

## Original Research Article

# SPUTUM MICROBIOLOGY IN ACUTE EXACERBATIONS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AT TERTIARY CARE HOSPITAL

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### ABSTRACT

**Background:** Chronic Obstructive Pulmonary Disease (COPD) is defined as a preventable and treatable disease with pulmonary component characterized by airflow limitation that is of not fully reversible which is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases and some significant extrapulmonary effects that may contribute to the severity in individual patients (1). It includes: Emphysema, Chronic Bronchitis, Small airway disease (2). The aim is to study the microbiology of sputum and BAL (bronchoalveolar lavage) samples in patients admitted at tertiary care hospital for acute exacerbations of COPD (AECOPD). The objective is to identify bacterial and fungal isolates from sputum and BAL sample in patients with AECOPD. To study the antimicrobial and antifungal susceptibility pattern of isolates from sputum and BAL samples in patients with AECOPD.

**Materials and Methods:** A cross sectional study was conducted on sputum samples collected from clinically suspected AECOPD patients admitted for acute exacerbations in COPD at government chest hospital, Hyderabad over a period of 1 year among n=100 inpatients above 30years of both gender after approval from institutional ethical committee. Inclusion criteria Clinically diagnosed AECOPD patients as per Anthonisen criteria. Both gender, smokers and non-smoker, age ranging greater than 30 years & Patients giving consent. Exclusion criteria is Patients with interstitial lung disease, Dyspnoea due to pulmonary thromboembolism, Ischemic heart disease. Patients clinically diagnosed as tuberculosis, malignancy. Previous admission or antibiotic treatment in the last 21 days. Patients managed in outpatient departments or requiring Intensive Care Unit admission. Patients who are not giving consent.

**Results:** 100 clinically diagnosed Chronic Obstructive Pulmonary Disease patients with acute exacerbations were included. 44 bacterial isolates were obtained, whereas no fungal isolates were found in the sample. The study group consisted predominantly males constituting 95%. COPD is leading cause of morbidity and mortality and early introduction of empirical antibiotics, can improve outcome and reduce mortality [3,4,]. Episodes of exacerbation add to the burden of the disease and are major reason for health care utilization including hospitalizations and intensive care admissions. [5]

**Conclusion:** Acute exacerbations due to infectious aetiology are frequent cause of morbidity in COPD patients. Isolation of aetiological bacterial agents using standard culture methods helps in management of AECOPD and its complications. Repeated antibiotic administration in management of AECOPD is associated with development of resistance in the causative agents. Thus, culture with antimicrobial susceptibility test report is necessary in the management of AECOPD. In the present study Streptococcus pneumoniae and Pseudomonas aeruginosa were commonest gram-positive and gram-negative bacterial isolates.

**Keywords:** Sputum Microbiology, Acute Exacerbations, COPD.

## INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is defined as a preventable and treatable disease with pulmonary component characterized by airflow limitation that is not fully reversible which is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases and some significant extrapulmonary effects that may contribute to the severity in individual patients.<sup>[1]</sup> It includes: Emphysema, Chronic Bronchitis, Small airway disease.<sup>[2]</sup>

Acute Exacerbation of COPD (AECOPD): This condition is defined as a sustained worsening of the patient's condition, from the stable state (in the patient's baseline dyspnea and cough or sputum, or both and beyond normal day to day variation, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD as per Gold guidelines.<sup>[3-6]</sup> It is characterized by presence of increased sputum volume, sputum purulence and dyspnea.<sup>[3]</sup>

Burden of the disease: Chronic obstructive pulmonary disease (COPD) is associated with significant morbidity and mortality, with the World Health Organization estimating its rise from being the fourth to the third leading cause of death by 2030. The mortality rates are supposed to increase by 30% every decade (4).

Almost 95% of mortality due to chronic respiratory disease in India can be assigned to COPD. Exacerbations of COPD have considerable impact on health care system at both primary and tertiary care levels as they are the major reason for antibiotic use and admissions. WHO has estimated that 600 million people worldwide have COPD. Additionally, exacerbations lead to indirect costs because of days lost from work. COPD affects 30% of patients seen in chest clinics and constitutes 1-25% of hospital admissions all over India.<sup>[7]</sup>

AE-COPD is a common cause of emergency room (ER) visits and is a major cause of morbidity and mortality.<sup>[8,9]</sup> COPD Prevalence had been estimated as 17 million and it is likely to increase by over 30% in next decade. Highest prevalence (9.4%) was reported from North Indian rural population from a study conducted by Jindal et.al from 1964-1995.<sup>[10]</sup>

**Causes of AE-COPD:** Exacerbations are caused or triggered by a variety of factors including viruses, bacteria, and air pollutants, and are associated with acutely increased worsening of existing (acute-on-chronic) airway inflammation and also due to defects in host defense mechanisms. Alterations produced in the bronchial epithelium by the damaging action of smoking favor bacterial adhesion and colonization. In turn, airway colonization and chronic infection contribute to progressive pulmonary damage via the action of proinflammatory substances in what is known as the "vicious circle theory."<sup>[11]</sup>

Infections are the important cause of acute exacerbation. Bacteria are responsible for causing 60% of exacerbations. Viral infections are the likely cause of approximately 30% of exacerbations, while PCR studies have suggested 40%. Fungal isolates have not been reported.

Exacerbations punctuate the clinical course of COPD in many patients, mostly of an infectious etiology, are a frequent cause of morbidity in COPD patients. Furthermore, infection was the most common observable cause of death in prospectively followed-up COPD patients. This condition is highly serious in our country as the prevalence of smoking and air pollution is very high which are the main cause for COPD and increase the frequency of exacerbation. Since it is a vicious cycle recurrent exacerbation will lead to rapid deterioration of lung parameters and early death due to respiratory failure and increased economic burden.<sup>[12]</sup>

Antimicrobial therapy: Over 90% of patients with AECOPD are treated with antibiotics, on empirical basis without proper sputum analysis. So the effectiveness of treatment is uncertain due to emerging new strains and their resistant pattern thereby leading to recurrent exacerbation.<sup>[13]</sup> It would be useful to find the proper etiology of COPD exacerbations, thereby facilitating the orientation of antibiotic treatment and reducing the high number of failures recorded with empiric treatment, which in some cases, is as high as 26%.<sup>[14]</sup>

This study is taken up to find out the Bacteriological & Fungal profile and their sensitivity pattern in AECOPD patients as the knowledge of possible bacterial & fungal etiology and sensitivity patterns of the isolates, facilitates the orientation of antibacterial and antifungal treatment so that timely institution of correct management is important for better prognosis of disease and helps in preventing development of antimicrobial resistance.

**AIM:** To study the microbiology of sputum samples in patients admitted at tertiary care hospital for acute exacerbations of COPD (AECOPD).

### Objectives:

1. To identify bacterial and fungal isolates from sputum sample in patients with AECOPD
2. To study the antimicrobial and antifungal susceptibility pattern of isolates from sputum samples in patients with AECOPD.

## MATERIALS AND METHODS

**Study Design:** A Cross-sectional study was conducted on sputum samples collected from clinically suspected AECOPD patients admitted for acute exacerbations in COPD (AECOPD) at Government General and Chest Hospital, Hyderabad.

**Period of Study:** The study conducted over a period of 1 year (November 2019 to November 2020)

**Sample Size:** The sample size is calculated using  $p = \text{Prevalence} = 40$

$$n = \frac{4pq}{i^2}$$

$$q = (100-p) = 60 \quad i = 10$$

According to Deepthi babu et al,<sup>[15]</sup> bacterial and fungal prevalence (P) was 40% and 10% allowable error, with power of the study being 80% and with 95% confidence interval, the sample arrived was 96.

**Study population:** The Study population consisted of 100 in-patients of both the sexes aged more than 30yrs presenting with signs and symptoms of AECOPD.

**Ethical clearance:** The study was taken up after the Institutional Ethical Committee approval. Informed consent was obtained from the study group.

**Inclusion criteria:**

- Clinically diagnosed AECOPD patients as per Anthonisen criteria
- Both gender
- smokers and non-smokers
- age ranging greater than 30 years
- Patients giving consent

**Exclusion criteria:**

- Patients with interstitial lung disease
- Dyspnea due to pulmonary thromboembolism, Ischemic heart disease.
- Patients clinically diagnosed as tuberculosis, malignancy.
- Previous admission or antibiotic treatment in the last 21 days
- Patients managed in outpatient departments or requiring Intensive Care Unit admission.
- Patients who are not giving consent.

**Collection of data:** Data was collected from patients who satisfied the inclusion criteria, using preformed structured questionnaire (Proforma). Demographic details like name, age, gender, address, date of admission, clinical data like presenting complaints, personal history, past medical history, history suggestive of immunization, chest radiographic findings, physical examination findings and details of clinical diagnosis were collected.

Basic blood investigation such as Hemoglobin estimation, Total leukocyte count, Differential count, ESR, Blood glucose level, Blood urea and Serum creatinine levels were documented (Master Chart).

**Sample collection and Transport**

- Sputum samples were collected immediately after admission before starting of antibiotics. When the

patient is unable to expectorate, aerosol induced sputum was collected using 15% sodium chloride nebulization.

- Bronchial wash samples collected in sterile containers at the time of bronchoscopic examination were received at microbiology lab.

**Processing of Specimen:** The sputum and bronchial wash samples were examined for physical appearance, Gram stain, KOH mount for fungal elements, culture for bacterial & fungal isolates.

**Bacterial culture:** The swab was inoculated on to 5% sheep blood agar and MacConkey agar. The plates were incubated at 37<sup>o</sup> C with 5-10 % CO<sub>2</sub> for 24 hrs. Culture plates were observed for growth after 24hrs and 48hrs. The organisms isolated were identified using standard microbiological techniques - the colony morphology, culture smear and biochemical properties. Antimicrobial susceptibility of the bacterial isolates to the commonly used antibiotics was done by Kirby Bauer disc diffusion method. For all Staphylococcus aureus detection of methicillin resistance was carried out. For all Gram-negative isolates, testing for ESBL and MBL detection was carried out.

**Fungal culture:** The specimen was inoculated on Sabouraud's dextrose agar with chloramphenicol or gentamycin and incubated at 25°C in a BOD incubator, observed for growth daily for first week and once every week for next 3 weeks.

If growth was present, colony morphology, rate of growth, texture, obverse and reverse pigment were noted. The colony was teased on a clean glass slide with a drop of Lactophenol cotton blue. Cover slip was placed over it, and then the preparation was examined under low power and high-power objectives. Depending upon colony morphology and microscopic appearance, the isolate was identified.

**For Gram positive organisms' identification:** Tube Catalase test done and catalase negative Gram-positive cocci (GPC) were further tested for Bacitracin and Optochin susceptibility (Annexure IX). Catalase positive GPC were further identified with Coagulase test (Slide coagulase and Tube coagulase methods)

**For Gram negative bacilli (GNB) identification:** Catalase test, Oxidase test, Motility test, Indole test, Methyl red test, Voges Proskauer test, Citrate test, Urease test, Triple sugar iron test, Nitrate reduction test, Sugar fermentation test were done and based on the results the organism was identified as shown in [Table 1].

**Table 1: Biochemical tests for identification of Gram-negative bacilli**

	<b>Klebsiella</b>	<b>Pseudomonas</b>
Motility	-	+
Indole	D	-
MR	-	-
VP	+	-
Citrate	+	+
H <sub>2</sub> S	-	-
Urease	+	-
Catalase	+	+
Oxidase	-	+
OF test	-	+

Antimicrobial susceptibility testing of the isolates was done on Mueller-Hinton agar using Kirby-Bauer disc diffusion method. Bacterial suspension was prepared by inoculating few isolated colonies of similar morphology into 4-5 ml of peptone water and incubated for 2-4 hours at 37°C. The turbidity of the broth was adjusted to 0.5 Mc Farland and lawn

culture was made on the surface of the medium using sterile cotton swabs. Antimicrobial discs were applied with the help of sterile forceps and the plates were incubated overnight at 37°C. Zones of inhibition were measured next day and reported following the CLSI guidelines [Table 2 and 3]

**Table 2: Antimicrobial discs used for Gram-Positive Cocci:**

S. No.	Antimicrobial Disc	Sensitive Zone	Resistant Zone
1	Cefoxitin (CX) 30µg	>22 mm	< 21 mm
2	Amoxycylav (AMC) 20/10µg	>20 mm	< 19 mm
3	Ofloxacin (OF) 5µg	>18 mm	<14 mm
4	Cefotaxime (CTX) 10µg	>23 mm	<14 mm
5	Cotrimoxazole (COT)(1.25/23.75µg	>16 mm	<10 mm
6	Linezolid (LZ) 30µg	>21 mm	<20 mm
7	Gentamycin (GEN) 10 µg	>15 mm	<12 mm

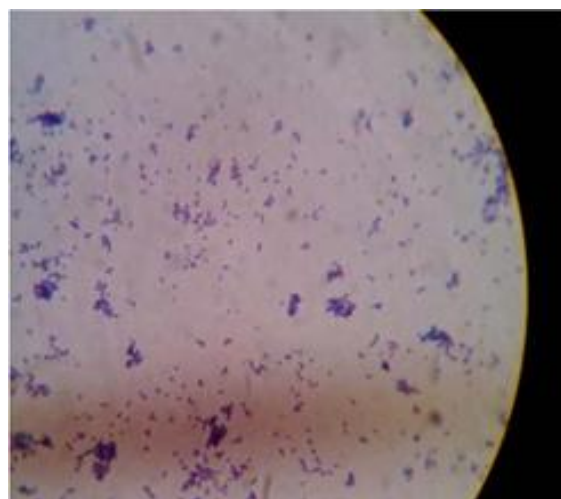
**Table 3: Antibiotic discs used for the Gram-Negative Isolates**

S No	Antimicrobial Disc	Sensitive Zone	Resistant Zone
1	Imipenem (IPM) 10µg	>23 mm	<19mm
2	Ciprofloxacin (CIP) 5 µg	>26 mm	<21 mm
3	Piperacillin+tazobactam (PIT) 100/10µg	>21 mm	<17 mm
4	Amikacin (AK) 30µg	>17 mm	<14 mm
5	Cefepime (CPM) 30µg	>25 mm	<18 mm
6	Ceftazidime (CAZ) 30µg	>21 mm	<17 mm
7	Cotrimoxazole (COT) 1.25/23.75µg	>16 mm	<10 mm
8	Cefotaxime (CTX) 5µg	>26 mm	<22 mm
9	Cefoxitin (CX) 30µg	>18 mm	<14mm

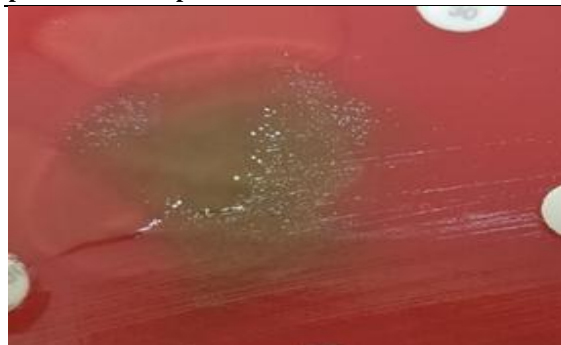
Data Entry and Analysis: The data was entered in Microsoft Excel 2010 version. Data was analyzed using Microsoft Excel 2010 and Epi Info 7.2.0. Descriptive and inferential statistical analysis were used in the present study.

## RESULTS

100 clinically diagnosed Chronic Obstructive Pulmonary Disease patients with acute exacerbations were included. 44 bacterial isolates were obtained, whereas no fungal isolates were found among the samples. The study group consisted predominantly males 95% and females 5% only. 58% of the study group was between 51-70 years. Most affected age group (32%) was 61-70yrs followed by 51-60yrs with 26% whereas 3% of patients were more than 80yrs age. Only 7% of study group were in the age group of 30-40yrs.



**Figure 1: Gram's stain from culture showing gram-positive cocci in pairs**



**Figure 2: Bile solubility by plate method for Streptococcus pneumoniae**

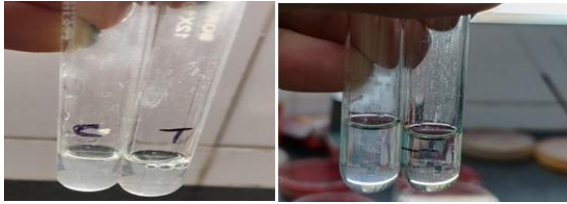


Figure 3: Bile solubility by plate method for *Streptococcus pneumoniae*



Figure 4: Optochin sensitivity and Antimicrobial susceptibility of *Streptococcus pneumoniae*

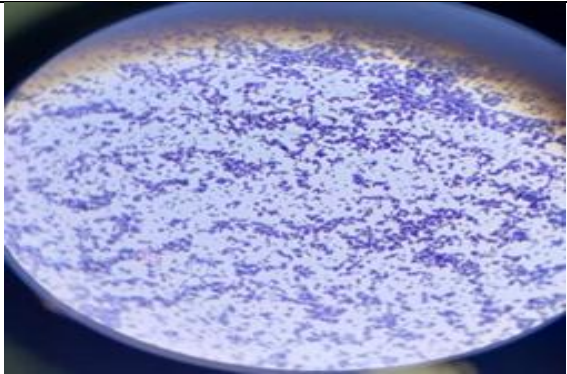


Figure 5: Gram's stain from culture showing gram-positive cocci in clusters



Figure 6: Golden yellow pigmentation of *Staphylococcus aureus* on nutrient agar



Figure 7: Antimicrobial susceptibility of *Staphylococcus aureus*

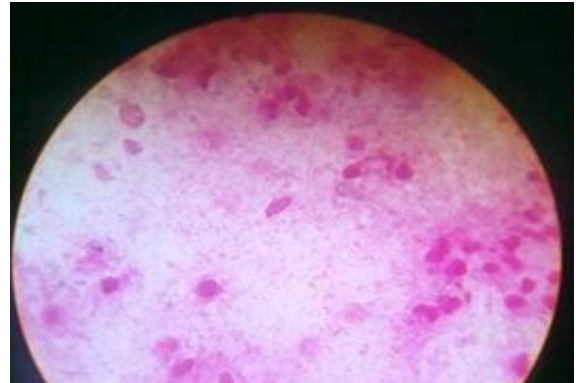


Figure 8: Direct gram stain of sputum showing plenty of pus cells and gram-negative bacilli.



Figure 9: MacConkey agar showing lactose fermenting colonies of *Klebsiella pneumoniae*



Figure 10: Antimicrobial Susceptibility of *Klebsiella pneumoniae*

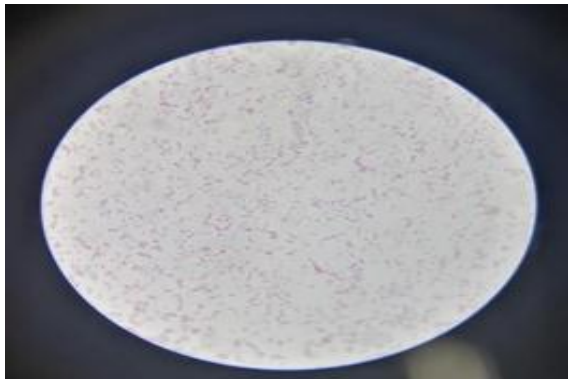


Figure 11: Gram stain of culture showing long slender gram-negative bacilli



Figure 13: *Pseudomonas aeruginosa* showing greyish moist colonies on blood agar

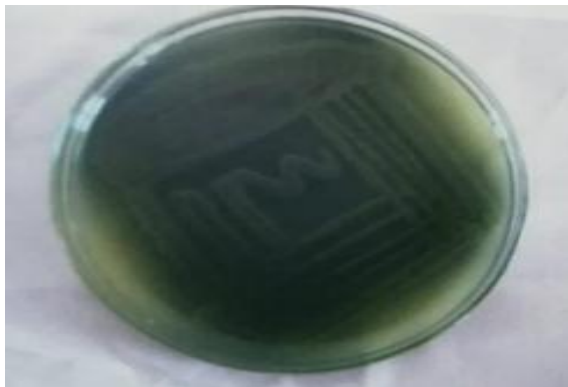


Figure 12: *Pseudomonas aeruginosa* showing greyish moist colonies on blood agar

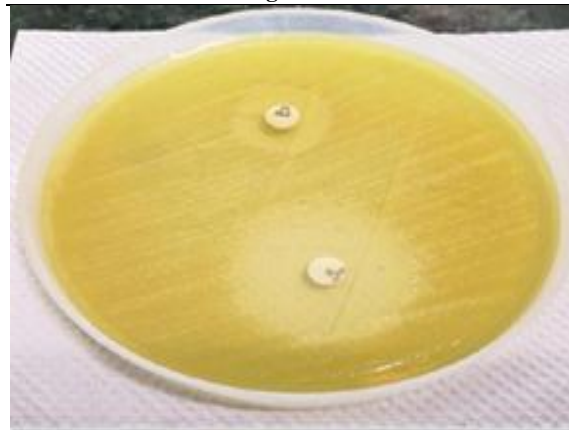


Figure 14: Combined disk test for detection of mbl production for *Pseudomonas aeruginosa*

Table 4: showing various variable in the study population

Symptoms	No	%
Increased Sputum volume	67	67%
Increased Sputum purulence	33	33%
Increased degree of breathlessness (dyspnoea)	100	100%
Fever >38°C	18	18%
wheeze/crepitations	76	76%
Gold criteria on severity of COPD	N	%
Mild(FEV1 >80%)	3	3.0
Moderate(FEV1 50 -79%)	58	58.0
Severe(FEV1 30 - 49%)	39	39.0
Type of exacerbation As per Anthonisen	N	%
Type 1	71	71.0
Type 2	8	8.0
Type 3	21	21.0

Table 5: Distribution of various isolated bacteria amount tested samples

Sample	No of cases	Culture Positive	Culture Positive %	Gram positive isolates			Gram negative isolates		
				Strep. pneumoniae	Staph. aureus	Total	Pseudomonas	Klebsiella	Total
SPUTUM	97	41	42.26%	16	11	27	8	6	14
BAL	3	3	100%	0	0	0	3	0	3
Total	100	44	44%	16	11	27	11	6	17

Among the 44 culture positive samples, 41 were sputum samples. All the 3 BAL samples were culture positive. Commonest bacterial isolates were gram-

positive bacteria (61.36%) followed by gram-negative bacteria (38.36%).

Table 6: Type of Bacterial isolates among 44 culture positive.

	Number of culture positive isolates	Percentage
Monobacterial	40	90.9%
Polybacterial	2	9.1%

Single bacterial isolate was observed in 90.1% of cases among 44 culture positives whereas polybacterial growth was observed in 9.1% of cases.

Polybacterial isolates were *Pseudomonas* and *Klebsiella* in 2 samples.

**Table 7: Antimicrobial susceptibility pattern of gram-positive isolates by Kirby Bauer disc diffusion test:**

Antibiotics µg	Streptococcus pneumoniae n =16				Staphylococcus aureus n =11			
	Sensitive	%	Resistant	%	Sensitive	%	Resistant	%
Amikacin(30)	-	-	-	-	11	100	-	-
Ampicillin(10)	11	68.8	5	31.3	6	54.5	5	45.5
Cefotaxime(30)	16	100	-	-	8	72.7	3	27.3
Cefoxitin(30)	-	-	-	-	7	63.6	4	36.36
Ciprofloxacin(5)	-	-	-	-	8	72.7	3	27.3
Chloramphenicol(30)	16	100	-	-	-	-	-	-
Cotrimoxazole (1.25/23.75)	16	100	-	-	4	36.4	7	63.6
Erythromycin(15)	16	100	-	-	9	81.8	2	18.2
Oxacillin(1)	12	75	4	25	-	-	-	-
Penicillin(10 units)	-	-	-	-	5	45.5	6	54.5
Tetracycline(30)	11	68.8	5	31.3	10	90.9	1	9.1
Vancomycin	16	100	-	-	11	100	-	-

All the *Streptococcus pneumoniae* isolates showed 100% sensitivity to Cefotaxime, Chloramphenicol, Cotrimoxazole, Vancomycin, whereas all *Staphylococcal* isolates showed 100% sensitivity to

Amikacin and Vancomycin. 25% of the *Streptococcal* isolates showed resistance to Oxacillin. 36.36% of the *Staphylococcal* isolates were MRSA showing resistance to Cefoxitin.

**Table 8: Antimicrobial susceptibility pattern of gram-negative isolates by Kirby Bauer disc diffusion test.**

Antibiotics µg	<i>Pseudomonas aeruginosa</i> n =11				<i>Klebsiella pneumoniae</i> n =6			
	Sensitive	%	Resistant	%	Sensitive	%	Resistant	%
Amikacin(30)	-	-	-	-	6	100	-	-
Cefepime(30)	10	90.9	1	9.1	-	-	-	-
Cefoperazone/Sulbactam(75/10)	10	90.9	1	9.1	4	66.7	2	33.3
Cefotaxime(30)	4	36.4	7	63.6	3	50.0	3	50.0
Ceftazidime(30)	4	36.4	7	63.6	4	66.7	2	33.3
Ciprofloxacin(5)	3	27.3	8	72.7	2	33.3	4	66.66
Cotrimoxazole (1.25/23.75)	-	-	-	-	1	16.7	5	83.3
Colistin(10)	11	100	-	-	-	-	-	-
Gentamicin(10)	6	54.5	5	45.5	6	100	-	-
Imipenem(10)	6	54.5	5	45.5	5	83.3	1	16.7
Piperacillin/Tazobactam(100/10)	6	54.5	5	45.5	4	66.7	2	33.3

In *Klebsiella pneumoniae*, resistance to Cefotaxime, Ciprofloxacin and Cotrimoxazole were shown in 3(50%), 4(66.7%) and 5(83.3%) isolates respectively. MDR strains were noted in 4(66.66%) of isolates of *Klebsiella pneumoniae*, whereas in *Pseudomonas aeruginosa* MDR strains were noted in 7(63.6%) of isolates. *Pseudomonas* isolates showed 100% sensitivity to colistin. Imipenem resistance was noted in one (16.7%) isolate of *Klebsiella pneumoniae*, whereas in *Pseudomonas* 6(54.5%) of isolates showed Imipenem resistance.

## DISCUSSION

This present cross-sectional study included study population of AECOPD category in the age group of 30-90 years. Majority of AECOPD patients were in the age group of 61-70 yrs (32%). This can be explained by the fact that COPD has the highest prevalence after fifth and sixth decade of life.<sup>[16]</sup> COPD is leading cause of morbidity and mortality and early introduction of empirical antibiotics, can improve outcome and reduce mortality.<sup>[3,4]</sup> Bacterial infections are considered to be the most important cause of AECOPD. It is estimated that roughly 40% of AECOPD are of bacterial origin. A

proper algorithm for antibiotic usage is therefore needed in such cases to avoid over usage of antibiotics.<sup>[17]</sup>

In the present there was a predominance of male population constituting 95% of the population over 5% female subjects which was similar to Shashibhushan et al [2016],<sup>[5]</sup> S kaviya sri et al 2020,<sup>[18]</sup> Avik Chakraborty et al 2016,<sup>[19]</sup> Ashok Kuwal et al 2018,<sup>[17]</sup> constituting 84%, 81%, 83.1% and 91.66% respectively.

The present study group were selected based on GOLD criteria and the most common type was moderate type (58%) followed by severe type (40%) in AECOPD patients. These findings were correlated with Karin et al. who reported the severity of COPD as 47% (severe) and 32% (moderate).<sup>[20]</sup>

The AECOPD was further categorized as per Anthonisen criteria. According to these criteria the present study showed Increased degree of breathlessness(dyspnoea) as the commonest symptom in AECOPD (100%) followed by increase in sputum volume (67%), and sputum purulence (33%). Type 1 exacerbation was the commonest type (71%) of AECOPD with all three cardinal symptoms followed by Type3 exacerbation (21%). The study of N. Arora et al,<sup>[21]</sup> showed that 100% of patients had

increase in sputum volume and 98.28% had various grades of dyspnoea. A single organism was identified in 90.91% whereas poly bacterial growth was observed in 9.1%. Gerard Rakesh et al,<sup>[22]</sup> in their study on bacterial agents in AECOPD revealed 37% of monobacterial isolates and 5% of polybacterial isolates. In a study done by Chawla K et al,<sup>[23]</sup> a single organism was isolated in most of the samples 92.85% and growth of two organisms was isolated in 7.14% cases.

In the present study, Gram positive and Gram-negative organisms were identified as 61.36% and 38.36% in 44 culture isolates. Similar findings were observed by Patel A K et al,<sup>[24]</sup> where Gram positive and Gram-negative isolation rate was 58.5% and 41.48% respectively.

The proportion of *Staphylococcus aureus* with Methicillin resistance was 36.36%. In a study done by Gerard Rakesh et al,<sup>[22]</sup> among AECOPD patients, the frequency of Methicillin - Resistant *Staphylococcus aureus* was reported as 11.90% which was lesser as compared to our study observation. Chawla K et al,<sup>[23]</sup> in their study observed that 50% of *Staphylococcus aureus* isolates were MRSA. All the MRSA isolates in our study was sensitive to Vancomycin.

## CONCLUSION

Acute exacerbations due to infectious aetiology are frequent cause of morbidity in COPD patients. Isolation of aetiological bacterial agents using standard culture methods helps in management of AECOPD and its complications. Repeated antibiotic administration in management of AECOPD is associated with development of resistance in the causative agents. Thus, culture with antimicrobial susceptibility test report is necessary in the management of AECOPD. In the present study *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were commonest gram-positive and gram-negative bacterial isolates.

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