



Original Article

Sputum bacteriology, antibiotic sensitivity pattern and C reactive protein levels in patients of COPD with acute exacerbations

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Abstract

Acute Exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD) are a common cause of hospitalization. It could have either an infective or non-infective etiology. Primary line of treatment is administration of empirical antibiotic therapy. CRP (C Reactive Protein) levels may be used as a biomarker to increase confidence about administering antibiotics. Periodic isolation and determination of antibiotic sensitivity pattern of causative bacteria is essential to formulate appropriate treatment strategies and avoid indeterminate use of antibiotics, to avoid rise in resistance. This cross sectional study was undertaken to determine the prevalence and bacterial etiological agents of AECOPD and their antimicrobial susceptibility pattern, and to correlate serum CRP levels with the causative factor. Sputum samples obtained from 50 AECOPD patients was subjected to bacterial culture and antibiotic sensitivity testing. Venous blood samples were also collected on admission and was subjected to a Rapid Latex Agglutination Test to determine CRP levels. Prevalence of sputum culture positivity was 62%. Commonest bacterial isolates were Klebsiella pneumonia (49%) and Pseudomonas aeruginosa (24%) . Extended spectrum beta lactamase was detected in 13.6% of enterobacteriaceae. Culture positivity was significantly higher in patients with purulent sputum as compared to non-purulent sputum. (p value=0.002). Serum CRP levels showed significant correlation with sputum purulence (p value=0.001) and culture positivity (p value=0.003). Thus sputum bacteriology results and the antibiotic resistance pattern provide guidance for the choice of empirical antibiotic treatment for COPD patients with clinical evidence of airway infection such as increased sputum purulence and elevated serum CRP levels.

Keywords: AECOPD, ESBL, CRP.

Introduction

Chronic Obstructive Pulmonary Disease is characterized by recurrent episodes of Acute Exacerbations. An acute exacerbation has the

three cardinal symptoms: dyspnoea, increase in sputum purulence, and increase in sputum volume –it requires immediate attention. They have an undeniably important impact on Health status,

worsen lung function and increase mortality. Modern diagnostic techniques show that most exacerbations have an infective aetiology with bacteria accounting for a substantial proportion^[1]. In clinical settings antibiotics remain the primary line of treatment. The choice of antibiotics depends on the local antibiotic policy and the pattern of local pathogens. Hence periodic isolation, identification and antibiotic sensitivity patterns of pathogens responsible for acute exacerbations will help us formulate appropriate treatment protocol which will be of immense use in reducing mortality. In AECOPD patients bacterial infections cause intense inflammatory response and levels of CRP is increased. Non-infective aetiology of COPD has been linked with lower systemic inflammatory response, as assessed by serum C Reactive Protein levels, than the infective kind^[2]. This can be used as an additional parameter to increase the confidence about the absence of bacterial pathogen and infection.

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. A gross underestimate of COPD Prevalence has been estimated as 17 million and is likely to increase by over 30% in the next decade. COPD affects 30% of patients seen in chest clinics and constitutes 1-25% of hospital admissions all over India.^[3]

This present study was done to identify the bacteriological profile and antibiotic sensitivity pattern in patients of AECOPD and to assess if serum CRP levels can increase confidence about the causal component of the disease.

Material and Methods

Study design & duration of study: This cross-sectional study was done over a period of five months.

Sample size: 50

Inclusion criteria: Patients satisfying Anthonisen criteria: Patients with history of chronic

bronchitis, emphysema and small airway disease with any two of the cardinal symptoms of acute exacerbation i.e. Dyspnoea, increase in sputum purulence, increase in sputum volume.

Exclusion criteria: Patients with evidence of congestive heart failure on admission and patients under treatment with antibiotics in the past 24 hours.

Before the commencement of the study, approval was obtained from the Institutional Ethical Committee. Informed consent was obtained from the study group. The patients were interviewed with a structured questionnaire.

Sputum samples were collected from study patients according to standard guidelines. The sputum was examined macroscopically and categorized into: purulent, mucopurulent and mucoid. All the sputum samples were pre-screened with Gram's stain, sputum specimens which yielded <10 squamous epithelial cells per low power field, or ≥ 25 polymorphonuclear leukocytes per low power field together with few squamous epithelial cells, were considered as representative of distal airways and were processed for culture^[4]. Sputum samples were mechanically homogenized with sterile glass beads using vortex machine. They were inoculated into blood agar, chocolate agar and Mac Conkey Agar plates and incubated at 37°C for 24 hours. Blood and Chocolate agar plates were incubated along with 5-10% CO₂. Colonies grown on culture plates were identified based on colony morphology, Gram staining, motility and standard biochemical tests. Antimicrobial susceptibility testing was done by disc diffusion method using Kirby Bauer technique on Mueller Hinton Agar using appropriate antimicrobial drugs according to CLSI guidelines.

Extended Spectrum β lactamase (ESBL) detection method:

Phenotypic Combined disc diffusion test:

Isolates of enterobacteriaceae which gave zone diameters of ≤ 27 mm to cefotaxime and ≤ 22 mm to ceftazidime in the initial screen tests were phenotypically confirmed. A culture suspension of

the test isolates was adjusted to 0.5 McFarland's standard and inoculated by using a sterile cotton swab on the surface of a Mueller Hinton Agar plate. Discs of cefotaxime (30 µg) and cefotaxime clavulanic acid (30/10 µg) were placed on one half of the plate. Discs of ceftazidime (30 µg) and ceftazidime-clavulanic acid (30/10 µg) were placed on the other half of the plate. The plate was incubated at 37°C for 24 hrs. Positive control *Klebsiella pneumoniae* ATCC 700603 and negative control *E. coli* ATCC 25922 were used as control strains. A ≥ 5 mm increase in zone diameter for cefotaxime-clavulanic acid and ceftazidime clavulanic acid versus the zone of cefotaxime and ceftazidime alone was confirmed as ESBL isolate.

Methicillin Resistance Detection in *Staphylococcus aureus*:

Cefoxitin Disc diffusion method:

0.5 Mcfarland's suspension of test isolate was lawn cultured on MHA plates. 30 µg cefoxitin disc was placed on the surface of lawn culture, incubated at 37°C for 18-24 hours. Isolates showing inhibition zone diameter ≤ 21 mm, were considered as Methicillin Resistant isolates. Positive control MRSA ATCC 43300 and negative control MSSA ATCC 25923 were used as control strains.

Venous blood samples for CRP measurement:

5ml of venous blood was collected from the patient under strict aseptic precautions on the day of admission in a sterile red top vacutainer. It was centrifuged to obtain serum and Latex Agglutination Slide Test for determination of CRP by semi quantitative method was performed using a commercially available kit. The test was performed with positive and negative controls provided with the kit as per test procedure to assure conformity and integrity.

Statistical Analysis

Done using Statistical Package for Social Sciences (SPSS) software 15.0 The proportional data of the study was tested using Pearson's Chi square analysis test χ^2 . Statistical significance was given if $P < 0.05$.

Results

The study population consisted of 50 patients of AECOPD in the age group from 34-87 years. 34(68%) were males and 16 (32%) were females. The study group had a definite male preponderance, with a male:female ratio of 68:32. The most prevalence was seen in the age group of 61-70 years.(44%) Smoking was associated with AECOPD in 43(86%) cases. There was statistical significance with p value < 0.001 . Increased level of breathlessness was the most common symptoms seen in 100% of the patients, followed by increased in sputum volume in 80% and increased sputum purulence in 76%. As per Anthonisen Criteria, Type 1 Exacerbation were most common with all three cardinal symptoms in 56%, Type 2 in 38% and Type 3 Exacerbation in 6% of patients.

Out of 38 patients who had purulent sputum 28 (73.7%) had culture positivity and out of 12 patients with non purulent sputum 3 (25%) had culture positivity. Totally culture positivity in 31 patients. 29 patients had monobacterial growth (93.5%) and 2 had polybacterial growth (6.5%). Out of the 2 mixed infections, one was *Klebsiella oxytoca* with *Acinetobacter baumannii*, and the other *Klebsiella pneumoniae* with *Pseudomonas aeruginosa*.

Bacteria isolated by sputum culture in AECOPD patients are given in (Chart I). Gram negative bacteria (97%) were predominantly isolated. Antimicrobial susceptibility pattern of Gram negative bacteria are shown in (Table I). ESBL production was phenotypically confirmed in 2/16 *Klebsiella pneumoniae* isolates (12.5%), 1/6 *Klebsiella oxytoca* isolates (16.7%). Thus 3 of 22(13.6%) of enterobacteriaceae isolates were ESBL producers. The single *Staphylococcus aureus* isolated was resistant to ciprofloxacin alone and sensitive to amikacin, penicillin, erythromycin, cotrimoxazole and cefoxitin. No MRSA was detected.

Correlation of clinical data of patients with their CRP level is shown in (Table II). 31 patients (62%) had a positive sputum bacteriological

profile and elevated serum CRP levels,11(22%) had elevated CRP but culture negative and 8(16%) had normal CRP and also culture negative. Patients with elevated CRP levels (cut-off value=

0.6mg/dl) showed higher mean serum CRP levels when associated with positive sputum bacteriological finding. This was statistically significant with p value < 0.001.

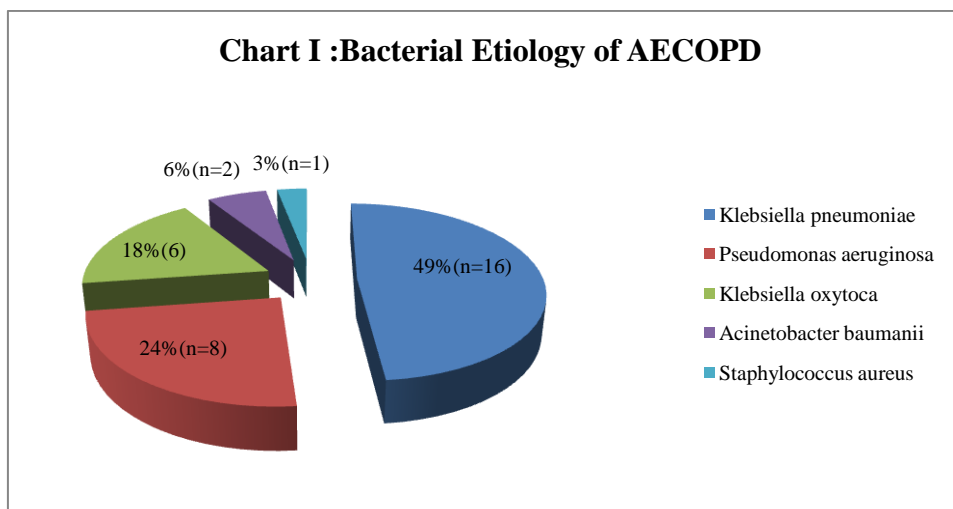


Table I: Antimicrobial Susceptibility Pattern of Gram Negative Bacilli

Antibiotic disc	Klebsiella pneumoniae (n=16)		Klebsiella oxytoca (n=6)		Pseudomonas aeruginosa (n=8)		Acinetobacter baumannii (n=2)	
	no	%	no	%	no	%	no	%
Amikacin	16	100%	6	100%	8	100%	2	100%
Ceftazidime	12	75%	3	50%	5	62.5%	1	50%
Cefotaxime	12	75%	3	50%	-	-	-	-
Cotrimoxazole	10	62.5%	2	33.3%	-	-	1	50%
Ciprofloxacin	16	100%	6	100%	3	37.5%	2	100%
Gentamicin	12	75%	4	66.7%	8	100%	2	100%
Piperacillin-Tazobactam	14	87.5%	4	66.7%	8	100%	2	100%
Imipenem	16	100%	6	100%	8	100%	2	100%

Table II: Clinical Data of Patients and their CRP

P-Positive N-Negative

Characteristic of patient	Elevated CRP n=42	Normal CRP n=8	p value
Age (Mean ±SD)	62.67±10.92	66.125±8.95	0.598
Sex M/F	30/12	4/4	0.409
Culture P/N	31/11	0/8	0.003
Smoking P/N	38/4	5/3	1
Sputum Purulence P/N	37/5	7/1	0.001

Discussion

This cross-sectional study had a sample size of 50 AECOPD patients. There was a predominance of males over females, with males accounting for 68% of the cases. This can be explained by the fact that men have pronounced smoking habits and are exposed more to the outside environment as compared to females. This was similar to a study done by Chawla K et al.^[5]

Smoking leads to decreased mucocilliary clearance and innate immunity thereby leading to increased bacterial colonization that can give rise to increased airway inflammation and thus exacerbation^[6]. In the present study smoking was associated with AECOPD in 86% of cases. This was similar to a study done by Jindal et al who stated that smoking is associated with COPD in 80-85% of cases^[7].

The AECOPD is further categorised as per Anthonisen criteria. Anthonisen criteria is based on 3 cardinal symptoms— increased sputum volume, increased breathlessness and increased sputum purulence.

There are 3 types of acute exacerbations which are based on the three cardinal symptoms:

- 1) Type 1: Severe exacerbation with all 3 cardinal symptoms
- 2) Type 2: Moderate exacerbation with 2 of 3 cardinal symptoms
- 3) Type 3: Mild Exacerbation with 1 of 3 cardinal symptoms plus 1 of the following:
 - a. Upper Respiratory Tract Infection in the past 5 days
 - b. Fever without other apparent cause
 - c. Increased Wheezing
 - d. Increased cough
 - e. Increased Respiratory rate or heart rate by 20% above baseline

According to Anthonisen criteria in this study, increased level of breathlessness was the commonest symptom in AECOPD (100%). Next most common symptom was increase in sputum volume (80%) and increase in sputum purulence (76%). This is in accordance with the study of N. Arora et al where 100% of patients had increase in sputum volume and 98.28% had various grades of dyspnoea.^[8] Type 1 exacerbation was the commonest (56%) type of AECOPD with all the three symptoms followed by Type 2(38%) exacerbations.

Out of the 50 sputum samples collected, 62% showed culture positivity and 38% showed normal throat commensals. Alamoudi OS et al obtained growth in 69.8% of sputum samples which is corresponding with our study^[9] A similar observation was made by Arora et al in which culture was positive in 72% cases.^[8]

In this study the correlation of sputum purulence with culture positivity was found to be statistically significant (p value =0.002). This correlates with the study done by Stockley RA et al^[10], Soler et al^[11] and Chawla et al^[5] who reported a culture positivity among 84%,84%, and 56% respectively

from purulent sputum samples of AECOPD patients. This proves that purulent sputum is the surrogate marker of bacterial infection as they yield positive bacterial cultures as compared to nonpurulent sputum.

Gerard Rakesh et al in their study on bacterial agents in AECOPD revealed 37% of monobacterial isolates and 5% of polybacterial isolates.^[12] In a study done by Chawla K et al^[5] monobacterial growth was found in most of the samples (92.85%) and polybacterial growth was found in 7.14%. This was corresponding to the present study.

The commonest organisms isolated were Gram Negative Bacteria (97%) as compared to Gram positive bacteria(3%). Niedermanmicheal S et al have mentioned in their study that Gram negative bacteria was the commonest bacteria isolated^[13]. Pradhan K.C et al reported *Klebsiella pneumoniae* as the predominant organism (40%)^[14] in his study which is similar to this study where *Klebsiella pneumoniae* was the most commonly (49%) and significantly isolated organism (p value<0.001) (Chart I) . In a study done by Chawla K et al^[5] *Pseudomonas aeruginosa* was the predominant isolate (25.92%) amongst the hospitalised patients followed by *Streptococcus pneumoniae* and *Acinetobacter baumannii* (18.51% each), *Klebsiella spp.* and *Moraxella catarrhalis*(14.8% each) Madhavi et al^[15] observed that the commonest isolate was *Klebsiella pneumoniae* (59%), followed by *Pseudomonas aeruginosa* 15%, *Staphylococcus aureus* 13.6%, *Streptococcus pneumoniae* 6.8% among AECOPD patients.

The antibiogram performed for various isolates were analysed (Table I). In *Klebsiella pneumoniae* resistance to cefotaxime/ceftazidime, cotrimoxazole and gentamycin was seen in 25%, 37.5% and 25% of isolates respectively. The other member of the enterobacteriaceae isolated was *Klebsiella oxytoca*. Resistance to cefotaxime/ceftazidime, cotrimoxazole and gentamycin was seen in 50%, 33%, and 66.7% isolates respectively. Studies performed by Madhavi et al observed resistance of *Klebsiella pneumoniae* to

cefotaxime 62%, ciprofloxacin 42% and cotrimoxazole 96%.^[15]

The frequency of ESBL producers among enterobacteriaceae was 13.6% amongst which 9.1% were *Klebsiella pneumoniae* isolates and 4.5% were *Klebsiella oxytoca* isolates. In a study done by Gerard Rakesh et al it was observed that 23.81% of *Klebsiella pneumoniae* were ESBL producers^[12].

The most common non-fermenter isolated from the sputum of AECOPD patients was *Pseudomonas aeruginosa*, which showed 37.5% resistance to ceftazidimide and 62.5% resistance to ciprofloxacin. Chawla et al^[5] reported a 60% resistance rate of *Pseudomonas aeruginosa* to third generation cephalosporins and fluoroquinolones. *Acinetobacter baumannii* isolates from AECOPD cases showed resistance to ceftazidimide 50% and cotrimoxazole 50%.

The age and sex of the patient showed no significant correlation with their serum CRP levels (Table II). This was in accordance with a previous study by Rajesh Gupta et al^[16].

No statistically significant correlation was found between smoking and CRP levels. This could be explained by the fact that cigarette smoking has a role in initiation of inflammatory process in COPD patients but it is not the leading cause of increased inflammatory markers and it should be noticed that not all cases develop inflammatory reaction following cigarette smoking and only some of them will show exacerbations following smoking. This reaction can be due to genetic differences^[17]. This was in accordance to a study done by Abdelsadek H et al.^[18]

CRP level had provided a reliable indicator of bacterial infection and some authors had reported CRP levels to be significantly elevated in patients with exacerbations of COPD and purulent sputum. This could be explained by the nature of COPD as a complex chronic inflammatory disease of the lungs involving several types of inflammatory cells and variety of inflammatory mediators. Although primarily affecting the lungs, the

chronic inflammatory process of COPD does have systemic repercussions.

Our study was compatible with the study by Dev et al^[19] and A.M Hussein et al^[20] in which, patients of AECOPD were split into 3 groups, two groups of patients showing elevated CRP levels of which one shows proven bacterial infection (by sputum culture – group I), the other in which there is no bacterial cause of infection (group II) and the third group which shows (group III) no deviation in CRP levels. Majority (62%) of the patients fell under Group 1 with a positive sputum bacteriological profile and elevated serum CRP levels in our study.

In our study Serum CRP levels showed significant correlation with sputum purulence and culture positivity, with 37 out of 44 patients with purulent sputum showing elevated serum CRP levels and 100% of those showing positive bacteriological profile of culture showed elevated CRP levels. (Table II)

At the time of acute exacerbation, the mean CRP level in patients whose sputum was negative for pathogens was 3.05 ± 1.46 mg/dl while in patients whose sputum yielded pathogens, it was 5.46 ± 2.75 mg/dl which is significantly higher (p value= 0.009). This is in accordance with the previous study by Arora et al.^[8]

Conclusion

The prevalence of bacterial infective aetiology of AECOPD was found to be 62%. Sputum purulence was found to be an important surrogate marker for culture positivity and will help in administering empirical antibiotic therapy. The commonest organisms isolated were Gram Negative Bacteria. ESBL production was detected in 13.6% of *Klebsiella* spp. isolates. Serum CRP levels showed significant correlation with sputum culture positivity. It can be used as an additional parameter to increase confidence about presence of bacterial pathogen in sputum and infective aetiology of AECOPD. Thus sputum bacteriology results and the antibiotic resistance pattern could provide guidance for the choice of empirical

antibiotic treatment for COPD patients admitted to the hospital with clinical evidence of airway infection such as increased sputum purulence and elevated serum CRP levels.

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