Bacteriological Profile of Chronic Obstructive Pulmonary Disease in Patients Admitted with Acute Exacerbation at a Tertiary Care Centre

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Abstract
Chronic Obstructive Pulmonary Disease (COPD) is the fourth leading cause of death in the world. COPD exacerbations constitute a significant burden on patients and healthcare systems. A study on ‘Bacteriological profile of Chronic Obstructive Pulmonary disease’ was conducted in the Department of Microbiology at Government medical college, Ernakulam for a period of 1 year from 1/3/2016 to 28/2/2017. Out of 105 sputum samples tested, 24 of 49 mucopurulent samples (49%) and 13 of 21 purulent samples (62%) were culture positive for pathogenic bacteria. The most common bacteria isolated was Pseudomonas aeruginosa (35%) followed by Acinetobacter species (20%), Hemophilus influenzae (17.5%), E.coli (10%), Klebsiella species (7.5%), Staphylococcus aureus (7.5%), Stenotrophomonas maltophilia (2.5%). Multi drug resistant bacteria comprised of 40% of the isolates. The mortality rate was 15% among culture positive patients.

Keywords: COPD (Chronic Obstructive Pulmonary Disease), Quantitative Sputum culture, Antibiotic susceptibility testing (ABST).

Introduction
Chronic Obstructive Pulmonary Disease is a disease state characterized by airflow limitation that is not fully reversible. COPD includes emphysema, an anatomically defined condition characterized by destruction and enlargement of the lung alveoli; chronic bronchitis, a clinically defined condition with chronic cough and phlegm; and small airways disease, a condition in which small bronchioles are narrowed. COPD is present only if chronic airflow obstruction occurs; chronic bronchitis without chronic airflow obstruction is not included within COPD. The natural course of COPD is punctuated by exacerbations which are defined as acute events characterized by a worsening of the patient’s symptoms that is beyond normal day to day variations and leads to a change in medication. Exacerbations may be precipitated by bacterial or viral infections of the lower respiratory tract, peaks of air pollution and in 1/3rd of cases no cause can be found. A study on ‘Bacteriological profile of Chronic Obstructive Pulmonary disease’ was conducted in the Department of Microbiology at Government medical college, Ernakulam for a period of 1 year from 1/3/2016 to 28/2/2017.
Objectives
1. To determine the proportion of patients admitted with acute exacerbation of COPD in which a bacterial etiology can be demonstrated.
2. To identify the common bacteria involved in the acute exacerbation of COPD, their antibiotic sensitivity pattern and the prevalence of multi drug resistant strains.

Materials and Methods
Study Design: Cross sectional descriptive study
Study Setting: Department of Microbiology and Department of Chest medicine, Government Medical College, Ernakulum.
Study Period: 1/3/2016 – 28/2/17 (1 year)
Study Population: Patients admitted with acute exacerbation of COPD in the department of Respiratory Medicine at Government medical college, Ernakulam during the study period
Age Group: 40 years and above
Inclusion Criteria: All patients with acute exacerbation of COPD, as defined by the Global Initiative of Obstructive Lung Disease (GOLD) who gave consent for the study and were able to give a satisfactory sputum sample (as determined by a Bartlett score >1 and requiring admission as determined by an experienced chest physician.
Exclusion criteria: Patients with features of Pulmonary tuberculosis, Bronchiectasis, Malignancy, Interstitial Lung Disease, Bronchial Asthma on chest X-ray/CT scan.

Sampling methodology: Universal sampling
Sample Size: 96, calculated using the formula 
Sample size = Z2 x p (1-p)/m2, where Z = 1.96 for 95% confidence interval, p=52, estimated prevalence from previous study by Arora et al, m= margin of allowable error (0.1)

Specimen Collection and Processing
All patients were advised by the nurse to collect sputum after rinsing their mouths and taking a deep breath. A single sample of spontaneously expectorated sputum was collected from each patient in sterile wide mouthed containers preferably before starting antibiotics. The sample was sent to the laboratory immediately after collection and was subjected to immediate processing.

Macroscopy
The macroscopic appearance of sputum was noted for the nature of the specimen as mucoid/ mucopurulent/purulent.

Microscopy
A smear was prepared from the mucoid/ purulent part of the sample and heat fixed. Gram staining was done on the smear which was graded according to Bartletts grading system for quality of sputum. Only samples with score greater than 1 indicating lower respiratory infection or a satisfactory specimen were included in the analysis. Presence of bacteria – types and density under oil immersion was also noted and the findings were graded and recorded in the proforma.

Culture
Culture was done directly with the sample collected by inoculating a loopful of sputum on to Blood agar, Chocolate agar and MacConkey agar plates. A heavy predominant growth of organisms other than normal flora of the upper respiratory tract were suggestive of bacterial infection.

Antibiotic Sensitivity Testing
Significant isolates obtained from culture were subjected to Antibiotic sensitivity testing by Kirby-Bauer disc diffusion method on Muller Hinton agar and interpreted according to CLSI guidelines.

Quantitative Culture
For quantitation of bacterial growth, quantitative cultures of sputum were done in addition to direct culture. This is based on the observation by Tebutt et al that infecting organisms are usually present in larger numbers than contaminants from the upper respiratory tract. Hence counts can be used
to assess the likely significance of any bacteria present.

Each sputum sample was homogenized using N-acetyl –l-cysteine (NALC) as mucolytic agent, diluted to 10⁻⁷ ml and quantified according to described methods as given below. To each specimen, a few glass beads and an equal volume of 2% (w/v) N-acetyl-l-cysteine (NALC) were added. The NALC solution was freshly prepared each day by dissolving 2 g NALC in 13 ml 1N NaOH and diluting to a final volume of 100 ml with Phosphate Buffered Saline (PBS). The pH of the solution was adjusted to 7.3 if necessary. The caps on the universal containers were securely tightened, the NALC-sputum mixtures were agitated on a vortex mixer for 10 seconds, allowed to stand at room temperature for 10 minutes, and finally vortex mixed for a further 15 seconds. Quantitative counts were made by adding 0.2 ml homogenized sputum to 0.8 ml of diluent (1 vol single strength nutrient broth + 9 vol PBS). Using a 1 ul disposable loop, a loopful of this diluted sputum was thoroughly mixed with 1 ml diluent, and from this a further 1 ul was spread onto Blood agar, Chocolate agar and MacConkey agar. Final dilution was 10⁻⁴ so than each colony represented 1 x 10⁷ CFU/ml of original sputum.

All the plates were incubated at 37 degrees in a moist atmosphere containing CO₂. The plates were examined after 24 hours and 48 hours to assess the colony characteristics on direct culture and quantitative culture (each colony on a dilution plate represent 1 x 10⁷ per ml original sputum). Bacterial colonies (other than those considered as normal flora) present in significant counts were considered as causative pathogens. Isolated bacteria were identified using conventional methods. The results were numerically coded and recorded in the proforma. History and other clinical details were obtained from patients during admission after obtaining informed consent and recorded in the proforma.

**Identification of the Isolate**

Identification of the isolates up to species level was done as per standard microbiological methods using

- Morphology of the isolate by Gram staining
- Culture characteristics of the isolates
- Biochemical reactions – Positive and negative controls for each reaction were included with each batch of tests.

**Results**

The present study was conducted in the departments of Microbiology and Chest Medicine, over a period of one year, from March 2016 to February 2017. A total of 105 samples were collected from patients admitted with acute exacerbation of Chronic Obstructive Pulmonary disease.

**Age Distribution**

The age of the youngest patient included in the study was 40 years and the age of the oldest patient was 89 years. The mean age of the patients included in the study was 65 years. Among the 105 admissions for acute exacerbations of COPD, the maximum number were in the age group 60-69 (51.5%).

**Gender Distribution among the Study Population**

Males were predominant in the study population compared to females. There were 88 (90.72%) males and 9 (9.27%) females in the study population. The male – female ratio was 9.5:1

**Sputum Microscopy**

Gram stain was done on all the samples. 55 samples (52.38%) showed predominance of bacteria other than normal commensals, the remaining 45 (47.62%) showed a mixture of bacteria suggestive of normal commensals. 13 samples showed Bartlett grade 2 and a single type of predominant bacteria, 42 samples showed Bartlett grade 1 and a single type of predominant bacteria.
Bacteria isolated in quantitative culture

Sputum Culture in Acute Exacerbation of COPD
Sputum culture (both direct and quantitative) was done in all 105 samples. Culture positivity (Single type of predominant bacteria) was demonstrated in 40 cases of acute exacerbation of COPD (38.05%) from 40 samples. From 65 (61.95%) cases of acute exacerbation of COPD only normal flora of the upper respiratory tract was isolated in sputum culture.

Single Type of Predominant Bacterial Growth (Direct V/S Quantitative Culture)
All samples were subjected to direct and quantitative culture of sputum. By direct culture of sputum, a single type of predominant bacterial growth was obtained in 40 samples (38.05%). However, only 32 (30.47%) samples showed counts equal to or exceeding 10³ CFU/ml of sputum by quantitative sputum culture. Of the 40 isolates detected by direct culture, 8 were not detected by quantitative culture. The organisms not detected on Quantitative culture were Hemophilus influenzae (all 7 isolates) and Pseudomonas aeruginosa (1 of 14 isolates).

Comorbidities
37 patients (38.14%) had Coronary Artery Disease, 12 patients (12.37%) had Cor pulmonale, 42 patients (39.17%) had previous history of Pulmonary Tuberculosis. Of the 20 cases admitted in the ICU, 9 had Coronary artery disease (45%), 3 had Cor Pulmonale (15%) and 10 had a past history of Pulmonary Tuberculosis (50%).

Bacterial Isolates in COPD
The most common bacteria isolated was Pseudomonas aeruginosa (14 isolates, 35%) followed by Acinetobacter species (8 isolates, 20%), Hemophilus influenzae (& isolates, 17.5%), E.coli (4 isolates, 10%), Klebsiella species (3 isolates, 7.5%), Staphylococcus aureus (3 isolates, 7.5%) Stenotrophomonas maltophilia (1 isolate, 2.5%).

Bacterial Isolates in ICU Patients
The isolates from ICU admitted patients were Acinetobacter species (4 isolates), Klebsiella species (3 isolates), Pseudomonas aeruginosa (2 isolates) and Stenotrophomonas maltophilia (1 isolate). 50% of the Acinetobacter species (4/8) obtained in the study were from ICU admitted patients. All of the Klebsiella species (3 isolates) and 1 isolate of Stenotrophomonas maltophilia was obtained from ICU admitted patients.

Bacterial Isolates from Patients Admitted in Wards
Pseudomonas aeruginosa (14.11%), Acinetobacter species (4.7%) and all the isolates of Hemophilus influenzae, E.coli and Staphylococcus aureus were from patients admitted in the wards.

Distribution of isolates (ICU/Ward admissions)

Bacteria Isolated in Readmissions
Among the 8 patients who were admitted twice, sputum culture of 3 patients grew normal flora of the upper respiratory tract on both occasions, 1 patient showed predominant growth of Pseudomonas aeruginosa, whereas 1 other patient...
showed a predominant growth of Acinetobacter species on both occasions.
Among the remaining 3 patients, there was a difference in sputum culture results during both admissions. In 1 patient who initially showed normal flora on sputum culture, predominant growth of Acinetobacter species was observed during the subsequent admission. In contrast, in another patient who initially showed predominant growth of Pseudomonas aeruginosa, normal flora was observed on sputum culture during the second admission. In 1 patient, predominant growth of Acinetobacter species was observed in the 1st exacerbation while predominant growth of E.coli was observed in 2nd one

Antibiotic Sensitivity Patterns
1. Pseudomonas aeruginosa
87.72% of the isolates were sensitive to Imipenem, followed by 71.42% to Tobramycin. 71.43% of the isolates were resistant to Ceftazidime and 71.43% were resistant to Piperacillin Tazobactam. 2 isolates were resistant to both Imipenem and Meropenem, indicating Carbapenemase production. 2 isolates were from ICU patients, both these isolates were sensitive to Imipenem but 1 was resistant to Meropenem and both were resistant to Gentamicin and Ciprofloxacin

2. Acinetobacter
Highest sensitivity was to Amikacin (62.5%). Resistance was 100% to Cephalothin, Cefuroxime, Cefotaxime, Ceftriaxone, Cefoperazone. Of the 8 isolates, 4 were from ICU patients

3. Hemophilus influenzae
Sensitivity was 100% to Cotrimoxazole, followed by 85.72% sensitivity to Chloramphenicol

4. E.coli
The sensitivity was 100% to Amikacin, Cefoperazone Sulbactam, Imipenem and Meropenem. The resistance was 100% to Cephalothin and Cefuroxime

5. Klebsiella
The sensitivity was 100% to Cefoperazone sulbactam and Meropenem, followed by 66.67% to Cotrimoxazole. The resistance was 100% to Ciprofloxacin and 66.67% to Imipenem.

6 Staphylococcus aureus
Only 1 isolate of Methicillin susceptible Staphylococcus aureus was obtained which was resistant to Penicillin and Erythromycin. 2 isolates of Methicillin Resistant Staphylococcus aureus were obtained. Both isolates were sensitive to Gentamicin, Linezolid. Both isolates were resistant to Penicillin, Cloxacillin, Erythromycin and Ciprofloxacin.

7. Stenotrophomonas maltophilia
Only 1 strain of Stenotrophomonas maltophilia was isolated. It was sensitive to Gentamicin, Cotrimoxazole, Tobramycin and Cefoperazone Sulbactam. It was resistant to Ampicillin, Cephalothin, Cefuroxime, Cefotaxime, Cefixime, Ceftriaxone, Ce�피메, Ceftazidime, Piperacillin Tazobactam, Imipenem and Meropenem.

MDR Isolates
Of the 40 isolates obtained by direct sputum culture, 40% (16/40) of the isolates were multidrug resistant. Of the 16 multidrug resistant isolates, 7 (43.75%) were from ICU admitted patients and 9 (56.25%) from ward admitted patients.
Pseudomonas aeruginosa - 35.7% (5/14) were multidrug resistant (1 from an ICU patient, 4 from ward admitted patients), Acinetobacter species – 62.5% (5/8) – 4 from ICU patients and 1 from a ward admitted patient, E.coli - 50% (2/4) – Both the isolates were from ward admitted patients, Klebsiella- 34% (1/3), which was from an ICU admitted patient, Staphylococcus aureus – 66.67 % (2/3) isolates were Methicillin resistant staphylococcus aureus. Both were from ward admitted patients, Stenotrophomonas maltophilia – the single isolate obtained was multidrug resistant and from an ICU patient.
Mortality
93.33% of the exacerbations were treated with antibiotics (oral/intravenous). The mean duration of hospital stay was 7 days. Of the 105 admissions for acute exacerbation of COPD, 8 patients expired. The overall mortality rate in our study was 7.61%. The patients who expired were all above 50 years of age, all were GOLD class 3 with MRC scores 2 and above. 6/8 (75%) of the patients had Coronary artery disease. 4/8 (50%) had Cor Pulmonale and 5/8 (62.5%) had old PTB. Among these patients, a predominant bacterial growth was detected in 6 i.e. 75%. The mortality rate among culture positive patients was 15% (6 out of 40). The mortality rate among patients with normal flora was 3.07% (2 out of 65).

Discussion
Age and Gender distribution
The majority (51.5%) of the patients belonged to the age group 60-69 years and were males (90.72%). Studies by Shashibhushan et al and Sharma et al showed a similar pattern with respect to age and gender of the study population. The prevalence rates of COPD in males varied from 2.12% to 9.4% in studies conducted in north India and from 1.4% to 4.08% in south India. The respective ranges for females were 1.33%–4.9% in north India and 2.55%–2.7% in south India. The median values of these prevalence rates are 5% for males and 2.7% for females. Thus, COPD is more common among males than females. The male to female ratio varied rom 1.32:1 to 2.6:1. This male preponderance was reflected in this study also.

Culture positivity and bacteriological profile
Sputum culture was done in all 105 samples and culture positivity was demonstrated in 40 samples (38.05%). Gram negative bacteria were predominant in our study. The most common isolates were Pseudomonas aeruginosa, (35%), Acinetobacter species (20%) and Hemophilus influenza (17.5%). The remaining bacteria isolated were E.coli (10%), Klebsiella species (7.5%), Staphylococcus aureus (7.5%) and Stenotrophomonas (2.5%). Culture positivity in our study was 38.05%. Arora et al and Sharma et al reported a higher percentage of culture positivity in their studies. i.e. sputum culture positivity of 50% and 48.7% respectively(9). The lower culture positivity may be attributed to the fact that 39.04% of the patients in our study were referred cases, already on antibiotics.

Commonly isolated organisms in COPD exacerbations include Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Haemophilus parainfluenzae, and Pseudomonas aeruginosa. Studies by Pela et al and Soler et al using bronchoscopic samples demonstrated that infections with Pseudomonas spp, Stenotrophomonas spp, and Gram negative bacteria occur in more severe exacerbations, affecting the most debilitated patients. A study by Arora et al in New Delhi demonstrated a bacterial etiology in approximately 50% of patients with acute exacerbation of COPD – Streptococcus pneumoniae in 25.8%, Pseudomonas aeruginosa in 12%, Klebsiella in 10%, Moraxella catarrhalis in 3.4% and Staphylococcus aureus in 1.7% cases. In another study by Shashibhushan et al in Bangalore the most common pathogenic bacteria isolated were Streptococcus pneumonia (42%), followed by Pseudomonas aeruginosa (23%), Klebsiella (15%), E coli (12%), Non fermenting Gram negative bacilli (7%) and Citrobacter (1%). Similar observations were made in a study by Sharma et al in Meerut wherein sputum culture positivity among patients with acute exacerbations of COPD was observed in 48.7% cases, the most commonly isolated bacteria being Streptococcus pneumonia (13%), followed by Ecoli (9.4%), Acinetobacter (8.1%), Pseudomonas aeruginosa (7.5%) and Klebsiella (6.3%).

Role of quantitative sputum culture
During the stable state and during exacerbations, standard sputum cultures may miss the presence of bacteria in the lung. An American study by
Bandi et al, demonstrated the presence of intracellular H influenzae in 87% of bronchial biopsy samples from patients with acute exacerbation of COPD, compared to 33% of patients with stable COPD and none in healthy controls.

In our study we have employed both a direct culture of sputum as well as a quantitative culture to isolate significant bacterial pathogens. Haemophilus influenza could not be isolated on quantitative culture as also one isolate of Pseudomonas aeruginosa. This could be because isolates of Hemophilus influenza did not withstand the rigorous processing involved in quantitative culture. Apart from this, the results of direct and quantitative culture yielded almost similar results. However, the possibility of missing important pathogens such as Hemophilus influenzae and the tedious process involved clearly outweigh the benefits of this procedure for routine laboratory use.

**Comorbidities**
39.05% of the patients had Coronary artery disease as a comorbidity, whereas 14.29% had Cor pulmonale and 40% had past history of Tuberculosis. There was no significant difference in the types of bacteria isolated in those with a past history of pulmonary tuberculosis compared to those who did not.

**Antibiotic susceptibility pattern**
Antibiogram of the isolates revealed that 40% of the isolates were multidrug resistant. Highest percentage of multidrug drug resistance was observed in Acinetobacter species (62.5%) whereas of the 14 isolates of Pseudomonas aeruginosa, 37.5% were multidrug resistant. 87.72% of the isolates of Pseudomonas aeruginosa were sensitive to Imipenem, 71.42% to Tobramycin and 64.28% to Meropenem. The recommended antibiotics for Pseudomonas infection include Piperacillin Tazobactam, Ceftazidime, Cefepime and Levofloxacain. However in our study 71.43% of the isolates were resistant to Piperacillin tazobactam, 71.43% to Ceftazidime, 64.28% to Cefepime and 42.86% were resistant to Levofloxacain. This suggests that the recommended antibiotics may not be effective in our hospital, thus reflecting the need for an antibiotic policy based on local susceptibility patterns. 62.5% of Acinetobacter isolates were multidrug resistant with 62.5% sensitive to Amikacin. All the isolates were resistant to Cephalothin, Cefuroxime, Ceftriaxone and Cefotaxime. 75% were resistant to Meropenem, 75% to Ciprofloxacin, 62.5% resistant to Gentamicin, 62.5% to Cefaperazone Sulbactam, 62.5% to Piperacillin Tazobactam, 62.5% to Imipenem and 37.5% resistant to Amikacin.

Isolates of Hemophilus influenzae were sensitive to Cotrimoxazole and Chloramphenicol, Cefotaxime, etc and there were no multidrug resistant isolates. Chawla et al reported 60% of Pseudomonas aeruginosa isolates being resistant to Cephalosporins. In a study by Elkorashy et al, 28.6% of isolates from patients admitted with AECOPD were multidrug resistant.

COPD is an important economic burden on the patient and the health care infrastructure of the country. Each patient was shown to spend on an average, up to 15 per cent of his annual income on smoking products and up to about 30 per cent on disease management.

**Conclusion**
In our study, direct microscopy was suggestive of a bacterial etiology in 55 (52.38%), the remaining 45 (47.62%) showed normal flora of the upper respiratory tract. Culture positivity was 38.05% i.e. 40 sputum samples showed a predominant bacterial growth, whereas 65 samples (61.95%) grew normal flora of the upper respiratory tract. 24 of 49 mucopurulent samples (49%) were culture positive and 13 of 21 purulent samples (62%) were culture positive for pathogenic bacteria.

The most common bacteria isolated from patients admitted with acute exacerbation of COPD in our hospital was Pseudomonas aeruginosa in 14
samples (35%). Drug resistant strains comprised 40% of the isolates. Among the isolates of Pseudomonas aeruginosa – 5 (35.7%) were Multidrug resistant.

Patients were treated based on the antibiotic susceptibility pattern, 8 patients (7.61%) expired. Among the culture positive cases (40), 34 improved on treatment and 6 patients expired despite treatment. The mortality rate was 15% among culture positive patients. Comorbidities associated with COPD also played a role in mortality of patients admitted with acute exacerbation of COPD.

**Conflicts of Interest** – Nil

**References**


