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BACTERIOLOGICAL STUDY OF ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Abstract

Background and objectives: Acute exacerbations are significant and frequent events in the natural history of Chronic Obstructive Pulmonary Disease (COPD). Majority of these exacerbations are of infectious etiology, bacteria are almost responsible for around 30-50% of cases. The purpose of this study was to determine the bacteriology of acute exacerbations of COPD in hospitalized patients in our institution and their antibiotic susceptibility pattern to formulate cost effective antibiotic strategy and reducing the emergence of drug resistance.

Methods: A prospective observational study was conducted among clinically diagnosed cases of acute exacerbations of COPD admitted in Medicine and Chest and Tuberculosis ward in Trichy SRM Medical College Hospital and Research Centre, Trichy. Direct gram stain was done for all sputum samples. The suitable sputum samples were cultured. Identification of organism and antimicrobial susceptibility testing was done by standard microbiological techniques.

Results: Significant growth of bacteria was obtained from 40.7% of sputum samples. Males (91.4%) were more affected than females (8.5%). The most common isolate was *Klebsiella pneumoniae* (39%), followed by *Pseudomonas aeruginosa* (29.7%), *Acinetobacter baumanii* (10.63%), *E. coli* (8.51%) and *Staphylococcus aureus* (7.8%). Drug sensitivity revealed that Gram negative isolates were sensitive to Levofloxacin (80.7%) followed by Amikacin (77.6%), Aztreonam (74.6%), Gentamicin (70.7%), Imipenem (68.4%) and Piperacillin-Tazobactam (66.9%).

Conclusion: Acute exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD) cases were common in males more than 50 years of age mainly due to smoking habits. Gram negative bacteria were more frequently isolated in our patients and appropriate antimicrobial therapy should be started early depending on the antimicrobial sensitivity results, in the wake of an increasing drug resistance.

Keywords: Acute exacerbations, Chronic Obstructive Pulmonary Disease, bacterial etiology, Antibiotic sensitivity.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a group of progressive debilitating respiratory condition, including chronic bronchitis and emphysema, which are characterized by cough, difficulty in breathing and limitations of airflow. Emphysema is a destruction and enlargement of the lung alveoli whereas chronic bronchitis is associated with chronic cough and phlegm along with narrowing of bronchioles of small airways[1]. Clinically, persistent reduction in forced expiratory flow rates is the most typical finding in COPD.

Exacerbations are a predominant feature in the course of COPD. Exacerbations are commonly considered to be episodes of increased cough and dyspnea and also change in the amount and character of sputum. Chronic Obstructive Pulmonary Disease (COPD), as defined by the Global Initiative for Obstructive Lung Disease (GOLD), is a disease state in which airflow limitation is not fully reversible and it is estimated that COPD will become the third leading cause of death worldwide by 2020[2]. Four to ten percent of adult male population of India and 3.5 to 6.5% population in Asia pacific countries suffer from this disease[3]. Males are more often affected than females. Cigarette smoking or inhalation of dust or fumes are important contributing factors.

The pathogens that have been associated with acute exacerbation of COPD by infecting the lower respiratory tract are mostly aerobic Gram positive bacteria like *Streptococcus pneumoniae* and Gram negative bacteria such as *Haemophilus influenzae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and also some of the respiratory viruses, and it is estimated that bacterial infections are almost responsible for more than 40% cases in India (Patel et al, Chawla et al)[4,5]. The prevalence of AECOPD varies from 1% in urban non-smoker to 21% in rural smokers and mortality rate has been increased to 30% if the patient is above 65 years and to 24% if patient is in Intensive care unit (Sharan,2015, Aleemullah, 2016)[6,3]. Several recent studies have reported the presence of multidrug-resistant (MDR) bacteria at hospital admission in patients with severe COPD exacerbations (ElKorashy et al,2014, Ewig et al,2000)[7,8]. Potential pathways by which bacteria could contribute to the pathogenesis of acute exacerbation include (1) primary infection of lower airways, (2) secondary infection of the airways after a primary viral infection, and (3) bacterial antigens inducing bronchial hyper reactivity and eosinophilic inflammation[9].

MATERIALS AND METHODS

A prospective observational study done at a tertiary care hospital for a total of 346 in-patients admitted with Acute Exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD) at Medicine and Chest and Tuberculosis ward between July to December 2016. All the sputum samples were processed at the Department of Microbiology, after getting Institutional Ethical Committee Clearance.

Inclusion criteria

Patients were diagnosed to have AECOPD depending upon the presence of any two of the following symptoms:

1. Increased cough
2. Increased purulence and/or volume of expectoration
3. Increased severity of dyspnea.
4. Fever and Leucocytosis.

Exclusion criteria

1.All cases who had evidence of pneumonia, lung abscess, bronchial asthma, lung cancer or bronchiectasis developed as a sequelae of other diseases, clinically or on chest radiography.
2. Those patients who were on antibiotic during the past one month.
3. All patients of AECOPD without fever and leucocytosis.

Specimen Collection and Transport

Early morning deeply coughed sputum sample was collected in a sterile wide mouth container, transported immediately and processed within 30 minutes.

Examination of Sputum

The quality of the sputum was assessed by both macroscopic and microscopic examination. Only mucoid or purulent samples (not the salivary samples) were subjected to Gram staining. According to Barlett's grading, on microscopic examination of sputum samples which showed 10-25/> 25 leucocytes (pus cells) and < 10 epithelial cells per 10x low power field were accepted for further processing. Sputum samples which do not fulfill these criteria were rejected. {Koneman, Text book of Diagnostic Microbiology,6th edition}[10]

Isolation of Bacteria from Sputum

For isolation and identification of organisms, the samples were inoculated into Mac Conkey agar, blood agar and chocolate agar plates and incubated at 37°C, while latter two plates were incubated in atmosphere containing 5% carbon dioxide. Plates were examined for growth after 24 and 48 hours of incubation. Culture isolates were identified according to standard techniques.

Antibiotic Susceptibility Testing

Antibiotic susceptibility test of the isolates was determined for the pathogenic organisms by Kirby-Bauer disc diffusion method on Muller Hinton agar plate, as described by Clinical and Laboratory Standard Institute guidelines (CLSI, 2016)[11].

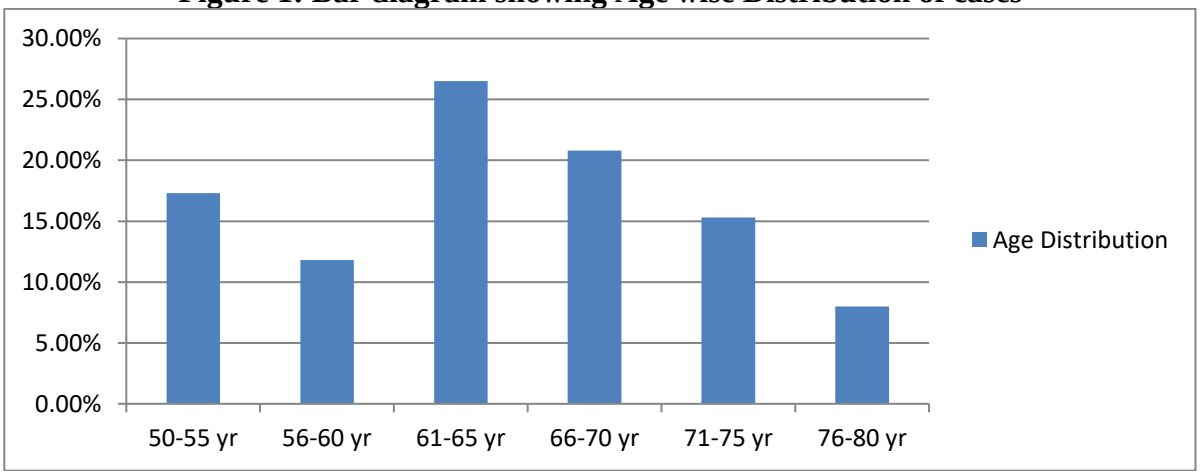
For Gram negative organisms, antibiotic discs used are –Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Cotrimoxazole (1.25/23.75µg), Amoxicillin-clavulanate (20/10µg), Piperacillin - Tazobactam (100/10µg), Imipenem (10µg), Ceftazidime (30µg), Ceftriaxone (30 µg), Cefepime (30µg) and Aztreonam (30µg).

For Gram positive organisms, antibiotic discs used are –Penicillin (10U), Ciprofloxacin (5µg), Gentamicin (10µg), Erythromycin (15µg), Clindamycin (2µg), Doxycycline(30µg), Cefoxitin(30µg), Teicoplanin(30µg), Vancomycin (E-strip) and Linezolid (30µg).

RESULTS

A total of 346 samples were included in the present study. Of them 299 (86.4%) were males and 47 (13.5%) females (M: F ratio 6.36:1). All males were smokers, while females were exposed to passive smoking or biomass fuel. All the 346 patients screened were between 50 to 80 years of age with maximum number (26.5%) being in the age group of 61 to 65 years.

Figure 1: Bar diagram showing Age wise Distribution of cases



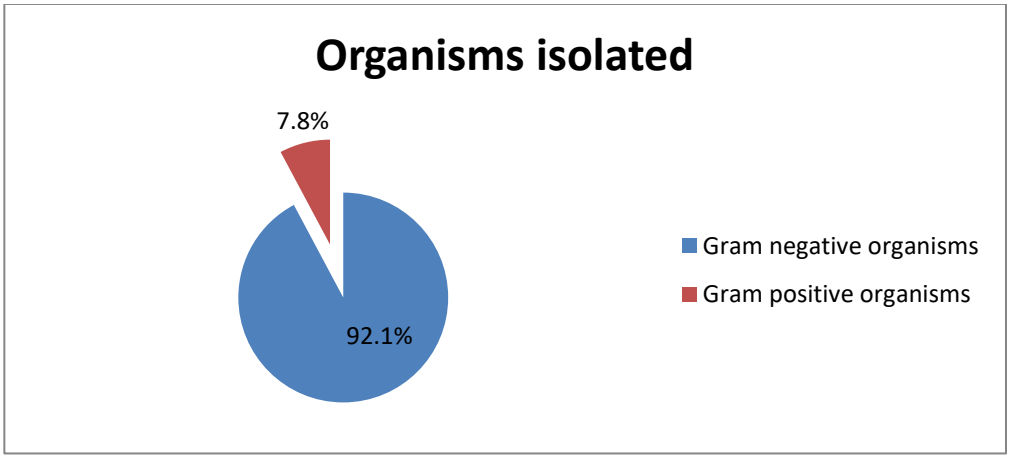
Of the 346 samples analyzed, 141(40.7%) showed significant growth of pathogenic bacteria. Among them, males (91.4%) were predominantly affected than females (8.5%).

Table 1: Profile of organisms isolated

Variables	Total number isolated	Percentage (%)
Normal flora (Polymicrobial/ Scanty growth)	205	59.3
Pathogenic bacteria	141	40.7

Single organism was isolated in most of the samples 95.7% (135/141) but in 4.2% cases (6/141) growth of two organisms (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) was noted.
In the present study, Gram negative organisms 92.1% (130/141) were isolated more than Gram positive 7.8% (11/141).

Figure 2: Pie diagram showing Gram negative vs Gram positive organisms among pathogenic bacteria



The most common Gram negative bacteria isolated in sputum culture was *Klebsiella pneumoniae* 55 (39%), followed by *Pseudomonas aeruginosa* 42 (29.7%), *Acinetobacter baumanii* 15 (10.63%), *E.coli* 12 (8.51%) and combination of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* 6 (4.2%). The only significant Gram positive bacterium isolated was Staphylococcus aureus 11 (7.8%) of which 4 were MRSA and 7 were MSSA based on Cefoxitin sensitivity.

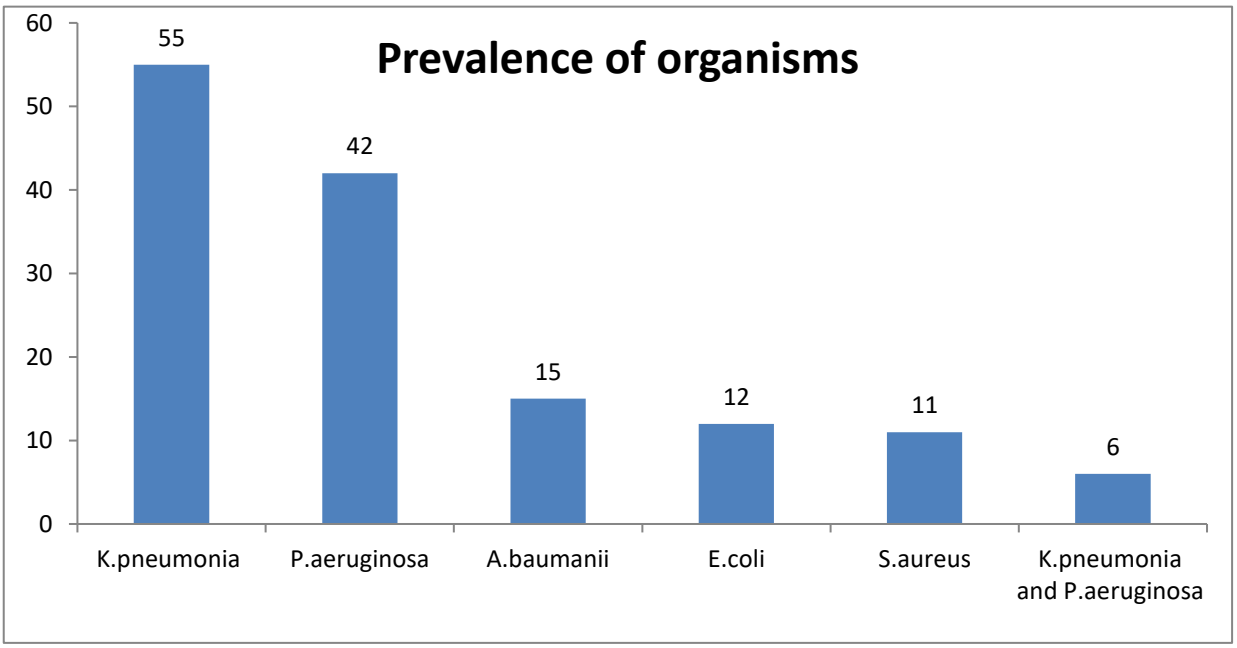


Figure 3: Bacteriological profile of patients with AECOPD

Among the Gram negative organisms, Levofloxacin showed the highest sensitivity (78.4%) followed by Amikacin (75.3%), Gentamicin (71.5%), Imipenem (70.7%) and Piperacillin-Tazobactam (67.6%).

Among MSSA patients in S.aureus, Doxycycline and Teicoplanin was 100% sensitive in all the patients whereas Erythromycin and Clindamycin were 85.7%, while Ciprofloxacin and Gentamicin were only 71.4% sensitive. In MRSA patients, Linezolid and Vancomycin was found to be 100% sensitive followed by 75% sensitivity to Gentamicin and Teicoplanin and only 25% sensitive to Erythromycin and Clindamycin.

Antibiotic Sensitivity pattern of isolates

Klebsiella pneumoniae, which was the most common isolate, was most sensitive to Amikacin (77.5%) followed by Gentamicin (74.1%), Levofloxacin (72.4%), Ciprofloxacin (65.5%) whereas Pseudomonas aeruginosa were 100% sensitive to most of the antibiotics.

Table 2. Sensitivity pattern of Gram negative organisms

	Klebsiella pneumonia {n=55+3 (58)}	Pseudomonas aeruginosa {n=42+3(45)}	Acinetobacter baumanii (n=15)	E.coli (n=12)
Amikacin	45(77.5%)	45(100%)	0	9(75%)
Ciprofloxacin	38(65.5%)	38(84.4%)	2(13.3%)	9(75%)
Levofloxacin	42(72.4%)	45(100%)	7(46.6%)	8(66.6%)
Gentamicin	43(74.1%)	45(100%)	0	8(66.6%)
Cotrimaxazole	20(34.4%)	NTˆ	0	4(33.3%)
Amoxyclavulanate	14(24.1%)	NTˆ	NTˆ	2(16.6%)
PiperacillinTazobactam	32(55.1%)	45(100%)	1(6.7%)	10(83.3%)
Imipenem	35(60.3%)	45(100%)	2(13.3%)	10(83.3%)
Ceftazidime	15(25.8%)	27(60%)	0	3(25%)
Ceftriaxone	15(25.8%)	NTˆ	NTˆ	4(33.3%)
Cefipime	17(29.3%)	27(60%)	0	3(25%)
Aztreonam	34(58.6%)	45(100%)	8(53.3%)	10(83.3%)

ˆNT- Not tested, as not recommended by CLSI.

Table 3. Sensitivity pattern of Gram positive organisms

Staphylococcus aureus (n=11)	MSSA (n=7)	MRSA (n=4)
Penicillin	0	0
Ciprofloxacin	5 (71.4%)	2 (50%)
Gentamicin	5 (71.4%)	3 (75%)
Erythromycin	6 (85.7%)	1 (25%)
Clindamycin	6 (85.7%)	1 (25%)
Doxycycline	7 (100%)	2 (50%)
Cefoxitin	7 (100%)	0
Teicoplanin	7(100%)	3 (75%)
Vancomycin	ND*	4 (100%)
Linezolid	ND*	4 (100%)

*ND – Not done

DISCUSSION

Bacterial infections are considered to be the most important cause of AECOPD. It is estimated that approximately 50 percent cases of AECOPD are of bacterial origin. As comparable with similar studies on AECOPD (Chakraborty et al, Madhavi et al, Saxena et al)[1,12,13] the age group of our study population affected with AECOPD was found to be above 50 years with more than 80 percent of the study population being males. A probable cause for this could be a much higher prevalence of chronic active smoking in males as well as prolonged exposure to smoking. The age group of patients ranged from 30-90 years. However, among them, maximum numbers of AECOPD cases (26.5%) were from 61 to 65 years. This is comparable with studies undertaken by Chakraborty et al. (61-65 years). Females on the contrary are much less exposed to active cigarette smoking although passive smoking

was found to be a possible cause for COPD in this population. Besides that, household smoke such as from biomass fuel was associated with many of the exacerbation in females.
In the present study, significant bacterial growth was found in 40.7 % of patients during exacerbation while in the remaining 59.3% of cases, the pathogenic organism could not be found and the reason may be due to inability to cultivate anaerobes, viral and mycological pathogens in our hospital and also similar results were obtained by Iyer et al[14] where 45% of bacterial pathogens from sputum was seen in patients with COPD during exacerbations. Culture positivity depends on nature of sputum, transportation time and the number of organisms present in the sample (Chawla K et al). In our study, the prevalence of Gram negative isolates was 92.1% as compared to 7.8 % of gram positive. Gram-negative bacilli were also the predominant organisms in studies done by Siripataravanit et al, Chakraborty et al and Vishwambar et al[15,1,16]. Only 6 patients out of 141(4.2%) were shown Mixed isolates, which is also similar to studies done by El Korashy et al and Saxena et al were 3.7% and 5% of cases were seen with mixed isolates respectively.

In our study, Klebsiella species was the predominant organism comprising of 39 % (55/141) which was in accordance with the study conducted by Saxena et al as well as Hariom Sharan whereas Pseudomonas aeruginosa was isolated in 29.7 % (42/141) of cases which is in contrary to the study conducted by Chawla et al where Pseudomonas aeruginosa was the most common isolate from the sputum of in-patients and the study by Anand K et al in which Streptococcus pneumonia was the commonest isolate[17].

In our study, 10 MDR and 6 ESBL strains of Klebsiella pneumoniae were obtained and overall it is mostly susceptible to Aminoglycosides, the sensitivity being 73.3% followed by ciprofloxacin (70.2%) and levofloxacin (69.5%) which is similar to the studies done by Viswambhar et al while it is discordant to the studies done by Saxena et al where they were sensitive to all drugs such as Imipenem, Meropenem, second and third generation of Cephalosporins while resistant only to Cotrimoxazole (65%).

In our study, Pseudomonas aeruginosa showed 100% sensitivity to Aminoglycosides and Levofloxacin, which is concordant with the study done by Madhavi et al. However, in a study done by Nseir et al[18], Pseudomonas spp. was found to be multi drug resistant. A total of 10.63% isolates of Acinetobacter baumanii were obtained and among them 86.5% was found to be multidrug resistant which is concordant with the study done by Ferrer et al[19]. E.coli showed 83.3% sensitivity to Carbapenem while 75% sensitive to both Amikacin and Ciprofloxacin which is similar to the study carried out by Chakraborty et al and also the resistance to Amoxicillin-clavulanate was found to be similar (83.3%).

In MSSA patients, Gentamicin and Ciprofloxacin showed 71.4% sensitivity, which is similar to the study done by Madhavi et al. As Vancomycin and Linezolid are given only for Methicillin or Oxacillin resistant isolates of S.aureus (MRSA strains), in our study they showed 100% sensitivity to both the drugs which is in accordance with the study done by Aleemullah et al, whereas it is dissimilar to the study done by Patel et al where they were 100% resistance to Linezolid.

LIMITATIONS

Anaerobic medium is not used for isolation of organisms.

CONCLUSION

Klebsiella pneumoniae is the most common organism responsible for AECOPD. In our study, overall, Levofloxacin has been found to show the best sensitivity amongst the Gram negative bacteria followed by Aminoglycosides, Carbapenems and Piperacillin-Tazobactam. As Carbapenems are reserve drugs, Levofloxacin, most preferably than Amikacin and Piperacillin-Tazobactam, can be used as the first empirical choice of antibiotic in treating Gram negative bacteria causing AECOPD.

Since, there is low rate of isolation of Gram positive isolate, no recommendation can be given. Hence, treatment depends on antimicrobial susceptibility pattern. Since the number of resistant strains is rising day by day, antimicrobial sensitivity pattern must be checked for the causative organism.

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