

## Bacterial etiology in acute exacerbations of COPD.

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### Abstract

**Background:** Chronic Obstructive Pulmonary Disorder (COPD) is a major cause of morbidity and one of the principal causes of death worldwide. The objective of this study is to identify the bacterial etiological agents in patients with acute exacerbations of COPDs by sputum culture and their antibiogram. **Methods:** This is a prospective study comprising 100 diagnosed patients of COPD taken as GOLD Grade 4 who were admitted in the Department of Pulmonary Medicine, TB and Chest Hospital which comes under King George Hospital, Visakhapatnam during a period from May 2015 to May 2016. **Results:** Out of 100 patients, 83 were males of which 87% were smokers. The most common organisms isolated were Gram negative bacilli in 51 patients and only 26 were Gram positive cocci. The most common Gram-negative isolate was *Klebsiella pneumoniae* 27 cases followed by *Pseudomonas aeruginosa* in 19 cases. The most common Gram-positive isolate was *Streptococcus pneumoniae* in 16 cases followed by *Streptococcus pyogenes* in 6 cases and *Staphylococcus aureus* in 2 cases.

**Keywords:** COPD, Bacterial aetiology, acute exacerbation, Smokers, Antibiogram.

### Introduction

Chronic Obstructive Pulmonary Disorder (COPD), a common preventable and treatable disease which is characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lungs to noxious particles or gases [1].

Cigarette smoking or inhalation of dust or fumes are important contributing factors. The disease is defined by the clinical symptoms in which excessive mucus production leads to coughing up sputum on most days during at least 3 consecutive months for more than two successive years. During episodes of acute exacerbations patient has increased expectorations and is usually associated with increased clinical symptoms, signs and functional disability [2].

Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD) is defined as an acute change in a patient's baseline dyspnea, cough and/or sputum beyond day-to-day variability sufficient to warrant a change in therapy. Exacerbation may be triggered by an infection with bacteria or viruses or by environmental pollutants [3].

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; additionally, exacerbations lead to indirect costs because of days lost from work [4].

A strong association exists between tobacco smoking and the occurrence of COPD. The reported smoker: nonsmoker prevalence ratio varied from 1.6 to 10.2. The difference in the prevalence of COPD among males and females could be due to the differences in their levels and type of smoking. Women, spending more time indoors for cooking than men who are exposed to biomass fuel combustion products and are prone to develop COPD [1].

Chronic clinical course of COPD is punctuated by periods of increased symptoms that are termed "acute exacerbations" [5].

COPD is classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria Potential pathways by which bacteria could contribute to the course and pathogenesis of AECOPD were identified. Bacteria cause a substantial proportion of acute exacerbations of chronic bronchitis which leads to considerable morbidity and mortality.

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Chronic colonization of the lower respiratory tract by bacterial pathogens amplifies the chronic inflammatory response present in COPD and leads to progressive airway obstruction (vicious circle hypothesis) [4]. Bacterial antigens in the lower airway induce hypersensitivity that enhances airway hyper reactivity and induces eosinophilic inflammation [4]. Studies have been performed and it has been shown that the growth of pathogenic bacteria during an episode of acute exacerbations has been more in terms of colony forming units (CFU) than during other times. Furthermore, the local inflammatory response of the host increases with increasing airway bacterial load [6]. Antibiotics are commonly prescribed empirically to patients presenting with COPD to treat presumed bacterial infection. The rise in bacterial resistance to antibiotics has focused our attention on the benefit of this practice, and more fundamentally the importance of bacterial infection in COPD, and its role in stimulating bronchial inflammation, which is the hallmark of this condition [7].

Still, many questions remain, and future studies will be needed to better define the mechanisms of bacterial invasion in the COPD patients and to develop effective vaccines to prevent exacerbation. In the meantime, we must rely on antibiotic therapy, and we will need prospective studies to corroborate preliminary findings showing that different patients may require different therapies; Thus, patient sub setting may be vital in the selection of antibiotic therapy for exacerbations of COPD.

## Materials and methods

All procedures performed in the study were by the ethical standards of the institutional and or national research committee and with the 1964 Helsinki declaration and its later amendments.

In this cross-sectional study, a total of 100 patients diagnosed as COPD Grade 4 as per GOLD guidelines were included in the study. The study was carried out at the Department of Microbiology with the help of Department of Pulmonary Medicine under the TB and Chest hospital, King George Hospital, Visakhapatnam, during the period from May 2015 to May 2016. The study comprises of all gender with their age group ranging from 45 to 80 years. For all the 100 patients admitted, detailed history was taken, those patients with increased cough, increased severity of dyspnoea, excessive sputum production and sputum sample was collected prior to the administration of antibiotics were included in the study.

Patients diagnosed with tuberculosis, bronchial asthma, lung abscess, lung carcinoma, ischemic heart disease and who were already on antibiotic treatment were excluded from the study.

Classification of severity of airflow limitation in COPD

In pulmonary function testing, a postbronchodilator FEV1/FVC ratio of <0.70 is commonly considered diagnostic of COPD. In patients with this ratio.

- GOLD 1 – mild: FEV1  $\geq$  80% predicted
- GOLD 2 – moderate: 50%  $\leq$  FEV1 < 80% predicted

- GOLD 3 – severe: 30%  $\leq$  FEV1 < 50% predicted
- GOLD 4 – very severe: FEV1 < 30% predicted

The GOLD guideline uses a combined COPD assessment approach based on patient's symptoms and previous history of exacerbations into 4 groups

- Group A: low risk (0-1 exacerbation / year, not requiring hospitalization) and fewer symptoms
- Group B: low risk (0-1 exacerbation / year, not requiring hospitalization) and more symptoms
- Group C: high risk ( $\geq$  2 exacerbations / year or one or more requiring hospitalization) and fewer symptoms
- Group D: high risk ( $\geq$  2 exacerbations / year or one or more requiring hospitalization) and more symptoms

Bacterial infections are one of the most common causes of acute exacerbation of COPD. They account for 25% exacerbations alone [6].

Patients were instructed and a deep coughed sputum sample was collected into a sterile wide mouth container with a screw cap. Smears were prepared and were stained by Gram staining technique (Figure 1-4) The smears were then examined and the quality of the sputum was assessed by Bartlett's grading (Table 1) and having a score of 1 or more were processed further [8]. A final score of 0 or less indicates either lack of inflammatory response or presence of significant salivary contamination, thus invalidating the specimen. Selected sputa were processed microbiologically by using a calibrated loop, 0.01 ml onto the following culture media: blood agar, MacConkey agar and chocolate agar.

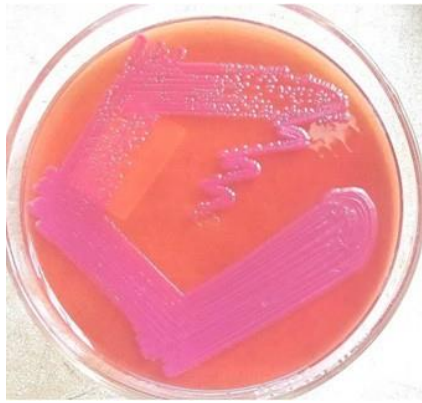
Incubated at 35  $\pm$  2°C under aerobic conditions. Chocolate agar was incubated in a candle jar. A first reading was taken after 24 hrs, and a second final one was taken after 48 hrs of culture.

## Results

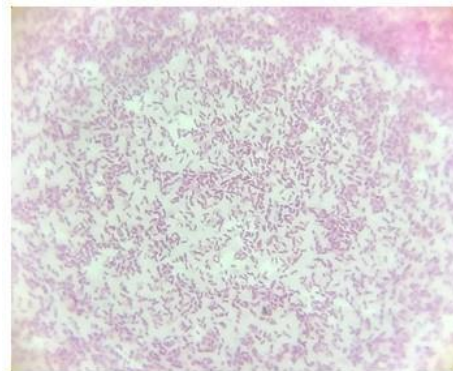
A Total of 100 patients clinically diagnosed and admitted with AECOPD were studied. Bacterial infections of AECOPD were analysed. Organisms grown were identified based on their morphology, cultural characteristics and biochemical reactions their culture & sensitivity patterns to various antibiotics were also recorded.

In 100 patients 83(83%) were males and 17(17%) were females. Male: female ratio was 4.8:1. As shown in (Table 2), among the 83 males 73 [88 %] were smokers, out of 17 females 9 [53 %], were smokers. 12 % were nons smokers. Majority of the patients in the study population were smokers. Out of the 100 specimens processed, 14 samples showed polymicrobial growth, 77 samples showed monomicrobial growth and the remaining ones were sterile. 77 samples that showed monomicrobial growth had Klebsiella pneumonia (Figure 1) the most common isolate, followed by Pseudomonas aeruginosa (Figure 5) and Streptococcus pneumonia (Figure 6).

The antibiotic discs which were tested for antimicrobial susceptibility upon Mueller Hinton agar by Kirby Bauer disc diffusion method.



**Figure 1:** Pink coloured mucoid lactose fermenting colonies on Mac Conkey agar resemble *Klebsiella* species.



**Figure 2:** Gram negative bacilli seen in smear from Mac Conkey agar showing short and stout bacilli resemble *Klebsiella* species showing short and stout bacilli resemble *Klebsiella* species.



**Figure 3:** Late lactose fermenting colonies on Mac Conkey agar resembling *Acinetobacter* species.



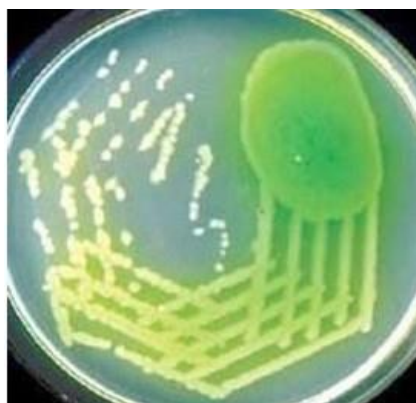
**Figure 4:** Tiny greyish colonies on chocolate agar resemble *Streptococcus* species

**Table 1: Bartlett's grading of sputum.**

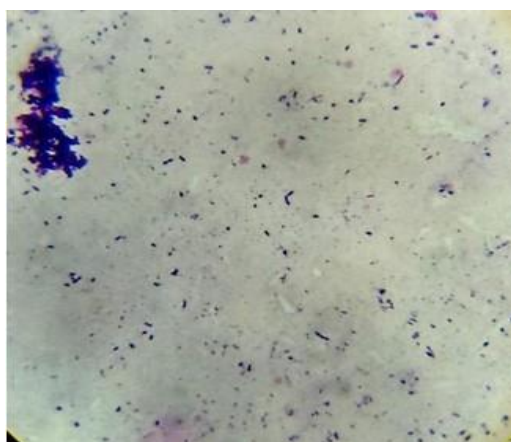
No. of neutrophils per 10x low power field	Grade
<10	0
25-Oct	1
>25	2
Presence of mucus	1
No. of epithelial cells per 10x low power field	Grade
25-Oct	-1

**Table 2: Incidence of smoking.**

Smoking status	Male	Female	Total [%]
Smoker	73	9	82%
Non-Smoker	10	8	18%



**Figure 5: Green pigmented colonies on nutrient agar resemble Pseudomonas species**



**Figure 6: Gram's staining smear from chocolate agar showing S. pneumoniae.**

**Table 3A: Isolates of gram negative bacilli growth.**

Different species of Gram negative bacilli isolated	Number of bacterial isolates
Klebsiella pneumoniae	27
Pseudomonas aeruginosa	19
Acinetobacter baumannii	5

**Table 3B: Isolates of Gram positive cocci.**

Gram positive cocci isolated	Number of isolates
Streptococcus pneumoniae	18
Streptococcus pyogenes	6
Staphylococcus aureus	2

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**Table 4: Antimicrobial susceptibility patterns in isolates of Gram negative bacilli.**

Antimicrobial susceptibility pattern						
Klebsiella pneumoniae		Pseudomonas aeruginosa		A. baumannii		Drug total sensitivity %
Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	
27	0	19	0	5	0	100%
17	10	15	4	3	2	68.60%
26	1	17	2	4	1	92.15%
25	2	16	3	2	3	84.31%
20	7	15	4	3	2	74.50%
26	1	15	4	3	2	86.27%
5	22	2	17	2	3	17.60%

**Table 5: Antimicrobial susceptibility patterns in isolates of in Gram positive cocci.**

Antimicrobial susceptibility pattern						
Antibiotic discs	S. pneumoniae		S. pyogenes		S. aureus	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Meropenem	16	0	8	0	2	0
Ceftriaxone	12	4	4	4	2	0
Levofloxacin	15	1	6	2	2	0
Azithromycin	14	2	6	2	1	1
Tigecycline	15	1	7	1	2	0
Teicoplanin	13	3	6	2	2	0
Amoxyclav	11	5	3	5	0	2

**Table 6: Shows total drug sensitivity percentage.**

Total drug sensitivity percentage			
Antibiotic	Gram negative bacilli sensitivity	Gram positive cocci sensitivity	Total sensitivity of the drug
Meropenem	100%	100%	100%
Ceftriaxone	68.60%	69.20%	68.90%
Levofloxacin	92.15%	88.46%	90.48%
Piperacillin tazobactam	84.31%	-	84.31%
Azithromycin	74.50%	80.76%	77.63%
Gentamycin	86.27%	-	86.27%
Amoxyclav	17.60%	53.80%	35.70%
Tigecyclin	-	92.30%	92.30%
Teicoplanin	-	80.70%	80.70%

With the help of antibiotic scale from HIMEDIA, the following sensitivity patterns were noted. The antibiotic susceptibility patterns of isolates of Gram negative bacilli are depicted in (Table 3A,3B). A total of 26 number of Gram positive cocci were isolated. (Table 4-6).

## Discussion

In spite of the multifactorial etiopathogenic nature of AECOPD, infection plays a very important and unique role, impairing not only ventilator function of the lung but also restricting patient daily routine activities.

The present study confirms previous data reporting a role of bacterial infection in AECOPD during hospital admissions for acute exacerbations. Presence of bacterial pathogens was found in 91 % of all admitted patients.

Remarkably, the incidence of positive sputum cultures in 91% of our patient population was quite similar to previous studies [9-11].

The present study also showed a significant number of higher males 88% which were chain smokers had COPD infection as compared to females which was 53% were non-smokers.

This finding was in conformity with other co-workers who reported similar findings [12]. The male predominance in the current study can be attributed to smoking and COPD being common among females can be due to indoor air pollution while domestic work. Aleemullah, et al. obtained pathogenic organisms from 109 (73.65%) sputum samples. Out of 148 samples processed Gram negative organisms were isolated predominantly 68(62.39%). While Gram positive organisms accounted for 41(37.61%) [13]. Saikat basu, et al. showed 21 positive sputum cultures. Among them, 76.20% were males and 23.80% were females. The prevalence of Gram negative bacteria was 71.42% and Gram positive bacteria was 28.58%. Klebsiella pneumoniae was the commonest isolated (33.33%) followed by P. aeruginosa (19.05%) and Staphylococcus aureus (14.30%) [14].

Chakraborty, et al. isolated pathogenic bacteria from 203 (60.1%) samples. Gram negative bacteria were isolated from 79.8 percent (162/203) while the rest were Gram positive. Klebsiella species was the commonest (49.2%; 100/203) Gram negative isolate from the sputum samples. Among the Gram negative organisms, carbapenem had the highest sensitivity (90.2%) followed by amikacin, ciprofloxacin and piperacillin

tazobactam. Linezolid was found to be 100 percent sensitive amongst the Gram positive organisms while both amoxicillin clavulanate and azithromycin showed a rather low sensitivity profile overall [15].

Pradhan K.C. et al, conducted a study on the sputum samples of 100 cases, suffering from chronic obstructive pulmonary disease among them 16 cases had chronic bronchitis. The isolates obtained were Klebsiella spp - 40%, Staphylococcus aureus - 26%, Pseudomonas aeruginosa - 8%, Escherichia coli - 2%, Proteus species - 1% [16].

In the current study Gram negative bacilli 51 out of 77 isolates (66.23%) outnumbered the Gram positive cocci 26 out of 77 isolates (33.76%) in monomicrobial isolates.

In the study by Aleemullah, et al. Saikat basu, et al. Chakraborty, et al. the isolates of Gram negative bacilli (62.39%) were more than the Gram positive cocci (37.61%) which is comparable to the present study [17-19].

In the studies like Pradhan et al, Chawla, et al. Soler, et al. Hariom Sharan et al, Chakraborty, et al. Alemullah, et al. Narayangowda, et al. Jayasimha, et al and Rakhee, et al. had similar findings [20-22]. Another study done by Mahmoud Ahmed et al showed a difference in bacteriological profile showing that there was a predominance of Gram positive cocci than Gram negative bacilli.

In this study, 3 commonest organisms isolated were Klebsiella pneumoniae in 27 cases (35.06%), Pseudomonas aeruginosa in 19 cases (24.67%) and Streptococcus pneumoniae in 18 cases (23.37%).

Studies by Chawla et al, Basu et al, Chakraborty, et al. showed that Klebsiella pneumonia (33.33%) was predominantly isolated followed by Pseudomonas aeruginosa (25.9%).

Similarly other studies done by Pradhan, et al. Hariom, et al. Saxena, et al. and Narayanagowda, et al. also suggested the higher isolation of K. pneumoniae as the predominant organism [23].

As the time has passed over, Enterobacteriaceae family organisms and Pseudomonas species have dominated the bacterial flora during acute exacerbations leading to increased severity of the disease. Multidrug resistance and biofilm formation by these organisms have further worsen the situation limiting the therapeutic option.

In the studies conducted by N. Arora, et al. (25.8%), Patel, et al. (32%), Rakesh, et al.(38.1%), Suseela, et al. (22%), Shashibhushan, et al. (42%) and Vishwamber, et al. (45%), the predominant pathogen isolated was Streptococcus pneumoniae [24-29].

Other pathogens isolated were A. baumannii 05 cases (6.49%), Streptococcus pyogenes 06 cases (7.79%) and S. aureus in 02 cases (2.59%).

In the study conducted by Hariom Sharan, et al. of (5.13%), Suseela, et al. 3% is the rate of isolation for A.baumannii comparable to the present study.

Investigation for diagnosis of AECOPD sputum culture is a very simple and useful technique. It offers easy detection, aetiology & complications due to bacteria causing the severe infection. If followed in a well-disciplined way, it can replace the costlier diagnostic methods like immune diffusion. Management of AECOPD can be achieved by the study of antibiogram which helps in creating a correct treatment protocol and hence antibiotics can be started empirically to treat the presumed bacterial infection. The mortality and morbidity can be reduced by finding out resistant pathogens and hence the treatment can be planned according to the organisms. Amino penicillins like ampicillin and amoxicillin were formerly the standard treatment in AECOPD. Due to emergence of resistance among respiratory pathogens, their utility had been limited. Beta lactams, amoxyclav has been used in the study to know about the resistance patterns. Aminopenicillins with beta lactamase inhibitor is a better choice.

Cephalosporin's demonstrated clinical efficacy and tolerability that can surpass the standard aminopenicillins. A third generation cephalosporin has been used to test its efficacy against pathogens. Quinolones like levofloxacin exhibit a broad spectrum of activity that includes both Gram positive and Gram negative organisms causing AECOPD. It was proven to be better than the other quinolones in treating Pseudomonas infection.

Macrolides are the most potent drugs used for treating sputum related infections Azithromycin has been used to see its efficacy. The newer and powerful drugs available are carbapenems and the drug used is meropenem.

The antibiotic meropenem, had 100% sensitivity to all the isolates tested was this was comparable to the study done by Chakraborty, et al. were carbapenems show 90.2% sensitivity. In the study conducted by Rakhee, et al imipenem had 85% sensitivity [22] In our study 3rd generation cephalosporin used was ceftriaxone which showed a sensitivity of 68.9%. In his study, Shashibhushan, et al. stated that ceftriaxone is the most effective drug [28]. In his study, Vishwambhar, et al. isolated Streptococcus pneumonia which was 100% sensitive to cephalosporins, a little higher than the present study [29].

In his study Chakraborty, et al. had 37% of sensitivity rate for ceftriaxone which is much less compared to the present study [15].

In the present study quinolones used were levofloxacin which showed a sensitivity of 90.48%, comparable to study by Vishwambhar, et al. which showed 89% efficacy with levofloxacin and 80% sensitivity to ciprofloxacin. Studies by Chakraborty, et al and Kumar, et al have efficacy of 62% and 76.9% for ciprofloxacin which are too low [30,31].

Much lower rates of sensitivity to seen with Narayanagowda, et al. with 54.55% and Saxena, et al. with 25.53% for ciprofloxacin and 22.34% for levofloxacin.

Periodic isolation, identification and resistant status of pathogens responsible for AECOPD will help us formulate appropriate treatment protocol and this will be of immense

use in reducing mortality and morbidity besides reducing the volume of antibiotics and development of resistance to antibiotics. The protective host immune responses that develop after exacerbation needs to be characterized to facilitate vaccine development. Further, the interaction among different etiologic factors such as environment, bacteria, viruses and atypical pathogens needs to be better understood to treat exacerbations and develop novel as well as cost effective preventive and therapeutic strategies.

## Conclusion

There have been significant advances in our understanding of aetiology of acute exacerbations of COPD (AECOPD). Frequent exacerbations appear to be associated with worsening health outcomes and effort should focus on prompt and effective treatment of each episode. Bacterial pathogens, mostly Gram negative bacilli are found to be the chief etiological agents in AECOPD. Antibiotic therapy should be initiated early depending on the culture. Susceptibility patterns of that particular region should be determined to prevent antibiotic resistance and to decrease health costs. However treatment options are limited and further research is needed to clarify the mechanisms that commence and sustain exacerbations and to identify new therapeutic agents. Better and more specific approaches to boost immune competence are currently under study.

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