* where significant association with severity in AECOPD patient was also found with influenza and rhinovirus.<sup>[8]</sup> Choi *et al.* have reported a higher bacterial identification rate with more advanced COPD.<sup>[13]</sup> *P. aeruginosa* has been

identified in severe COPD patients possibly related to the high prevalence of bronchiectasis in moderate-to-severe COPD patients.<sup>[13]</sup>

Our study found concomitant infection with one case of *M. tuberculosis* and is in agreement with a study from Hong Kong, in which *M. tuberculosis* was isolated from sputum in 1.1% of the cases.<sup>[25]</sup> Past history of pulmonary tuberculosis is shown to be an independent predictor of prolonged intensive care in AECOPD patients in two Indian studies.<sup>[26,27]</sup> The influence of COPD and TB on the natural course of each other needs to be elucidated further.

Atypical bacterial agents have been reported as relatively uncommon in AECOPD (5%–10%).<sup>[3,28]</sup> Our study is in agreement with published literature as only one *M. pneumoniae* was detected by the Multiplex PCR FTD respiratory pathogen diagnostic panel.<sup>[28]</sup>

In the present study, *A. flavus* was isolated in 3 (3/74, 4%) of AECOPD patients. The clinical significance of a fungal culture alone from sputum samples in the absence of corroborative histopathological and radiological findings is uncertain and therefore cannot be assumed to be a triggering factor for AECOPD.

There are a few limitations of this study. We have done only a qualitative molecular detection method. Further studies to detect viral load at time of acute exacerbation versus stable state are necessary to establish their role as pathogens in AECOPD patients. Finally, as the present study was single centric, further multicentric studies are needed to come to definitive conclusions regarding the rate of infections with viral, bacterial, and their coinfection in AECOPD.

## CONCLUSION

Viral infections were found to be the major cause of AECOPD. The use of molecular methods for the detection of viral agents helped to reveal the real epidemiology of viral infections. A high proportion of AECOPD patients with only viral infection in our patients signify the importance of timely institution of antiviral therapy, avoidance of unnecessary antibiotics, and implementation of influenza vaccination.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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