Original Article

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Aerobic Bacteriological Study of Acute Exacerbations of Chronic Obstructive Pulmonary Disease

Microbiology Section

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ABSTRACT

Background: The natural history of chronic obstructive pulmonary disease is characterized by frequent exacerbations. Majority of exacerbations are infectious and bacteria responsible for 30-50% of these cases. The purpose of this study was to determine the bacteriology of acute exacerbations of chronic obstructive pulmonary disease in hospitalized patients in our institution and their antibiotic susceptibility pattern to formulate cost effective antibiotic strategy and reducing the emergence of drug resistance.

Materials and Methods: One hundred and seven clinically diagnosed cases of acute exacerbations of chronic obstructive pulmonary disease admitted in medicine, tuberculosis and chest wards were selected for the study. 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: Our study showed growth of pathogenic organisms in 41.12% cases. Males (67.29%) are more affected than females (32.71%). Gram negative bacilli were more isolated than gram positive cocci. The commonest isolate was *Klebsiella pneumoniae* 15 (38.46%), followed by *Staphylococcus* aureus 9 (23.08%), *Streptococcus* species 6 (15.39%), *Pseudomonas aeruginosa* 4 (10.26%), *E.coli* 2 (5.13%), *Acinetobacter* species 2 (5.13%) and *Enterobacter* species 1(2.56%). The antibiotic susceptibility reveals that vancomycin, linezolid, azithromycin and clarithromycin were most effective drugs for gram positive cocci, meropenem & piperacillin-tazobactam for gram negative bacilli and amikacin & levofloxacin for both gram positive cocci & gram negative bacilli.

Conclusion: In developing country like India acute exacerbations of chronic obstructive pulmonary disease is common in adults more than 50 years of age due to smoking habits and high indoor pollution. This leads to a major impact on the quality of life of patients with the condition. They are a major cause of hospital admission and health care utilization.

Keywords: Bacterial aetiology, COPD, Antibiotic sensitivity pattern

INTRODUCTION

Acute exacerbation of COPD (AECOPD) is defined as a sustained worsening of the patient's condition, from the stable state and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD [1].

The prevalence of AECOPD varying from 1% in urban non-smoker to 21% in rural smokers [2] and mortality rate of 24% if the patient required ICU admission. This mortality rate increased to 30% if the patient was above 65 years [3].

The clinical guidelines have included Winnipeg criteria, which are based on increased breathlessness, sputum purulence and sputum volume, to diagnose the patients and grade the severity of acute exacerbations of chronic obstructive pulmonary disease.

The Winnipeg criteria

Type of Exacerbations Criteria

Type 1	All the 3 symptoms.
Type 2	Any 2 symptoms.
Type 3	Any 1 symptom plus at least 1 of the following: Upper respiratory tract infection lasting ≥ 5 days, fever, increase in wheezes, increase in cough and increase in heart rate 20% [4].

Three classes of pathogens have been implicated as causing acute exacerbation of COPD by infecting the lower respiratory tract: respiratory viruses (Influenza, Para influenza, Rhinovirus, Corona virus, Adenovirus & RSV), atypical bacteria (*Mycoplasma pneumoniae* & *Chlamydia pneumoniae*) and aerobic gram positive & gram negative bacteria. The variable bacteriological profile in poor lung function gives evidence that they are involved in the progression of the disease.

Early diagnosis and knowledge of local bacteriological profile & antibiogram help us to reduce the number of failure cases recorded with empirical treatment during AECOPD. The present work was done to find out the aerobic bacteria and their antibiotic sensitivity pattern in AECOPD as very little reports are available from India.

MATERIALS AND METHODS

One hundred and seven patients (51, 30 & 26 subjects were type 1, 2 & 3 respectively) of AECOPD admitted in medicine, tuberculosis and chest wards over a period of 6 months from 1st October 2014 to 31st March 2015 were selected for the study, after permission of the ethical committee of our institute.

Variables included for the study were age, sex, smoking, immunization for Haemophilus influenazae & *Streptococcus pneumoniae*, signs and symptoms of the patient. The information regarding these variables was collected by using a pretested questionnaire.

Inclusion criteria: All clinically diagnosed cases of AECOPD formed the subject of the study group.

Exclusion criteria: Patients admitted in emergency department, attending out patient department, sign & symptoms suggestive of coronary thrombosis, localized suppurative infection in the lung, allergic origin pulmonary disease and use of antibiotics before hospitalisation were not included in the study.

Specimen collection: Early morning samples were obtained from cases that were clinically diagnosed as AECOPD. Patients

were instructed to collect deep coughed sputum into a sterile wide mouth container with a screw cap after rinsing the mouth twice with plain water.

Specimen transport: The samples were brought to Microbiology laboratory of Sri Aurobindo Medical College and Post Graduate Institute Indore, immediately and processed within 30 minutes of collection.

Culture methods: Direct gram stain was done from sputum sample and reported according to Bartlett's grading system. A score of 1 and above was considered suitable sample [5]. The suitable sputum samples were inoculated onto Mac Conkey's agar, chocolate agar and two blood agar plates. On one blood agar streaking with *Staphylococci* was done to facilitate growth of *Haemophilus influenzae*. All the plates were incubated at 37°C for 24 hours in 7-10 % CO_2 concentration. The isolated organisms were identified by standard microbiological techniques [6]. All the isolates were tested for antimicrobial susceptibility (Hi-Media Mumbai) by Kirby-Bauer disk diffusion method on Mueller-Hinton agar [7].

RESULTS

A total of 107 cases were included in the present study, 72 (67.29%) were males and 35 (32.71%) females (M:F ratio 2.06:1). Amongst them 43 (40.19%) were aged 51-60 years and 3 (2.80%) were aged more than 80 years [Table/Fig-1]. Out of 72 males, 45 (62.5%) were smokers and 27 (37.5%) were non-smokers. None of the patients had received immunization for *Haemophilus influenae* or *Streptococcus pneumoniae* in the past.

Gram staining findings were noted in 90.65% (97/107) cases. Growth of pathogenic organisms was obtained in 41.12% (44/107) cases.

Age group	Males		Females		Total	
	No.	%	No.	%	No.	%
41-50 years	7	9.72	6	17.14	13	12.15
51-60 years	30	41.67	13	37.14	43	40.19
61-70 years	24	33.33	11	31.43	35	32.71
71-80 years	9	12.50	4	11.43	13	12.15
> 80 years	2	2.78	1	2.86	3	2.80
Total	72	100	35	100	107	100

[Table/Fig-1]: Age & Sex wise distribution

Monomicrobial growth yielded in 88.64% (39/44) and polymicrobial in 11.36% (5/44) sputum samples. Among the 39 single pathogenic microbial growth gram negative bacilli were more isolated (24/39) than gram positive cocci (15/39). Out of 39 monomicrobial growths, *Klebsiella pneumoniae* was the commonest bacteria 38.46% followed by *Staphylococcus aureus* 23.08%, *Streptococcus* species 15.39%, *Pseudomonas aeruginosa* 10.26 %, *E.coli* 5.13%, *Acinetobacter* species 5.13% & *Enterobacter* species 2.56% cases. Out of 44 sputum samples 5 yielded polymicrobial growths. The pattern of polymicrobial was *Staphylococcus aureus* with *Candida albicans*, *Klebsiella pneumoniae* with *Citrobacter freundii*, *Klebsiella pneumoniae* with *E.coli*, *Klebsiella pneumoniae* with *Staphylococcus aureus*, *Klebsiella pneumoniae* with *Streptococcus pneumoniae* in each case. It was observed that purulent or mucopurulent sputum gave better isolation of pathogens than mucoid or mucosalivary sputum.

Klebsiella pneumoniae which was the most common isolate, were mainly sensitive to amikacin, meropenem & levofloxacin. *Staphylococcus aureus* which was the prevalent gram positive isolate, were sensitive to vancomycin, linezolid, amikacin, clarithromycin, cefotaxime & levofloxacin. 44.44% (4/9) of *Staphylococcus aureus* were methicillin-resistant (MRSA). *Streptococcus* species were sensitive to levofloxacin, linezolid, vancomycin, azithromycin & clarithromycin. *Pseudomonas aeruginosa* were mainly sensitive to amikacin, meropenem & polymyxin B. *E.coli* were sensitive to amikacin and meropenem [Table/Fig-2].

DISCUSSION

It was observed that AECOPD prevalent in 41-80 years age group. However among them, 51-70 years age group constituted 73%. Thus, AECOPD was common in advance age group as respiratory tract is more susceptible due to impairment of immunological defence mechanism, associated co-morbid illness, increased duration of seasonal variation & tobacco smoking. Males were affected more than females because they were more involved in smoking & start it in younger age group, therefore more chance of inhalation and increased environmental exposure or temperature variation [8]. In non-smokers, especially among women, exposure to indoor air pollution was an important factor [2].

In our study gram staining findings were in correlation with the culture findings in 90.65% cases; therefore it remained a time honoured

Antibiotics	Conc.	Staph.	Strept.	Kleb.	Pseudo.	*E.coli*	Acinet.	Entero
	µg	N=9(%)	N=6(%)	N=15(%)	N=4(%)	N=2(%)	N=2(%)	N=1(%)
Amikacin	30	9(100)	NT	13(86.67)	4(100)	2(100)	2(100)	1(100)
Amoxy-clavulanic acid	20/10	6(66.67)	NT	9(60)	NT	1(50)	NT	0
Azithromycin	15	7(77.78)	5(83.33)	NT	NT	NT	NT	NT
Cefotaxime	30	8(88.89)	NT	6(40)	NT	1(50)	1(50)	1(100)
Ceftazidime	30	NT	NT	5(33.33)	1(25)	0	0	0
Clarithromycin	15	8(88.89)	5(83.33)	NT	NT	NT	NT	NT
Ciprofloxacin	5	3(33.33)	NT	5(33.33)	0	0	0	0
Cotrimoxazole	23.75/ 1.25	3(33.33)	1(16.67)	1(6.67)	NT	0	0	0
Gentamicin	10	5(55.56)	NT	7(46.67)	2(50)	1(50)	1(50)	0
Levofloxacin	5	8(88.89)	6(100)	12(80)	3(75)	1(50)	1(50)	1(100)
Linezolid	30	9(100)	6(100)	NT	NT	NT	NT	NT
Ofloxacin	5	5(55.56)	4(66.67)	7(46.67)	1(25)	1(50)	NT	0
Piperacillin-Tazobactam	100/ 10	NT	NT	11(73.33)	2(50)	1(50)	1(50)	1(100)
Polymyxin –B	300 Units	NT	NT	NT	4(100)	NT	NT	NT
Meropenem	10	NT	NT	15(100)	4(100)	2(100)	2(100)	1(100)
Vancomycin	30	9(100)	6(100)	NT	NT	NT	NT	NT

[Table/Fig-2]: Antibiotic sensitivity pattern of bacteria
Staph.=*Staphylococcus aureus*, Strept.=*Streptococcus species*, Kleb.=*Klebsiella pneumoniae*, Pseudo.=*Pseudomonas aeruginosa*, *E.coli*=*Escherichia coli*, Acinet.=*Acinetobacter* species, Entero=*Enterobacter* species, NT= Not Tested

method for sputum samples. Aerobic culture positivity was 41.12% which is similar to other study [9]. The prevalence of gram negative isolates was 61.54%, as compared to 38.46% of gram positive isolates corresponding to other studies [10,11].

Klebsiella pneumoniae and *Staphylococcus aureus* were common bacteria in our study. The bacterial isolates depend on prevalence of bacteria in hospital environment, in the community, antibiotic prophylaxis & severity of exacerbation. *Haemophilus influenzae* was not isolated in our study which can be explained by the fact of temperature variations & use of antibiotics either self or prescription of unqualified medical practitioners.

Clinicians prescribed the antibiotics on the basis of antibiogram. Based on sensitivity pattern, effective drugs like azithromycin & clarithromycin are available for treatment of gram positive isolates, meropenem & piperacillin-tazobactam for gram negative isolates and amikacin & levofloxacin for both gram positive & negative isolates. Vancomycin & linezolid are to be given only for methicillin/oxacillin resistant isolates of *Staphylococcus aureus*. Least sensitivity was shown with cotrimoxazole & ciprofloxacin. Though sensitivity to levofloxacin was excellent for majority of pathogens, it can't be considered as the first option for starting treatment, as its frequent usage can lead to emergence of the resistant strains.

CONCLUSION

Amikacin and levofloxacin, were the most active antibacterial agents and therefore, the drug of choice in treating AECOPD in our setting. Bacteria causing AECOPD are different in India as compared to western studies. More studies like this are required at regular interval, to formulate an antibiotic policy which helps in preventing mortality & morbidity due to acute exacerbations of chronic obstructive pulmonary disease.

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