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# SPUTUM BACTERIOLOGY AND ANTIBIOTIC SENSITIVITY PATTERN IN PATIENTS HAVING ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN KMCT MEDICAL COLLEGE HOSPITAL, CALICUT, INDIA

**Pharmacy**

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## ABSTRACT

**Background:** Chronic obstructive pulmonary disease (COPD) is a common disease characterized by persistent airflow obstruction with an enhanced chronic inflammatory response in the air passages and the lung to noxious particles or gases. The intent of the present study was to obtain comprehensive insight into the bacteriological profile and antibiotic sensitivity pattern in sputum culture of AECOPD patients in our region.
**Methods:** A prospective observational study was held out in KMCT Medical College Hospital, Calicut which comprised of 240 patients diagnosed with acute exacerbation of chronic obstructive pulmonary disease from October 2016 to June 2017. Sputum culture and sensitivity reports were prospectively examined for the bacteriological profile and their antimicrobial sensitivity and resistance pattern.
**Results:** Our study shows 103 positive sputum cultures out of total 240 cases. Males (76.7%) are more often affected than females (23.3%). The prevalence of bacteria was 42.9%.
Klebsiella pneumoniae was the commonest bacteria isolated (30.09%) followed by Pseudomonas aeruginosa (29.1%). The drug sensitivity reveals that 61.2% of the isolates were sensitive to amikacin followed by ceftriaxone 51.45% and 48.54% of the isolates were sensitive to meropenem. High resistance rates were observed against ampicillin (42.8%). Increased severity of acute exacerbation of chronic obstructive pulmonary disease was related to increased prevalence of antibiotic resistance.
**Conclusion:** AECOPD is more usual in adults more than 60-79 years of age due to smoking habits and high indoor pollution. Klebsiella pneumoniae was the most common pathogen in AECOPD patients, and amikacin was most effective antibiotic against most of the organisms. Amikacin should be the first line empirical antibiotic.

## KEYWORDS

Acute Exacerbation Of Copd (aecopd), Culture Sensitivity, Sputum Culture, Antibiotic.

## INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a spectrum of disorders that results in airflow obstruction. At one end of the spectrum is chronic bronchitis, which is characterized by airway inflammation, mucus hyper secretion and airway reactivity[1]. Acute exacerbation of COPD (AECOPD) is defined as a sustained worsening of the patient's condition, from the stable state and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD[2]. Cigarette smoking or inhalation of dust or fumes are important contributing elements [3].

### *Staging of AECOPD*

The severity of AECOPD without respiratory failure can be classified traditionally according to Winnipeg criteria. The three-stage system is based on three principal symptoms:

1. Increase in sputum volume
2. Increase in sputum purulence
3. Increase in shortness of breath[4]

Bacterial infections are the most usual cause of AECOPD, it is estimated that bacterial infections are responsible for more than 40% of all exacerbations in India [5]. It has been observed that the use of antibiotics used to treat AECOPD has an impact on the failure rate[6]. More than 90% of patients with AECOPD are treated with antibiotics, due to the emergence of resistant strains [7]. Nowadays investigators need culture studies for proper selection of antibiotic, but it is a time consuming process. The choice of the antibiotic should be constituted on the local bacterial resistance pattern.

The aim of this study is to identify the causative bacteria in AECOPD and to determine the antibiotic susceptibility and resistance patterns among these pathogens at KMCT Medical College Hospital, Calicut, which will assist us to design a proper antibiotic regimen to treat the AECOPD patients, which will induce a beneficial result in morbidity and mortality of the disease.

The fact that compelled us to carry up this study is the empirical use of antimicrobials, rise in bacterial resistance, the need to evaluate the benefit of the practice, and more fundamentally the importance of bacterial infection in AECOPD.

Early diagnosis and knowledge of the local bacteriological profile & antibiogram help us to scale down the number of failure cases recorded with empirical treatment during AECOPD.

The result may help the physicians to choose optimum antibiotics therapy.

## AIMS AND OBJECTIVES

1. To evaluate the proportion of patients having bacterial infected acute exacerbation of COPD in the cases admitted to KMCT Medical College Hospital, Calicut.
2. To assess the culture sensitivity and organism isolated.
3. Selection of appropriate antibiotics.
4. Preparation of Antibiogram.

## MATERIALS AND METHODS

This prospective observational study aimed to identify the causative bacteria, antibiotic sensitivity, and antibiotic resistance in hospitalized patients due to AECOPD. All patients previously diagnosed for COPD and admitted with AECOPD in the Department of Pulmonary Medicine, KMCT Medical College Hospital, Calicut during the period October 2016 to June 2017, were candidates for inclusion in the study. The protocol of the present research study was approved by the institutional ethical committee at the Department of Microbiology, KMCT Medical College Hospital, Calicut. The study included 240 inpatients with AECOPD.

### Inclusion criteria for the case

- Previously diagnosed patients with COPD having acute exacerbation with increased dyspnea, increased sputum volume, increased sputum purulence.
- In patients.

**Exclusion criteria for the case**

- Mentally retarded patients.
- Pregnant and lactating women.
- Patients having bronchiectasis, TB, pneumonia, malignancy and other evident disease on chest x-ray.
- Patients previously admitted within 3months and taken antibiotics.

**SPUTUM ANALYSIS**

Expectorated sputum is the most commonly used sample for diagnosis of AECOPD. Culture studies help for proper choice of antibiotics. The choice of the antibiotic should be based on the local bacterial resistance pattern, thus there is an urgent need of a better approach for the management of this morbid disease. The objective of this study is to analyse the bacteriological profile of the patients with AECOPD and also to study their antimicrobial sensitivity pattern, which will assist us to design a proper antibiotic regimen to treat the AECOPD patients[8].

**Specimen Collection**

The specimen for culture was collected before antibiotic therapy was started. The patient was instructed to wash his or her mouth with water to decrease mouth bacteria and dilute saliva. Patients were taught to take a deep breath, hold it momentarily, and then cough vigorously into a cup. Sputum was collected in sterile sputum cups[5]. Mostly, while preparing the sputum smear, purulent part of the sputum is taken[9].

**Gram stain**

A Gram stain of the sputum was examined for polymorph nuclear leukocytes and epithelial cells[5]. Bartlett has proposed a scheme for grading the quality of sputum specimens after a slide of the specimen has been examined at 100x magnification.

The interpretation of this criteria is if the total score is 1 and above, the sputum is positive and can be cultured. Whereas if the total score is 0 and below, the sputum is negative and cannot process[9]. Only if the sputum is positive (score 1), the further culture of sputum is carried away.

**Sputum Culture**

The culture and susceptibility of sputum samples may help a lot to identify those who require antibiotics. The knowledge of the patterns of antimicrobial resistance in the respiratory pathogens will help to prevent treatment failures. So the bacteriological analysis of the sputum samples in exacerbation is essential.

After collecting the sputum[3],these inoculated plates were then incubated for a period of 24 hours, after which they were examined for evidence of bacterial growth. Fortunately, at AECOPD, there is an increased chance of detecting bacteria, particularly if the exacerbation is associated with the presence of purulent sputum. In case of bacterial growth along the medium, the bacteria were further identified using standard tests.

The bacteria found will be either Gram-positive or Gram negative.

**Antibiogram**

Antibiotic sensitivity test of the isolates were performed on Mueller-Hinton agar plates by the disc diffusion method of Kirby-Bauer (KB). The Kirby-Bauer testing method determines the sensitivity of microorganisms to specific antimicrobial drugs. Using specifically targeted antibiotics helps decrease the frequency of drug resistant bacteria developing.

The isolated micro-organisms were tested for their antimicrobial susceptibility pattern using disc diffusion method according to CLSI guidelines[10].

KB tests are executed under standard conditions, so the minimum inhibitory concentration of a given antibiotic can be computed by comparing the observed zone of inhibition[11].

**Statistical analysis**

All the data were entered into Microsoft Excel 2013 spreadsheet and analysed using SPSS software.

**RESULT**

A prospective observational study was held among 240 patients, selected from a total of 300 patients admitted to the pulmonary medicine department.

Bacterial infections of AECOPD were analysed. The individual bacterial isolates and their sensitive and resistance pattern to several antibiotics were also recorded.

**Sex Wise Distribution Of Aecopd**

**Table 1: Sex-wise distribution of AECOPD patients**

| SEX | FREQUENCY | PERCENTAGE |
|---|---|---|
| Male | 184 | 76.7% |
| Female | 56 | 23.3% |
| | 240 | 100% |

Out of 240 patients with acute exacerbation of COPD, 184 (76.67%) were males and 56 (23.3%) were females.

**Age Wise Distribution Of Aecopd**

The age group of the patients included in the present study ranged from 30-90 years. Out of 240 patients, maximum number (178) belonged to the age group of 60-79 years. The next most common age group was 40-59 years (42).

**Table 2: Age-wise distribution of AECOPD**

| AGE | FREQUENCY | PERCENTAGE |
|---|---|---|
| 30-40 | 5 | 2.08% |
| 40-59 | 42 | 17.5% |
| 60-79 | 178 | 74.17% |
| 80-90 | 15 | 6.25% |
| Total | 240 | 100% |

**Smoking Pattern Of Aecopd Patients**

In our study, out of 240 patients, 131 (54.59%) were current smokers, 70 (29.16%) patients were ex-smokers and 39 (16.25%) were non-smokers.

**Table 3: Smoking pattern of study population**

| SMOKING STATUS | FREQUENCY | PERCENTAGE |
|---|---|---|
| Non-Smoker | 39 | 16.25% |
| Smoker | 131 | 54.59% |
| Ex-Smoker | 70 | 29.16% |
| Total | 240 | 100% |

**Proportion Of Organisms Isolated**

The sputum samples of 240 patients were subjected to culture study. Out of which 103 (42.9%) were positive for pathogenic bacteria and 137 (57.1%) were normal flora.

**Table 4: Proportion of Organism isolated**

| | FREQUENCY | PERCENTAGE |
|---|---|---|
| Normal flora | 137 | 57.1% |
| Bacteria | 103 | 42.9% |

**Organism Isolated In Aecopd**

Among the pathogenic bacterial growth, there are 9 types of bacterial strains, out of which 7 were Gram-negative bacteria (77.78%) and 2 were Gram-positive bacteria (22.22%).

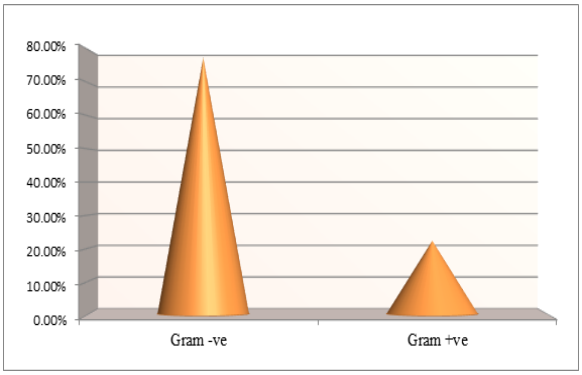


**Figure 1: Organism isolated**

**Specific Organism Isolated In Aecopd**

The most common pathogenic bacteria isolated in sputum culture was Klebsiella pneumoniae 31 (30.09%), followed by Pseudomonas aeruginosa 30 (29.1%), Acinetobacter 15 (14.56%). Other common

organisms isolated were Haemophilus influenzae & E-coli in 7 cases, Streptococcus pneumoniae in 5 cases, Staphylococcus aureus & Moraxella catarrhalis in 3 cases, and Burkholderia cepacia in 2 cases.

**Table 5: Organism specific isolation**

| ORGANISMS | FREQUENCY | PERCENTAGE |
|---|---|---|
| Klebsiella pneumoniae | 31 | 30.09% |
| Pseudomonas aeruginosa | 30 | 29.1% |
| Acinetobacter | 15 | 14.56% |
| Haemophilus influenzae | 7 | 6.79% |
| E. coli | 7 | 6.79% |
| Streptococcus pneumoniae | 5 | 4.86% |
| Staphylococcus aureus | 3 | 2.93% |
| Moraxella catarrhalis | 3 | 2.93% |
| Burkholderia cepacia | 2 | 1.95% |
| Total | 103 | 100% |

**Percentage Sensitivity of Organism Isolated**

**Table 6: Percentage sensitivity of organisms**

| Organism | Antibiotics | % Sensitivity |
|---|---|---|
| Klebsiella pneumoniae | Amikacin | 76.2% |
| Pseudomonas aeruginosa | Amikacin | 89.47% |
| Acinetobacter | Amikacin | 66.67% |
| Haemophilus influenzae | Ceftriaxone | 85.71% |
| E.coli | Amikacin | 100% |
| Streptococcus pneumoniae | Linezolid | 100% |
| Staphylococcus aureus | Linezolid | 100% |
| Moraxella catarrhalis | Ceftriaxone | 100% |
| Burkholderia cepacia | Ceftazidime | 100% |

Klebsiella pneumoniae, Pseudomonas aeruginosa, E.coli and Acinetobacter was mainly sensitive to amikacin. H.influenzae was mainly sensitive to ceftriaxone. M.catarrhalis was most sensitive to ceftriaxone and B.cepacia towards ceftazidime

Streptococcus pneumoniae and Staphylococcus aureus shows sensitivity to linezolid.

**Percentage Resistance of Organisms**

**Table 7: Percentage resistance of organisms**

| Organism | Antibiotic | % Resistance |
|---|---|---|
| Klebsiella pneumoniae | Ampicillin | 66.67% |
| Pseudomonas aeruginosa | Cefepime | 40% |
| Acinetobacter | Ampicillin | 80% |
| Haemophilus influenzae | Cotrimoxazole | 42.86% |
| E.coli | Ampicillin | 85.71% |
| Streptococcus pneumoniae | Erythromycin | 33.33% |
| Staphylococcus aureus | Erythromycin | 60% |
| Moraxella catarrhalis | Ciprofloxacin | 100% |

**Antibiogram Of Aecopd**

K. pneumoniae, which was the most commonly isolated gram negative bacteria is sensitive to amikacin (76.2%), followed by gentamycin (66.67%) and meropenem (66.67%). They were extremely resistant to ampicillin (66.67%) followed by cefuroxime (61.90%).

P.aeruginosa was next mostly isolated bacteria and were primarily sensitive to amikacin (89.47%), followed by ciprofloxacin (84.21%) and piperacillin + tazobactam (84.21%) and they were highly resistant to cefepime (40%).

S. pneumoniae, which were the most common isolated gram positive bacteria is 100% sensitive to linezolid followed by ceftriaxone (80%). They were extremely resistant to erythromycin (33.33%) and aztreonam (33.33%).

Staphylococcus aureus isolates showed sensitivity to linezolid (100%), followed by cefazolin (66.67%). They were highly resistant to erythromycin (60%), azithromycin (66.67%).

**Antibiogram (Sensitivity) of organisms**

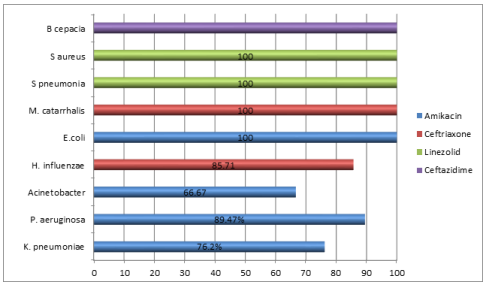


**Figure 2: Antibiogram Sensitivity % of Organisms**

**Empirical Antibiotic Selection Based on Sensitivity and Resistance Pattern**

**Table 8: Empirical antibiotic selection**

| Choice of drug before getting culture report | Choice of drug after getting culture report |
|---|---|
| **For gram positive isolates**<br>Streptococcus pneumonia<br>1st choice : Linezolid<br>2nd choice : Ceftriaxone<br>Staphylococcus aureus<br>1st choice : Linezolid<br>2nd choice: Cefazolin | Give most appropriate sensitive drug based on culture report |
| **For Gram Negative isolates:**<br>1st choice : Amikacin<br>2nd choice: Ceftriaxone | |

## DISCUSSION

A prospective observational study was conducted to identify the causative bacteria and to investigate the sensitivity pattern of antibiotics in patients admitted to pulmonary medicine department with acute exacerbation of COPD.

Three classes of pathogens have been implicated as causing acute exacerbation of COPD, by infecting the lower respiratory tract. 1) Respiratory viruses - <10% ,2) Atypical bacteria – 30% 3) Aerobic Gram positive and gram negative bacteria – 50%.

Fortunately, at acute exacerbation of COPD, there is an increased chance of detecting bacteria, especially if the exacerbation is associated with the presence of purulent sputum. The culture and susceptibility of sputum samples may help a lot to identify those who need antibiotics. The knowledge of the patterns of antimicrobial resistance in the respiratory pathogens will help to prevent treatment failures. So the bacteriological analysis of the sputum samples in exacerbation is essential. Optimal and effective antimicrobial therapy for acute exacerbation episodes can significantly diminish healthcare costs and maintain quality of life in the elderly patient.

The age group of patients ranged from 30-90 years. However among them, maximum numbers of AECOPD cases (74.17%) belongs to 60-79 year of age. This is comparable to studies undertaken by ***Gurumayum P et al*** (61-75 years), ***DR. A.K. Borthakur et al*** (50-60 years) [12, 13]. Thus, AECOPD was common in advance age group, as respiratory tract is more susceptible due to impairment of immunological defense mechanism, associated co-morbid illness, increased duration of seasonal variation & tobacco smoking.

Out of 240 patients, clinically diagnosed as acute exacerbation of chronic obstructive pulmonary disease, 184 (76.7%) were males and 56 (23.3%) were females. A prospective study was made by ***Madhavi S et al*** who had 79% males and 21 % females [14]. Predominance of male over female patients as shown in this study can be explained by the fact that in our country males are exposed more to outside environment because of their more mobility as compared to females. Moreover smoking habits are more pronounced in males that constitute one of the predisposing factors for the development of COPD.

In our study, 54.59% of patients were smokers and 16.25% were non-smokers. A study conducted by ***Devanath S N et.al*** showed AECOPD was more common among smokers 62.5% than non-smokers 37.5% [4]. Smoking and air pollution are responsible for decrease in mucociliary clearance and innate immunity. It leads to increased bacterial colonization that can give rise to increased airway inflammation and thus exacerbations. We found that ex-smokers (30.56% of patients)

had experienced exacerbation attacks, which imply that smoking cessation was too late and the disease progression continued even after smoking cessation.

In the present study, a significant bacterial growth was found in 42.9% of patients during exacerbation attack ***Iyer et al*** isolated 45% bacterial pathogens from sputum in patients with COPD during exacerbations [15]. ***Erkan L et al*** observed bacterial pathogens in 55% AECOPD cases [16]. Culture positivity depends on nature of sputum, transportation time and the number of organism present in the sample. The remaining 57.1% of the cases could be due to prior antibiotic administration or due to inability to include cultivation methods for viral and mycological pathogens.

The prevalence of Gram negative isolates was 77.78%, as compared to 22.22% of gram positive. Gram-negative bacilli were also the predominant organisms in the study done by ***Siripataravan et al*** [17].

To obtain high susceptibilities to antimicrobial agents, we tested the susceptibilities of the isolated bacterial strains to major groups of antibiotics that have effect against both gram-negative and gram-positive bacteria.

In our study, the most common organism isolated was Klebsiella pneumoniae (30.09%) followed by Pseudomonas aeruginosa (29.1%), Acinetobacter (14.56%), Haemophilus influenzae (6.79%), E coli (6.79%), Streptococcus pneumoniae (4.86%), Staphylococcus aureus (2.93%), Moraxella catarrhalis (2.93%), Burkholderia cepacia (1.95%). This finding is contrary to other studies reported by ***D. Seshagiri Rao et al***. in 2017 who had found Streptococcus pneumoniae (28%) while ***Anand K. Patel et al*** had found Klebsiella pneumoniae (59%) as commonest isolate [18,1].

A master antibiogram for our region would allow tertiary care institutions to consider resistance patterns in hospitals referring patients and to select appropriate antimicrobial therapy or change drugs in non-responding patients. Implementing continued local surveillance programs for antibiotic resistance is essentially important. Moreover, further local studies should be carried out to elucidate the mechanisms of resistance of different pathogens in AECOPD. Judicious use of antimicrobials is essential to prevent the emergence of resistant and/or MDR bacteria in AECOPD

**Antimicrobial susceptibility of the isolates:**

In our study it was found that Amikacin was the most effective antibiotic for gram-negative bacteria followed by Ceftriaxone which is similar to the study conducted by ***DR.A.K. Borthakur et al.*** in 2017 who found Amikacin to be most effective whereas ***Anand K. Patel et al*** have shown Piperacillin +Tazobactam as more effective [13,1].

Klebsiella pneumoniae showed maximum sensitivity to Amikacin (76.2%), Meropenem (66.67%), and gentamycin (66.67%). They were highly resistant to Ampicillin (66.67%) followed by cefuroxime, which is similar to study conducted by ***Madhavi S et al*** [14].

Pseudomonas aeruginosa is mainly sensitive to Amikacin (89.47%) and ciprofloxacin (84.21%). H. Influenzae was found to be highly sensitive to Ceftriaxone (85.71%).

S.pneumoniae was 100% sensitive to linezolid followed by ceftriaxone (80%). Staphylococcus aureus isolates showed sensitivity to linezolid (100%), followed by cefazoliṇ (66.67%) ***Shashibhushan BL et al*** showed Beta lactam antibiotics as most effective antibiotic against Streptococcus pneumoniae [8].

## CONCLUSION

In this prospective observational study we analyzed the bacteriological profile of the patients with AECOPD and also their antimicrobial sensitivity pattern. Bacterial infections play a major role in AECOPD and were seen more in the age group of 60-79 years.

Sputum culture is a good and simple tool to study the etiology & complications due to bacteria in AECOPD. Direct Gram stained smear of sputum specimens has a good correlation with culture results.

Our study offers a reference for the spectrum of antibiotics that are most effective, hence will be of high value at the tertiary care hospital.

Antibiogram helps in the correct treatment protocol during management of AECOPD. It also helps in screening resistant pathogens and better drug for treatment, thereby helping to decrease the mortality and morbidity.

Klebsiella pneumoniae is responsible for the majority of AECOPD cases in our study. Amikacin followed by meropenem and gentamycin can be used for empirical treatment of these infections.

P. aeruginosa was next mostly isolated bacteria and were mainly sensitive to Amikacin, followed by ciprofloxacin

Most commonly isolated gram positive organism were S. pneumoniae, which was sensitive to linezolid followed by ceftriaxone (80%).

Staphylococcus aureus isolates showed sensitivity to linezolid (100%), followed by cefazolin (66.67%). They were highly resistant to erythromycin (66.67%), azithromycin (66.67%)

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