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Gram Negative Bacterial Pathogens and Their Sensitivity Pattern in Patients with Acute Exacerbation of COPD

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ABSTRACT

We studied 90 patients who were admitted due to acute exacerbation of chronic obstructive pulmonary disease. Aim of our study was to isolate bacterial pathogens in sputum culture of these patients and to find the sensitivity pattern of these pathogens.73 males, 17 females aged between 45 to 85 years were included inthis cross sectional study. The sputum specimen was collected using sterile sputum cups prior to empiricalantibiotic administration and subjected to Gram's stain, culture & sensitivity.Out of 90 cases 81% were males and 19% werefemales. The prevalence of Gram negativebacteria was 79% and Gram Positive bacterium was 21%. Pseudomonasaueroginosa was the commonest bacteria isolated (28%) followed by Klebsiellapneumoniae (16%). 79.55% of isolates were sensitive to a combination of Ciprofloxacin with gentamicin. The best single antibiotic was piperacillin with tazobactam(68.18%).Sensitivity to Amoxicillin with clavulanic acid was observed in 54.55% of isolates.As an empirical therapyin AECOPD patients the best antibiotic that can be started in our hospital would be Ciprofloxacin with gentamicin or cefotaxime withgentamicin. Amoxicillin with clavulanic acid is not very effective as resistance is high. Other best antibioticwould be pipericillin+tazobactam as monotherapy.

Keywords: COPD,Acute exacerbation,Bacterial pathogens,Sensitivity pattern.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause for morbidityand one of the principal causes of death worldwide [1].Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) isdefined as an acute change in a patient's baseline dyspnea, cough and/or sputum beyond day-to-day variability sufficient to warrant a change in therapy.Exacerbations of COPD have considerable impact on health care system at both primary and tertiary care levelsas they are the major reason for antibiotic use and admissions.Exacerbationslead to indirect costs because of days lost from work [2].COPD affects 30% of patientsseen in chest clinics and constitutes 1-25% of hospital admissions all over India[3].The disease is also associated with working and social incapacity and has tendencyfor repeated exacerbations, both infective and non-infective. Cigarette smoking orinhalation of dust or fumes are important contributing factors for acute exacerbation of COPD.

The role of bacterial infection in exacerbations of COPD and the useof sputum cultures to reach an etiological diagnosis to guide clinical managementare subjects of current debates. The role of infection in exacerbations of COPDremains controversial and incompletely understood. Although some investigatorsbelieve that bacteria are not important for patients with exacerbation, wedisagree and believe that patients with at least two of the three cardinal symptomsof exacerbation should receive antibiotic therapy with an open-mind.We reviewed the data, showing the bacteriologic studies, pathologicinvestigations, and clinical trials, all support role for bacteria and appropriate antibiotic therapy in AECOPD[4,5,6,7,8].Causes of exacerbation can be both infectious and noninfectious[4].Infectious causes arebacterial pathogens *Haemophilusinfluenzae*, *Streptococcus pneumoniae, Moraxella catarrhalis,Enterobacteriaceae*spp*., Pseudomonas* spp.Viral pathogens *Rhinovirus* spp*., influenza.*Non infectious causes are smoking, environmental conditions (low temperatures), air pollution exposure, lack of compliance with long-term oxygen therapy, failure to participate in pulmonary rehabilitation.

According to the ministry of health and family welfare, India has 17 millionpersons living with chronic obstructive pulmonary disease, a number that is estimated torise to 22 million by 2016.According to recent findings, there are 1.1 billion smokers globally, and around three billion people are exposed to biomass fuel.In a study conducted by Chest Research Foundation India, 40% of the COPD patients are non-smokers they were exposed to biomass fuel rather. The smoking associations with COPD were high from most countries *i.e.,* 2.65 in India, 2.57 in China and 2.12 in Japan. In a large, multicentric study from India, the population prevalence of COPD was4.1 per cent of 35295 subjects with a male to female ratio of 1.5:1. Almost all forms ofsmoking products such as cigarettes and 'beedis' used in different States were found to besignificantly associated with COPD[3].

In non-smokers, especially women, exposures to indoor air pollution fromdomestic combustion of solid fuels was an important factor. More significantly theexposure to environmental tobacco smoke (ETS) was an established cause for COPD.The odds ratio for risk from ETS exposure in non-smokers (1.535) was on significant riseduring both the childhood and

the adulthood. On an average, an Indian COPD patientspent about 15 percent of his income on smoking products and up to 30 per cent ondisease management. Tobacco smoking was also the most frequent cause of chronic cor-pulmonale which occurred as a long term complication of COPD both amongst men andwomen.Patients with COPD show significant impairment of their lung defencemechanisms. The effects of tobacco smoking on the ciliated bronchial epithelium and excessive production of mucus hamper normal drainage of secretions. In addition, impairment of the phagocytic function of macrophages and neutrophilsexists, which makes it difficult to eliminate microorganisms that may reach thelower airways.

Studies performed using specific invasive techniques have shown that boththe number of patients with pathogenic bacteria in their respiratory secretions andthe number of colony-forming units of such bacteria increase duringexacerbations. Furthermore, the local inflammatory response of the host increaseswith increasing airway bacterial load[5].Current knowledge indicates that thepresence of green (purulent) as opposed to white (mucoid) sputum is one of thebest and easiest methods of predicting a high bacterial load in respiratory tract secretions and the need for antibiotic therapy[6] . The production of green sputum isa surrogate marker for exaggerated bronchial inflammation associated with thepresence of bacterial pathogens in increasing concentrations [7].It can be speculated that, for symptoms of acute exacerbation to appear, there must be a minimum bacterial load in the airways, a threshold above which the inflammatory reaction is severe enough to elicit clinicalsymptoms of exacerbation. This threshold may be difficult to establish and may varyfrom patient to patient owing to different modifying factors decreased FEV1 hasbeen shown to correlate with greater neutrophilic inflammation in BAL fluid. Thisis related to the common observation that patients with more severe impairment oflung function suffer greater number of exacerbations.In acohort of 1,016 severeCOPD patients, infection was the cause of51% ofexacerbations,whereas heart failurewasthe second commonestcause with26% ofcases; however, in asmuch as 30% of cases,thecause was unknown [8]. Amongthese cases of unrecognisedcause,the importance ofenvironmental factorssuchas lowtemperature[9]and airpollution[10]must be stressed.

Bronchial hyperresponsiveness may also have additive effects with bacterial colonisation, and hyperresponsive patients may react with exaggerated bronchial obstruction and respiratory symptoms. By favouringcolonisation, chronic mucus hypersecretion may facilitate the growth of bacteria and rapid achievement of a colony forming units concentration above the threshold Impairment of host defences. Any impairment in host defences, either locally within the bronchial mucosa orsystemically, such as impairment of antibody responses, may facilitate bacterialadhesion and faster growth. Elderly patients or patients with significant comorbid conditions may feel worse with lower levels of bronchial inflammation and thus thethreshold for these patients may be lower[11]. Several potential contributions of bacterial infection to the etiology, pathogenesisand clinical course of COPD can be identified. Three classes of pathogens have beenimplicated as causing acute exacerbation of COPD by infecting the lower respiratory tract[4]. They are respiratory viruses, atypical bacteria,aerobic Gram-positive andGram-negative bacteria.The relative contributions of these three different classes of pathogens may change depending on the severity of the underlying obstructive airway disease. Such changes may also happen within a class, especially for bacterial pathogens.In the

last decade with the increasing use of fiber optic bronchoscopy, newer sampling methods like tracheobronchial aspirated sample, broncho alveolar lavage fluid, and protected specimen brushing have emerged.This has renewed interest in the area of bacteria and COPD, and this should lead to a precise delineation of the contribution of bacterial infection to the disease.

The precise role of bacteria remains controversial as determining the contribution of bacteria to exacerbations is difficult as COPD patients are often colonised withbacteria even when clinically stable. Studies using bronchoscopic sampling have isolatedbacteria from 50% of patients during an exacerbation, but also from 25 -30% of stablepatients. In 40% of COPD exacerbations in which the three symptoms of increaseddyspnoea, sputum purulence and sputum volume were present and there was a significantbenefit of antibiotics over placebo[4].

MATERIALS AND METHODS

This is a hospital based cross sectional study, conducted over a period of 1year, comprising of 90 patients diagnosed withacute exacerbation of COPDfrom A.J. Institute of Medical Sciences Hospital Mangalore. Patients admitted with acute exacerbationof COPD were included in this study wherein the sputum culture and sensitivity was sent prior to starting the antibiotictreatment.Only in patients were organisms were isolated and sensitivity testing was done were included in the study. The exclusion criteria are known case of Pulmonary tuberculosis, all cases who had evidence of pneumonia or bronchiectasis, bronchial asthma,lung abscess, lung cancer, patients who were already taking antibiotics before selection, patients with Ischemic heart disease, cases of Acute exacerbation of COPD not yielding organisms on culture.

Two early morning sputum samples were collected in sterile containers fromall patients after rinsing the mouth twice with plain water. Patients were instructed to collect sputum after taking a deep breath and cough out the sputum into a sterilewide mouth container with a screw cap.Samples were labeled and numbered andprocessed in the laboratory by conventional methods.After culture depending on the organism isolated sensitivity testing was done.

Sensitivity to following antibiotics was tested:ciprofloxacin, levofloxacin, ceftriaxone, cefotaxime, ceftazidime,cefepime,amoxicillin+clavulunate, piperacillin+tazobactam, cefoperazone+sulbactam,gentamicin, amikacin, erythromycin, azithromycin ,amoxicillin, penicillin.

Statistical Analysis

The data was entered into the Microsoft Office Excel 2007 and analysiswas done by chi square test using Statistical Package for Social Sciences (SPSS) version 17.P value of ≤0.05 was considered statistically significant.

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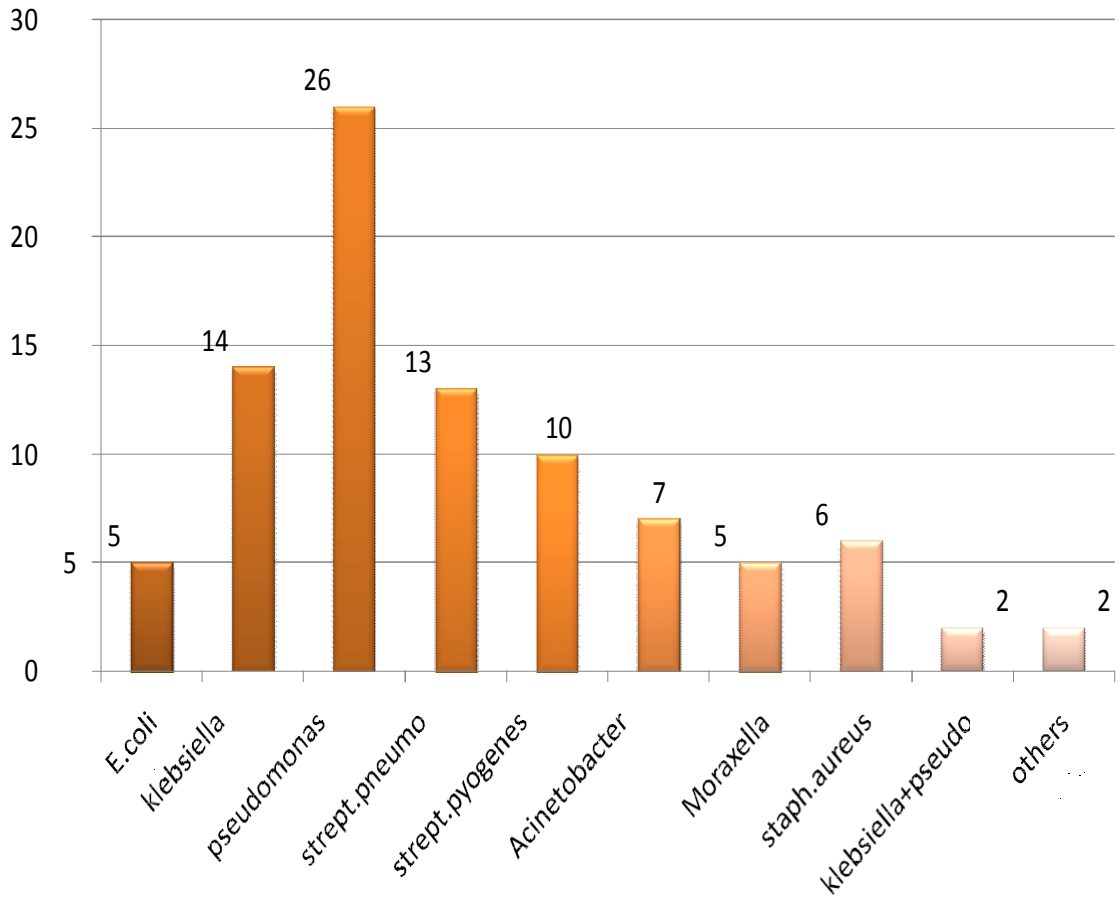
RESULTS

A total of ninety (90) patients, diagnosed as cases of acuteexacerbation of chronic obstructive pulmonary disease were studied.Bacterial infections of AECOPD were analyzed. The individual bacterial isolatesand their culture & sensitivity patterns to various antibiotics were recorded.

Bacteriological profile: Out of ninetycases pathogenic bacteria isolated, eighty six cases (86) had singlemicrobial infections and four (4) had polymicrobial infections.On gram staining 61 organisms(68%) were gram positiveand 29 organisms(32%) were gram negative.

The 3 commonest organisms isolated in our study were pseudomonas aeruginosa in 26 cases, klebsiella pneumonia in 14 cases, and streptococcus pneumonia in 13 cases.



Graph 1: SHOWING THE COMMONEST OCCURANCE OF GRAM NEGATIVE ORGANISM IN THE STUDY

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Table1: ANTIBIOTICSENSITIVITYPATTERNOFPSEUDOMONAS

ANTIBIOTICS	SENSITIVE	RESISTANT	TOTAL
CEFOTAXIME	11(58%)	8(42%)	19
CEFTRIAXONE	10(55%)	8(44%)	18
CEFTAZIDIME	7(70%)	3(30%)	10
CEFEPIME	6(86%)	1(14%)	7
LEVOFLOXACIN	24(92%)	2(8%)	26
CIPROFLOXACIN	22(85%)	4(15%)	26
AMOXYCLAV	4(23%)	14(77%)	18
PIPPERACILLIN+ TAZOBACTUM	22(91%)	2(9%)	24
CEFAPERAZONE+SULBACTUM	2(100%)	0(0)	2
GENTAMICIN	24(89%)	2(11%)	26
AMIKACIN	0(0)	4(100%)	4
PENICILLIN	0(0)	11(100%)	11
ERYTHROMYCIN	0(0)	10(100%)	10
AMOXYCILLIN	0(0)	13(100%)	13

Pseudomonas auroginosa was isolated in a total of 26 subjects. Table 1 shows Levofloxacin andCiprofloxacin were used in all the culture and sensitivity of all subjects and their sensitivity was 92% & 85%respectively. P=0.033 (P ≤0.05 is statistically significant,Confidence Interval 95%). Piperacillin+tazobactum was used in 24 cultures and its sensitivity was 91%. Gentamicin was used in 24 cultures and its sensitivity was 89%. The sensitivity forcephalosporins ranged from 55% -86%(cefepime). Amoxicillin was used in 13 culturesand it was resistant in all cultures.

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Table2:ANTIBIOTICSENSITIVITYPATTERNOFKLEBSIELLA

ANTIBIOTICS	SENSITIVITY(%)	RESISTANT(%)	TOTAL
CEFOTAXIME	10(60%)	4(40%)	14
CEFTRIAXONE	7(50%)	7(50%)	14
CEFTAZIDIME	4(44%)	5(55%)	9
CEFEPIME	6(86%)	1(14%)	7
LEVOFLOXACIN	10(91%)	1(9%)	11
CIPROFLOXACIN	11(79%)	3(21%)	14
AMOXYCLAV	4(33%)	8(67%)	12
PIPPERACILLIN+ TAZOBACTUM	8(80%)	2(20%)	10
CEFAPERAZONE+SULBACTUM	6(86%)	1(14%)	7
GENTAMICIN	12(92%)	1(8%)	13
AMIKACIN	9(90%)	1(10%)	10
AZITHROMYCIN	0(0)	1(100%)	1
PENICILLIN	0(0)	5(100%)	5
ERYTHROMYCIN	0(0)	4(100%)	4
AMOXYCILLIN	0(0)	7(100%)	7

Klebsiella was isolated in a total of 14 subjects. Table 2 shows the culture and sensitivity of klebsiella is most susceptible to Aminoglycosides sensitivity being 92% (p=0.0001,CI 95%) followed by ciprofloxacin and levofloxacin at 79%&91%, respectively. p=0.013 (P ≤0.05 is statistically significant,Confidence Interval 95%). The best among the cephalosporins was cefepime with 86% sensitivity. The newer antibiotic combinations of pepperacillin+tazobactum&cefoperazone +sulbactum showed a sensitivity of 80% & 86% respectively

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Table 3:ANTIBIOTIC SENSITIVITY PATTERN OF STREPTOCOCCUSPNEUMONIA

ANTIBIOTICS	SENSITIVE	RESISTANT	TOTAL
CEFOTAXIME	13(100%)	0	13
CEFTRIAXONE	13(100%)	0	13
CEFTAZIDIME	1(100%)	0	1
CEFEPIME	3(100%)	0	3
LEVOFLOXACIN	8(89%)	1(11%)	9
CIPROFLOXACIN	8(80%)	2(10%)	10
AMOXYCLAV	10(77%)	3(23%)	13
PIPPERACILLIN+ TAZOBACTUM	9(100%)	0	9
CEFAPERAZONE+SULBACTUM	10(100%)	0	10
GENTAMICIN	12(93%)	1(7%)	13
AMIKACIN	9(100%)	0	9
AZITHROMYCIN	6(55%)	4(45%)	10
PENICILLIN	2(15%))	11(85%)	13
ERYTHROMYCINN	3(37%)	5(63%)	8
AMOXYCILLIN	8(61%)	5(39%)	13

Streptococcus was isolated in a total of 13 subjects. Table 3 shows that 100% of Streptococcus were sensitive to 3rd generation cephalosporins P=0.0001.(P ≤0.05 is statistically significant,Confidence Interval 95%) 89% of streptococci were susceptible to Levofloxacin. 80% of streptococci were susceptible to ciprofloxacin. 77% of streptococci were susceptible to Amoxyclav. 100% of streptococci were susceptible to pepperacillin+tazobactum. 93% of streptococci were susceptible to Aminoglycosides. 61% of streptococci were susceptible to Amoxicillin.

DISCUSSION

The 3 commonest organisms isolated in our study were pseudomonasaeruginosa in 26 cases, klebsiella pneumonia in 14 cases, and streptococcuspneumonia in 13 cases.According to western literature the causative organisms for theacute exacerbation ofCOPDwere H.influenza, strept.pneumonia, pseudomonas aeruginosa.In a study by HallettWibur [12]streptococcus pneumoniae and H.influenza werepredominant.In a study by Eller Jorg, Anja Ede et al[13]showed that the predominant organismcausing AECOPD include strept.pneumonia, non

typableH.influenza and to some extentMoraxella.In a study by De Abate Andrew C., Dan Henry et al[14]shows that thepredominant causative pathogens in AECOPD include H.influenza, para influenzaand Moraxella.In a study by Miravitlles Marc, Cristina Espinosa et al[15]shows H.influenza,pseudomonas and streptococcus pneumonia as the most common organisms causingAECOPD.A study done by Hui DS, Ip M, et al[16]shows that gram-negative bacteriaincluding Klebsiellaspp, P. aeruginosa and Acinetobacter spp. constitute a largeproportion of pathogens identified in with AECOPD in some Asian countries.Surveillance on the local prevalence and antibiotic resistance of these organisms isimportant in guiding appropriate choice of antimicrobials in the management of AECOPD.

In contrast to western literature Indian Literature review shows no isolates ofH. influenza in AECOPD patients[16].In a study by Pradhan K.C et al[17] in 100 cases, shows klebsiella to be themost predominant followed by staphylococcus aureus and pseudomonas.In a study by kamatsr et al[18] showed staphylococcus aureus, streptococcuspneumoniae, and klebsiella were most predominant organisms.A study conducted by Arorausha et al[19]shows the predominant organism isolatedin AECOPD were staphylococcus aureus ,pseudomonas, streptococcus pneumonia andklebsiella.In our study of 90 patients the most predominant organisms causing AECOPDwere gram negative organisms mainly pseudomonas and klebsiella.There were no isolates of H.influenza.Pseudomonas aeruginosa was isolated in 26(29%), klebsiellapneumoniae in14(15%). This is in similarity with most of the Indian studies that show a predominance ofgram negative organisms.

Bacterial isolates in Acute exacerbation of COPD depend on severity of COPD,severity of exacerbation, prior antibiotic therapy[19].In mild and moderate COPD and in cases were prior antibiotic is sparingly used Streptococcus pneumonia,Moraxella cataralis and H.Influenza are the common isolates.As the severity of COPD increases and when patient receives repeated antibiotics, and in severe exacerbations gram negative organisms-pseudomonas and klebsiella are the common isolates[13].

Bacterial isolates in acute exacerbation of COPD may vary depending on the geographical area,prevalence of bacteria in the community and hospital setting in particular geographical area,antibiotics used in the hospital and at the community level.Hence it is important to carry out hospital based as well as community based studies to determine the empirical antibiotic therapy as appropriate early antibiotic therapy in acute exacerbation of COPD will decrease the morbidity and mortality in these patients.

Antibiotics have to be started empirically to treat the presumed bacterialinfection in AECOPD.Aminopenicillins like ampicillin and amoxicillin were formerly the standardtreatment in AECOPD. Due to emergence of resistance among respiratory pathogenstheir utility had been limited.Aminopenoicillins with beta lactamase inhibitor is a better choice.Cephalosporins demonstrated clinical efficacy and tolerability that can surpass thestandard aminopenicillins[13].

The quinolones like ciprofloxacin exhibit a broad spectrum of activity thatincludes both gram positive and gram negative organisms causing AECOPD.Ciprofloxacin was proven to be better than the newer quinolones in treating pseudomonasinfection[14].In our study we found that aminopenicillins were not effective against bothklebsiella and pseudomonas.Klebsiella was sensitive to a combination of ciprofloxacin and gentamicinsensitivity being 85% or levofloxacin and gentamicinsensitivity being 92%.Pseudomonas aeruginosa was sensitive to a combination of ciprofloxacin andgentamicin sensitivity being 87% or a combination of levofloxacin and gentamicin withsensitivity of 91%.

In a study done by Moellering, R. C they found that Aminopencillins witbeta-lactamase inhibitors were better than aminopencillins alone but were noteffective in controlling severe infection in AECOPD due to beta lactamse producingstrains[20].A study done by Vogel, F Cephalosporins have demonstrated clinical efficacyand tolerability that compare well with or surpass those of the standardaminopenicillins with or without a beta-lactamase inhibitor[21].
A Study by sanjaysethi[4]shows that the ciprofloxacin has excellent efficacyagainst the gram negative organisms.

Intravenous administration of third generation cephalosporins and ciprofloxacin[22]were the best antibiotics for treating less severe AECOPD patients empirically. Most ofthe organisms were susceptible to these antibiotics in our study also.In severe infections as the organisms causing were likely to be gram negativeorganisms, a combination of ciprofloxacin with gentamicin is the best antibioticcombination, alternatively a combination of intravenous third generation cephalosporinwith gentamicin can be used.But we have not correlated the severity with the organism isolated but it ispossible that majority of our patients had severe or very severe COPD as we hadincluded patients who needed hospital admission.Hence in future studies correlation with the severity of COPD, priorantibiotic use, comorbid illness needs to be correlated with the organism isolated.The newer antibiotics like pipericillin+tazobactam and cefoperazone+sulbactamwere very effective in treating very severe exacerbations of COPD. Routine use ofthese has to be limited to prevent the emergence of resistanceStill, many questions remain, and future studies will be needed tobetter define the mechanisms of bacterial invasion in the COPD patients and todevelop effective vaccines to prevent exacerbation. In the meantime, we must rely onantibiotic therapy, and we will need prospective studies to corroborate preliminaryfindings showing that different patients may require different therapies; thus,patient subsetting may be vital in the selection of antibiotic therapy forexacerbations of COPD.

CONCLUSION

The commonest organisms causing acute exacerbation of COPD in our study weregram negative organisms. Most common gram negative organisms isolated were pseudomonas aeruginosa followed by klebsiella pneumonia. They were sensitive to ciprofloxacin and genatmicin. So initial empirical anti biotic therapy canbe started with a combination of Ciprofloxacin or Levofloxacin with gentamicin in our hospital. In our study most of the organisms were resistant to Amoxicillin, Ampicillin, &Co-amoxyclav. Hence these antibiotics

may be avoided in the initial empiricaltherapy.Single antibiotic therapy either with ciprofloxacin or intravenous cefotaximecan be given if the exacerbation is not severe.

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