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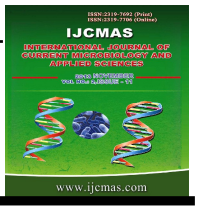

Original Research Article

Bacterial agents causing acute exacerbations in Chronic Obstructive Pulmonary Disease (COPD) patients, their antibiograms to Extended Spectrum Beta-Lactamases (ESBL) production in a tertiary care hospital, India

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Keywords

Chronic Obstructive Pulmonary Disease; antibiotic sensitivity; pathogenic microbes; ESBL producers.

A B S T R A C T

Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of morbidity and mortality worldwide. The natural history of COPD is characterized by frequent exacerbations. Majority of exacerbations are infectious and knowledge on the pattern of infectious bacteria and their antibiotic sensitivity is essential to formulate cost-effective antibiotic strategy and reducing the emergence of drug resistance. One hundred sputum samples from clinically diagnosed COPD cases suffering from acute exacerbation were collected to isolate and identify the aerobic bacterial agents causing acute exacerbations in COPD patients, to study the antibiotic sensitivity patterns of the isolates and phenotypic detection of ESBL production by the isolates. Preparation of media, reagents, Gram staining, identification of culture isolates, different tests including antibiotic sensitivity tests were carried out following standard laboratory procedures. Among the 37single pathogenic microbial growth 19 were Gram negative bacteria and 18 were Gram positive bacteria. *Streptococcus pneumonia* was the commonest bacteria isolated followed by *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus.* All the isolates of *K.pneumoniae* were ESBL producers. *Staphylococcus aureus* strains were mainly sensitive to erythromycin, gentamicin, amikacin, co – trimoxazole, vancomycin. All the five *S. aureus* strains isolated showed resistance to oxacillin. Periodic isolation and identification and resistant status of pathogens causing AECOPD will help us to formulate appropriate treatment protocol and this will be of immense use in reducing mortality and morbidity besides reducing the volume of antibiotics and development of resistance to antibiotics

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of morbidity and mortality worldwide. The World Health Organization (WHO) estimated that COPD is currently the seventh leading cause of death and disability worldwide (Schulman, *et al.*, 2000; Global Strategy for the diagnosis, management and

prevention of COPD, 2011; Chan Yeung et al. 2004). It is estimated that COPD will become the third leading cause of death worldwide by2020, next to heart and cerebro-vascular diseases (Murray CJL, Lopez, 1996). WHO estimates 600 million people worldwide have COPD (World Health Report, 1998). The prevalence of smoking is slowly decreasing in the industrialized world and rising in the developing countries especially in Asia and Africa (Chan Yeung et al. 2004). Although coronary heart disease and stroke show consistent and substantial reduction, COPD shows increasing incidence and prevalence (National Heart, Lung and Blood Institute, 1998). This increase is largely driven by two factors namely the rise in tobacco – related mortality and morbidity and the ageing population (Chan Yeung et al. 2004). Higher prevalence rates for COPD are found in men than in women globally (Murray CJL, Lopez, 1996). The natural history of COPD is characterized by frequent exacerbations with an increase of cough, purulent sputum production and dyspnoea (Nestor Soler *et al.,* 1998). Exacerbations punctuate the course of COPD in many patients (Jan V. Hirschmann, 2000). Exacerbations of COPD increase the rates of hospitalization and mortality and decrease the quality of life (Iyer Parameswaran and Murphy).

Majority of exacerbations are infectious in aetiology. Three classes of pathogens responsible for acute exacerbation of COPD by infecting the lower respiratory tract: respiratory viruses, atypical bacteria, and aerobic Gram-positive and Gram-negative bacteria (Murray amd Lopez, 1996).

The present study of the aerobic bacterial causes associated with acute exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD) and their in vitro antibiotic sensitivity pattern with special reference to Extended Spectrum Beta - Lactamases (ESBL) production is very pertinent for the clinician to plan a general outline of treatment for the patient with an episode of acute exacerbation of COPD. Knowledge on the pattern of local micro organisms and their antibiotic sensitivity is then essential to allow for effective and cost saving antibiotic strategy and reducing the emergence of drug resistance. Therefore a study was undertaken to isolate and identify the aerobic bacterial etiological agents causing acute exacerbations in COPD patients, to study the antibiotic sensitivity patterns of the isolates and phenotypic detection of ESBL production by the isolates.

Materials and Methods

Sources of the material

One hundred (100) sputum samples from clinically diagnosed COPD cases suffering from acute exacerbation were collected for the study. Sputum samples from hundred healthy subjects (100) were included as control in the study. Patients suffering from bronchial asthma, bronchiectasis, bronchial carcinoma, pneumonia, the subjects who were recently undergoing antibiotic therapy and known cases of pulmonary tuberculosis were not included in the present study.

Data collection

Complete data about the patient viz: Name, Age, Sex, Hospital number, Date of collection of the sputum, History of present illness, symptoms were collected from the patient. Past history of smoking, Occupational history was also collected. Any history of exposure to indoor air pollution was collected from the female patients.

Sample collection

Patient was educated about the difference between sputum and oral secretion. Deeply expectorated sputum sample after an oral gargle with water (to produce a sample from the lower respiratory tract and not getting contaminated with the secretions of the upper respiratory tract) was collected directly into a sterile and wide mouthed disposable universal container. Sample containers were labeled immediately after the collection with patients name, age, sex, IP No, and ICU ward along with date and transported to the Microbiology laboratory. Using these system, negative numbers are assigned to a smear when squamous epithelial cells are observed indicating contamination with oropharyngeal secretions (saliva).

Direct smear study

Direct smears where made from the most purulent part of the sputum samples on a clean grease free microscopic slide and stained with GRAM's STAIN. Under oil immersion objective, the stained sputum smear was observed for the presence of the bacteria, their gram reaction, size, shape, arrangement, any yeast cells, polymorph nuclear leukocytes and squamous epithelial cells. An ideal sputum sample must contain 8-10 polymorphonuclear leucocytes for every 2-3 squamous epithelial cells per high power field. Sputum samples fulfilling these criteria were processed further.

Culture and identification of the isolate

Sputum samples were plated onto Nutrient agar, Blood agar with *Staphylococcus aureus* streaks, Mac Conkey's agar, Chocolate agar, Filde's agar. Preparation of media, reagents, Gram staining, identification of culture isolates and different tests were carried out following standard laboratory procedures (Mackie and McCartney Practical Medical Microbiology, 14 th edition 1996). The plates are incubated overnight at 37^0C. After 24 hours of incubation colony morphology was studied.

A single well separated colony is identified. Preliminary tests like Gram's staining of the colony, Hanging- drop preparation, Catalase test and Cytochrome oxidase test were done. Biochemical tests like Indole test, Methyl red test, Voges proskauer test, Citrate utilisation test, Urease test, Triple sugar iron agar, Nitrate reduction test, Hugh-Leifson's oxidation-fermentation test, coagulase production (for Staphylococci), Optochin Sensitivity (for *Streptococcus pneumoniae*)were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol, Xylose, Arabinose and Dulcitol, inositols were done to identify the isolate according to standard laboratory procedures.

Antibiotic Sensitivity Test

Antibiotic sensitivity test of the isolates were performed by Kirby Bauer Disc Diffusion method using Mueller Hinton agar and antibiotic discs (obtained from HI-MEDIA). Broth suspension of the organisms was made and adjusted to 0.5 Mc Farland's turbidity standards. A lawn culture was made over the surface of the media using a sterile swab, then appropriate antibiotics disc were placed

Analysis of sample

Sputum samples from Hundred (100) clinically diagnosed cases of AECOPD were analyzed using standard techniques.

Bartlett's grading of sputum.

No of Neutrophils Per 10x Low Power Field	Grade
<10 10-25 >25 Presence of mucus	0 +1 +2 +1
No. of Epithelial cells per 10x low power field	
10-25 >25 TOTAL *	-1 -2

* Average the number of epithelial cells and neutrophils in about 20 or 30 separate 10x microscopic fields and then calculate the total. A final score of 0 or less indicates lack of active inflammation or contamination with saliva.

Table.2 Antibiotic sensitivity chart according to CLSI Guidelines

Antibiotics	Symbol	Disc potency	Sensitive (in mm)	Intermediate sensitive(in mm)	Resistant (in mm)
Amikacin	AK	30 mcg	17	15-16	14
Ampicillin	AMP	10µg	≥17	14-16	13
Ceftazidime	CAZ	30 mcg	18	15-17	14
Ceftazidime/ Clavulanic acid	CAC	30/10mcg	27-34	-	-
Ceftriaxone	CTR	30 mcg	21	14 – 20	13
Ciprofloxacin	CIP	5 mcg	21	16-20	15
Co-trimoxazole	COT	1.25/23.75 mcg	16	11-15	10
Gentamicin	GEN	10 mcg	15	13-14	15
Tetracycline	TE	30 mcg	19	15-18	14
Piperacillin+ Tazobactam	PIT	100/10 mcg	21	18-20	17
Vancomycin	VA	30 mcg	17	15-16	14
Oxacillin	OX	1 mcg	13	11-12	13
Aztreonam	AT	30mcg	22	16-21	15
Imepenem	IPM	10µg	≥16	14-15	13
Polymyxin – B	PB	300 units	12		11

Klebsiella pneumoniae ATCC 700603, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. The different antibiotics used for sensitivity test following CLSI guidelines are presented (Table.2).

ESBL production by the isolates is detected phenotypic ally by using ceftazidime disc and ceftazidime/clavulanic acid (combination) disc. Discs were placed 24 mm apart. Increase in zone diameter of 5 mm or greater of the combination disc when compared with the ceftazidime disc alone indicates the ESBL production by the isolate.

Result and Discussion

The age group of the patient in the study, ranged from forty five to eighty years. Out of one hundred (100) patients, the most common age group was fifty five years (43%). The next common age group was sixty five years (32%). (Table No.1) .Among clinically diagnosed COPD patients who were suffering from acute exacerbations, 68 were males and 32 were females. Out of 68 males, 48 (70%) were smokers and twenty (20) (29.41%) were non-smokers. Chronic history of cough with expectoration, exertional dyspnoea was the common clinical manifestations in the patients. Majority of the patients had mucopurulent sputum and had a history of aggravated cough with expectoration when exposed to cold climate.

Bacteriological Profile

Forty two percent of the sputum samples were positive for pathogenic bacteria and 58% were culture sterile. Out of the forty two sputum samples which grew pathogenic bacteria, thirty seven sputum samples yielded mono microbial growth and five yielded polymicrobial growth (Table.3). Among the thirty seven single pathogenic microbial growth 19 (51.35%) were Gram negative bacteria and 18 (48.64%) were Gram positive bacteria. Out of 37 mono microbial growths, *Streptococcus pneumoniae* was the commonest bacteria isolated in 16 cases, followed by *Moraxella catarrhalis* in 7cases, *Pseudomonas aeruginosa* in 7 cases, *Klebsiella pneumoniae* in 5 cases and *Staphylococcus aureus* in 2 cases.

Out of the forty two (42) sputum samples 5 yielded polymicrobial growths. Two cases showed *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and 3 cases exhibited *Klebsiella pneumonia* and Staphylococcus aureus (Table.4).

Antibiotic Sensitivity Pattern of the Isolates

Streptococcus pneumoniae was sensitive to ampicillin, centamicin, amikacin, co-trimoxazole and erythromycin. *Moraxella catarrhalis* was mainly sensitive to ciprofloxacin, ceftazidime, imipenem, aztreonam, piperacillin + tazobactam. It was resistant to penicillin (due to beta lactamase production) and tetracycline. *Pseudomonas aeruginosa* was mainly sensitive to ceftazidime, amikacin, piperacillin + tazobactam, polymyxin – B, imipenem, aztreonam and resistant to penicillin and ceftriaxone. *Klebsiella pneumonia* strains were mainly sensitive to piperacillin+ tazobactam, imipenem, ceftazidime + clavulanic acid. They were resistant to ampicillin, ceftazidime, amikacin, tetracycline and ciprofloxacin. Among the 10 isolates of *K.pneumoniae* all the isolates were ESBL producers. *Staphylococcus aureus* strains were

mainly sensitive to erythromycin, gentamicin, amikacin, co – trimoxazole, vancomycin. All the five *S. aureus* strains isolated showed resistance to oxacillin.
Sputum samples from the control groups showed non- pathogenic growth or commensal flora in all the cases.

COPD is a heterogeneous disease affecting the older and often socially disadvantaged population. It is likely that different insults or susceptibility in combination with smoke exposure are responsible for this heterogeneity. They are a major cause of hospital admission and health care utilization. Large numbers of COPD patients have never been diagnosed or indeed have been misdiagnosed. Indeed the existing patients known to have COPD represent the tip of the very deep ice berg. Further, there has been a dramatic rise in antibiotic resistance among common respiratory pathogens in recent years
Current evidence indicates that chronic infection probably contributes to the pathogenesis of COPD in a substantial subset of patients (Sethi *et al.,* 2009). Low socio-economic conditions, poor diet, environmental pollution and childhood infections are not only responsible for the development of COPD but also for continued decline in lung functions, disease complications and an early mortality (American Lung Association, 2011).

It was observed that AECOPD was prevalent in 45-80 year age group. However among them, 45-65 year age group constituted 75%. Thus AECOPD was common above 40 years **(**Mohan *et al.,* 2006**;** Chronic Obstructive Pulmonary Disease (COPD). The probable reason may be because of expression of deterioration in host defenses at the bronchial mucosal level in patients with advanced lung disease (Jorg *et al.,* 1998).
AECOPD was higher in males 68 (68%) than females 32 (32%). This finding is in conformity with the observation of other workers (Jindal *et al.,* 2001). The male preponderance was seen in our study because most of them were smokers. In non smokers, especially among women, exposure to indoor air pollution was an important factor (Arora *et al.,* 2001). Studies conducted in very different environments have constantly observed that admissions to COPD increased on days with high pollution levels **(**Mohan *et al.,* 2006). In the present study AECOPD was more common among smokers 70%

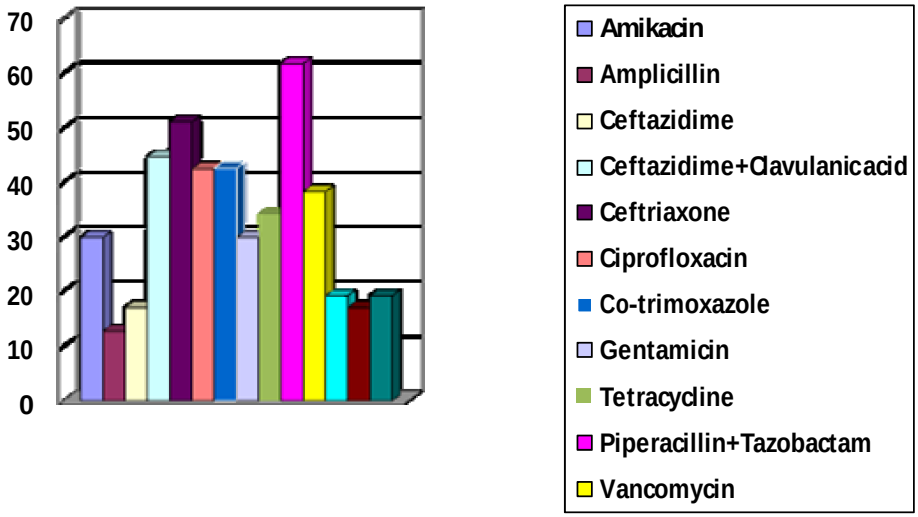
Table.3 Showing the growth pattern in 100 sputum samples

Growth	No. of samples	Percentage
Single isolate	37	37%
Multiple isolates	5	5%
Non- pathogenic	58	58%

Table.4 Showing organism isolated from the Sputum culture (42 culture positives)

S.No	Name of the organism	Number of isolates	Percentage
1	*Streptococcus pneumoniae*	16	38.10%
2	*Klebsiella pneumonia*	10	23.81%
3	*Pseudomonas aeruginosa*	9	19.14%
4	*Moraxella catarrhalis*	7	16.67%
5	Methicillin Resistant *Staphylococcus aureus*(MRSA)	5	11.90%

Fig.1 Antibiogram (sensitivity) of 47 isolates in sputum culture



than non-smokers 29.41% which was similar to other studies (Arora *et al.*, 2001). It is well known that the frequency of infection resulting in AECOPD by various microorganisms varies from one geographical area to another. Our country has a wide climatic variation and COPD is more common in northern India because of long cold winters, small houses and high levels of indoor pollution (Kamat Sudhakar, 1991).

Pathogenic bacteria were found in 42% of patients with AECOPD. This could be due to declining lung function (Jorg *et al.*, 1998). The prevalence of Gram negative isolates was 61.90%, as compared to 50% of Gram positive isolates. The Gram negative organisms were more common in the patients with the most severe lung dysfunction, where as the Gram positive bacteria predominated in the exacerbations of the patients with the mildest degree of lung function abnormalities (Niederman Michael, 2000).

Among the isolates, *Streptococcus pneumoniae* was the predominant organism isolated (31.10%) followed by *Klebsiella pneumoniae* (23.81%),

Pseudomonas aeruginosa (19.14%), *Moraxella catarrhalis* (16.67%), Methicillin - Resistant *Staphylococcus aureus* (11.90%) *H. influenzae* was not isolated in the present study. This is in concordance with previous studies conducted elsewhere (Allegra *et al.,* 1996; Wilson *et al.,* 1999). *Streptococcus pneumoniae* (25.86%) was the commonest organism isolated in our study which correlated with a previous study (Arora and Daga, 2001). *Haemophilus influenzae* could not be isolated in our institute inspite of using Filde's agar, Chocolate agar, Blood agar with Staphylococcus aureus streaks onto which the sputum samples were plated. It could have occurred because of prior antibiotic use or seasonal variations in causation. It has been reported that *Haemophilus influenzae* was the most frequent bacterium isolated followed by *Streptococcus pneumoniae*, and *Moraxella catarrhalis,* and *Pseudomonas aeruginosa*.(Erkan *et al.,* 2008).

In the present study *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed a higher percentage than other pathogenic bacteria. It is because both the organisms are nosocomial pathogens and large number of patients in our study was the regular patients to the Hospital Out Patient Departments. Hence the higher percentage of both the organisms could be because of increased oropharyngeal carriage by these patients. It has been shown that the organisms persist in the sputum after apparently effective therapy of acute exacerbations of chronic bronchitis and the same phenotypic isolates were responsible for subsequent relapses (Arora *et al.,* 2001).

Bacteria play either a primary role in the development of exacerbations of COPD or represent a secondary infection following an initial viral process. The role of bacteria in exacerbations of COPD remains controversial, since bacterial species are present in the airways of between 25–50% of patients with COPD even when in a stable condition (Nee, 2003).

Antibiotics are important in treatment of AECOPD. The most common decision a physician has to make when treating a patient with AECOPD is to prescribe the right antibiotic therapy. Antibiotics are commonly prescribed empirically to patients presenting with AECOPD to treat presumed bacterial infection. The rise in bacterial resistance to antibiotics has focused our attention to study the ESBL production by the isolated bacteria from cases of AECOPD. Multi drug resistant strains of the bacteria infect the lower respiratory tract and cause acute exacerbations of chronic bronchitis. This may result in increase in morbidity and mortality of the patient thereby increasing the economic burden of a nation.

The choice of antibiotics depends on the local antibiotic policy and the pattern of local pathogens. Based on the sensitivity pattern, the common antibiotic that can be used are Amikacin, Ceftazidime-Clavulanic acid, Ceftriaxone, Ciprofloxacin, Co - trimoxazole and Imipenem. In case of mild to moderate AECOPD the patients are to be treated with a short course of antibiotics for a minimum of 5 days than traditional longer treatment (Sanjay and Murphy, 2001).

In this study concluded that *Streptococcus pneumonia* was the predominant organism isolated from the sputum of patients suffering from AECOPD followed by *Klebsiella pneumonia,* Pseudomonas *aeruginosa*, *Moraxella catarrhalis* and,

Methicillin - Resistant *Staphylococcus aureus.* Among the 10 isolates of *K.pneumoniae* all the isolates were ESBL producers. *Staphylococcus aureus* strains were mainly sensitive to erythromycin, gentamicin, amikacin, co – trimoxazole, vancomycin. All the five *S. aureus* strains isolated showed resistance to oxacillin.

Periodic isolation and identification and resistant status of pathogens responsible for AECOPD will help us to formulate appropriate treatment protocol and this will be of immense use in reducing mortality and morbidity besides reducing the volume of antibiotics and development of resistance to antibiotics. The protective host immune responses that develop after exacerbation needs to be characterized to facilitate vaccine development. Further, the interaction among different etiologic factors such as environment, bacteria, viruses and atypical pathogens needs to be better understood to treat exacerbations and develop novel as well as cost effective preventive and therapeutic strategies.

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