ORIGINAL ARTICLE

Study of Sputum Bacteriology of Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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Abstract:

Background: Chronic Obstructive Pulmonary Disease (COPD) would be the third leading cause of death by 2030 as per WHO. India has close to 30 million COPD patients at present. Acute exacerbations in these patients are associated with worse outcomes and are responsible for a decline in lung function. Bacterial infections cause exacerbations in 30-50% cases and new bacterial strains are often isolated; they are different in India than what Western studies report. Hence, it is useful to know the bacteriological profile of the prevalent strains as per geography so as to manage the exacerbations better. *Aim and Objectives:* The study aimed to determine the bacteriological profile and sensitivity to antibiotics in sputum of patients with Acute Exacerbation of COPD (AECOPD). *Material and Methods:* Ninety patients presenting with Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD) who met the inclusion criteria were included and their sputum was sent for Gram stain, Ziel-Neelson stain and bacterial culture and antibiotic sensitivity testing. *Results:* Culture was positive for pathogens in 34 (37.7%) patients. *P. aeruginosa* 14 (41.1%), *K. pneumoniae* 11 (32.35%) and *E. coli* 9 (26.47%) were the organisms isolated. The isolates were sensitive to Beta-Lactam/Beta-Lactamase Inhibitor (BL/BLI) combinations, carbapenems and colistin and polymyxin B. *P. aeruginosa* and *E. coli* were also sensitive to aminoglycosides and fluroquinolones while *K. pneumoniae* was variably resistant to them. Age, gender and leucocyte count were not significantly associated with the sputum positivity rate. *Conclusion:* Cefoperazone/sulbactam with an aminoglycoside and/or levofloxacin may be considered for initiation of therapy in AECOPD patients.

Keywords: Acute Exacerbation, Beta-Lactam/Beta-Lactamase Inhibitor, Chronic Obstructive Pulmonary Disease, Exacerbations

Introduction:

The World Health Organization (WHO) estimates 65 million people to be suffering from moderate to severe Chronic Obstructive Pulmonary Disease (COPD) globally. It has also predicted that COPD would become the third leading cause of death worldwide by 2030 [1]. Smoking and exposure to biomass fuels in poorly ventilated dwellings are some of the important risk factors associated with development of chronic lung disease in India. Crude estimates suggest that India has 30 million COPD patients [2]. In India, COPD is the second leading cause of death today and is among the top eight leading causes of disabilities in all states [3]. Severe exacerbations of COPD are related to a significantly worse outcome in patients.

Exacerbations are defined as an acute worsening of respiratory symptoms in the form of acute change in the patient's dyspnea or breathing difficulty, cough and/or sputum production requiring change in treatment and/or hospitalization [4].

Microorganisms including bacteria and viruses

have been identified as triggers and risk factors for COPD exacerbations [5]. In India, bacterial infections are found be responsible for more than 40% of COPD exacerbations [6]. During exacerbations often new bacterial strains have been identified in the respiratory tract of COPD patients [7-8]. It has been found that exacerbations accelerate the decline in lung function in COPD patients, which makes it all the more necessary to prevent them [9]. A difference in kind of risk factors and exposure, environmental and genetic factors and patterns of antibiotic resistance results in distinct bacteriological profiles and antibiotic response and resistance patterns in Acute Exacerbations of COPD (AECOPD) in Asian patients [10]. Bacterial etiology in AECOPD in India differs from that found in western literature [11]. The current study aims to study the bacteriological profile during acute exacerbations of COPD along with their antibiotic sensitivity profile so as to aid health authorities and institutions frame antibiotic protocols for these patients as per the local bacteriological prevalence and sensitivity pattern.

Material and Methods:

Study Population:

A prospective open label study was conducted on 90 patients of previously diagnosed COPD who presented with acute exacerbations requiring hospitalization. The study was carried out during the period of 1st January 2018 to 31st December 2018 at a tertiary care institute of Ahmedabad after getting approval from the Institutional Ethics Committee (GCSMC/EC/TRIAL/APPROVE/2016/172-A). Informed consent was taken from all study participants. COPD was diagnosed on the basis of clinical history, risk factors and clinical findings and supported by spirometry which was done at time of diagnosis. GOLD criteria were used to establish the severity of disease in these patients [12]. The following inclusion and exclusion criteria were followed to select study patients:

Inclusion Criteria:

- Patients diagnosed with COPD on basis of history, risk factors, clinical features, examination and spirometry.
- Acute exacerbation was defined as an acute event characterized by a worsening of the patients' respiratory symptoms that is beyond day-to-day variations and requires change in medication and/or requiring hospitalization. This would usually present as.
  1. Increased dyspnea
  2. Increased sputum volume
  3. Increased sputum purulence

Exclusion Criteria:

- Patient having any other evidence of chest disease on chest X-ray like consolidation, bronchiectasis, mass lesion, cavitary lesion, interstitial lung disease or any other such disease.
- Patients with sputum positive for acid fast bacilli.
- Patients who have taken antibiotics in the previous 21 days.

Collection and Processing of Sputum Samples:

Deeply expectorated sputum samples were sent to Department of Microbiology after collection in sterile containers. Appropriate sputum samples were selected using Bartlet's criteria [13]. These criteria take three parameters into consideration

for the same and give a scoring system to each of the parameters as mentioned below:

1. No. of neutrophils/ low power field: if <10, score is 0; if 10-25, score is 1+; if >25, score is 2+
2. Presence of mucus: Score is 1+
3. Epithelial cells/low power field: if 10-25, score is -1; if >25, score is -2.

If the total score of all the three parameters was 0 or less it indicated lack of inflammation or contamination with saliva. Hence those samples were rejected and the ones with scores higher than 0 were considered for the study.

After proper collection, sputum samples were transported immediately to microbiology laboratory of our institute for further processing by conventional methods. Sputum samples were examined for physical appearance, Gram stain, acid fast bacilli smear, pyogenic culture for bacterial organisms and drug sensitivity testing. Sputum samples were first examined under low power field and only those having less than 25 epithelial cells per low power field were considered for further analysis.

Incubation and Culture of Samples:

Appropriate sputum sample was inoculated on nutrient agar, 5% sheep blood agar, chocolate agar and MacConkey's agar. These inoculated plates were then incubated at 37°C for a period of 18-24 hours after which they were examined for evidence of bacterial growth. The isolated organisms were identified by standard microbiological techniques.

Identification of Pathogens:

A single well separated colony was proceeded for preliminary tests like Gram's staining, catalase test, cytochrome oxidase test, motility test. Biochemical tests like indole test, methyl red test, voges proskauer test, citrate utilization test, urease test, triple sugar iron agar, Hugh-Leifsons oxidation fermentation test were performed. Sugar fermentation tests with sugars viz: glucose, lactose, sucrose, maltose, mannitol, were done to identify the isolate. These tests were performed according to standard methods.

Antimicrobial Susceptibility Testing:

All the isolates were tested for antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to CLSI guidelines (2015).

The following antibiotics were tested by the disk diffusion method:

Ampicillin (10µg),
Ampicillin/ Sulbactam (20µg),
Cefuroxime (30µg),
Cefotaxime (30µg),
Ceftazidime (30µg),
Cefoperazone/Sulbactam (105µg),
Cefepime (30µg),
Ciprofloxacin (5µg),
Levofloxacin (5µg),
Doxycycline (30µg),
Amikacin (30 µg),
Cotrimoxazole (25µg),
Azithromycin (15µg),
Piperacillin/Tazobactam (100/10µg),
Gentamicin (10µg),
Aztreonam (30µg),
Imipenem (10 µg)

Disk diffusion testing was done using 300 U polymyxin B disk and Colistin disk (10 µg) (BBL, BD, USA), according to the CLSI Guidelines January 2015. The disk zone diameters were interpreted according to the CLSI Guidelines (2015) for colistin (resistant ⩽ 10 mm and

susceptible ⩾ 11 mm) and polymyxin B (resistant ⩽ 11 mm and susceptible ⩾ 12 mm). Antibiogram was provided after studying the zone of inhibition after 24 hours of further incubation with the discs according to CLSI Guidelines. Appropriate statistical tests in the form of ratios, percentages, and means as well as the chi square test were utilised to analyse the data collected and results were accordingly obtained. Confidence level was set at 95% and 0.05 significance level.

Results:

Demographic Profile of Study Population:

All patients in our study needed hospitalization for their exacerbations. Our study had 74 males and 16 females which came out to a ratio of M: F=4.6:1, 82.2% of patients were males and 17.8% were females. The mean age of the participants was 60.82 ± 11.18 years. 68 (75.5%) patients belonged to age group of 50-69 years out of which 46 (51%) patients belonged to the age group of 60-69 years (Fig.1). Symptoms of the patients are presented in Table 1. Seventy-six patients had grade 4 COPD while 14 patients had grade 3 COPD as per GOLD criteria. Thirty out of 90 patients (33.33%) patients showed evidence of fibrosis from old lesions on their chest radiograms. The rest had normal X rays. Sixty-five participants had a history of past or present smoking which comprised of 72.22% of the study sample. All the smokers happened to be males. None of the participants gave a history of exposure to biomass fuels perhaps because of their urban residence.

Table 1: Symptoms of Exacerbation in Study Patients

Symptoms	No. of patients (%)
Dyspnea	90(100)
Cough	83(92.2)
Fever	14(15.5)

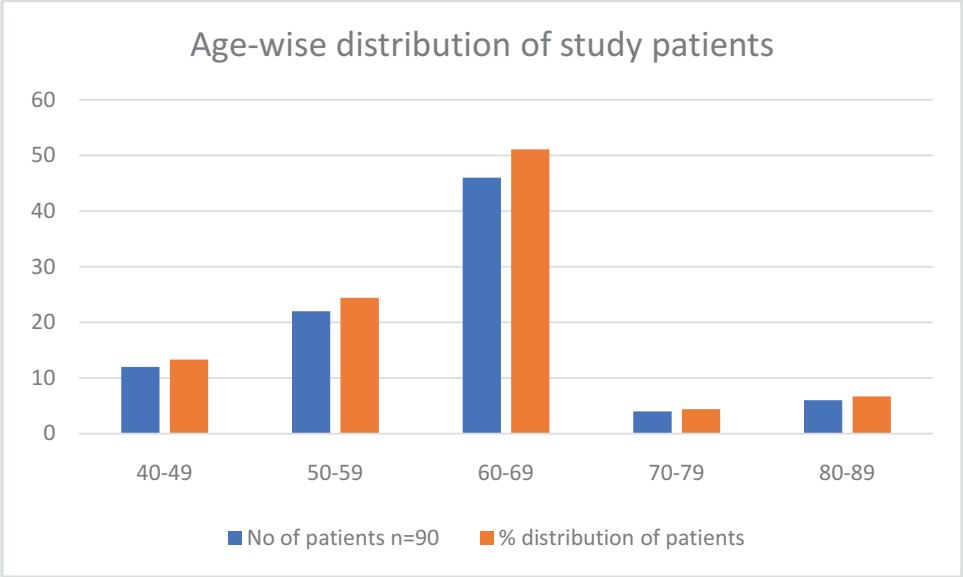

Fig. 1: Age-wise Distribution of Study Patients

Pathogens Isolated in the Study:

In our study, 34 (37.7%) patients were found to be positive for potential pathogens all being Gram negative isolates in their sputum culture. 30 (88.23%) were males and 4 (11.76%) were females. Out of these 34 isolates 14 (41.1%) isolates were *P. aeruginosa* followed closely by *K. pneumoniae*, 11 (32.35%) patients' isolated *K. pneumoniae*. *E. coli* was isolated in the remaining 9 (26.47%) patients. Three isolates out of these (% of *E. coli* isolates) were found to produce extended spectrum β-lactamases. The rest of the patients had normal microbial flora.

Antibiotic Susceptibility Results for Microbial Isolates:

On sensitivity testing all the isolates were found to be sensitive to cefoperazone/sulbactam, cefipime/tazobactam, imipenem, meropenem, colistin and polymyxin B. All *P. aeruginosa* isolated were fully sensitive to fluroquinolones as well as aminoglycosides. *E. coli* isolates were sensitive to aminoglycosides but partially resistant to fluoroquinolones. There was varying degrees of resistance for the fluroquinolones and aminoglycosides among *K. pneumoniae* isolates (Table 2).

Table 2: Antibiotic Resistance Pattern of Bacterial Isolates in AECOPD Patients

Antibiotics	*P. aeruginosa* (n=14)	*E. coli* (n=9)	*K. pneumoniae* (n=11)
Cefotaxime	14(100%)	5(55.5%)	7(63.6%)
Ceftazidime	7(50%)	4(44.4%)	6(54.5%)
Cefepime	7(50%)	4(44.4%)	4(36.3%)
Aztreonam	5(35.7%)	4(44.4%)	Not Done
Cefoperazone	9(64.2%)	5(55.5%)	6(54.5%)
Ceftriaxone	10(71.4%)	4(44.4%)	7(63.6)
Cefpirome	5(35.7%)	4(44.4%)	4(36.3%)
Piperacillin/tazobactam	0(0%)	0(0%)	Not Done
Gentamicin	0(0%)	0(0%)	5(45.4%)
Tobramycin	0(0%)	0(0%)	4(36.3%)
Amikacin	0(0%)	0(0%)	5(45.4%)
Netilmicin	0(0%)	0(0%)	5(45.4%)
Ciprofloxacin	0(0%)	4(44.4%)	5(45.4%)
Ofloxacin	0(0%)	4(44.4%)	5(45.4%)
Levofloxacin	0(0%)	0(0%)	5(45.4%)

All isolates were resistant by varying degrees to third generation β-Lactams (BL) alone without combination of β-Lactamase Inhibitors (BLI). All our patients except two patients had monomicrobial growth.

Relation of Sputum Positivity to Leucocyte Count:

We also found in our study that the positivity of sputum culture was not related to a high leucocyte count (chi square 0.2363, p-value 0.62) (Table 3). Also, the relation of gender to sputum positivity was not found to be significant (chi square 1.356, p-value 0.24). Age of the patient was also not found to be related to sputum positivity significantly (chi square 2.1037, p-value 0.71)

Discussion:

COPD remains an important cause of morbidity and mortality in the elderly population. The prevalence of COPD is on the rise as is evident from the literature. Bacterial and viral infections are the most common cause for acute exacerbations of COPD [14]. Bacterial pathogens are isolated between 30-50% patients of acute exacerbation of COPD, a finding that is consistent with the present study [11]. A study done in Hong Kong reported a 37.8% of sputum positive rate in patients of AECOPD, which is close to our study [15]. A meta-analysis of 118 studies of bacteriological profile of patients with acute COPD exacerbations found 49.59% of prevalence of bacterial infection in these patients [16].

In our study *P. aeruginosa* was the predominant organism isolated followed by *K. pneumoniae*. This is consistent with the study performed by Kuwal and Joshi where they found 38.23% isolates to be *P. aeruginosa* and 29.41% isolates to be *K. pneumoniae*. In the same study they concluded that Gram-negative bacilli were more frequently isolated in more severe forms of COPD, a finding consistent with our study [11]. Similar results have been found in studies done by Raveendra *et al*. [17] and Elkorashy *et al*. [18]. Common bacterial infections identified in these patients are *Hemophilus influenzae (NTHi), Moraxella catarhallis*, *Streptococcus pneumoniae* or *P. aeruginosa* [19].

These findings thus suggest a changing profile of bacteriology of COPD patients and a need to adjust therapy accordingly. Also, in our study most patients had severe COPD to start with and all needed hospitalisation.

Table 3: Distribution of Sputum Positivity in Relation to Leucocyte Counts

Total leucocyte count (/cmm)	⩾11000	<11000	Total	p-value
Sputum positive	14	20	34	0.62
Sputum negative	26	30	56	
Total	40	50	90	

p=0.62, significance level 0.05 at 95% confidence interval

It has been suggested that patients with severe COPD may have a weaker immune system leading to colonisation by these virulent pathogens. The microorganism type also gets determined by COPD severity [20]. Also, the increasing inadvertent use of antibiotics may be the reason for increasing incidence of Gram-negative pathogens in AECOPD patients. However, the incidence of sputum positivity did not relate significantly to either gender or age in our study. Sharma *et al*. also found similar findings in their study [21]. Their study also had similar age and gender distribution as our study. The sputum positivity also did not significantly relate to leucocyte count.

An elevated leucocyte count was however found in 44.44% of our patients which is consistent with the study of Fanny *et al*. [15]. The same study also reported a fever in 16.7% of patients which is close to our study (15.5%). This indicates that an elevated leucocyte count may be an inflammatory marker in AECOPD rather than a marker of infection. A study by Fattouh *et al*. demonstrated a highly significant difference ($p<0.001$) between leucocyte counts found at exacerbation to that of remission in COPD patients [22].

Serial measurements of inflammatory markers like total leucocyte count and C-reactive protein may help predict exacerbations and thus prevent them although this needs to be explored in the future.

Our study also demonstrated varying degrees of resistance to commonly utilised antibiotics in practice. Similar findings to our study have been demonstrated in study conducted by Hassan *et al.* [23]. However, many studies from India have shown varying degrees of antibiotic susceptibility and resistance patterns in comparison to ours [11, 19, 21]. This further emphasizes the need to know about local bacteriological and antibiotic susceptibility and resistance patterns as these are also related to the antibiotic use practices in different study populations. In one study conducted on multi-drug resistant *K. pneumoniae*; it was found that 88.2% of the isolates were resistant to three or more classes of anti-microbial agents [24]. Inadvertent and frequent use of antibiotics may be an important cause for infections with Gram negative resistant organisms as found in our study.

In conclusion, bacterial infection remains an important cause for AECOPD. Gram negative bacilli predominance was found in our patients of AECOPD and several other similar studies. Interestingly, a similar study conducted recently for urinary tract infections also showed preponderance of resistant Gram-negative organisms [25]. In the said study, the most common organism isolated was *E. coli* (38.48%) which was followed by *K. pneumoniae* (14.85%) and *P. aeruginosa* (10.30%).

Leucocytosis was not associated with sputum positivity in the current study but may be treated as an inflammatory marker to look out for. Increasing antibiotic resistance to commonly used antibiotics is a serious cause of concern and needs to be addressed. Carbapenems and BL/BLI combinations were effective across the entire spectrum of isolated organisms and may be recommended to be used as first line agents with aminoglycosides and/or levofloxacin in patients hospitalised with COPD in our institutional guidelines, depending upon the severity and respiratory status of the patient.

The limitations of our study were a small sample size and a single centre study. Also, bronchoscopy

and collection of bronchoscopic samples might have resulted in an increased yield of micro-organisms in our study. This needs to be explored in future studies. The results of our study need to be tested in a larger population and extrapolated so as to establish regional guidelines for antibiotic use to treat such patients.

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How to cite this article:
Shah SJ, Mehta N, Shah S. Study of Sputum Bacteriology of Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease. *J Krishna Inst Med Sci Univ* 2021; 10(2):130-138

Submitted: *15-Jan-2021* ***Accepted:*** *15-Mar-2021* ***Published:*** *01-Apr-2021*