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Original Research Article

A Study of Aerobic Bacteriological Profile & Antibiotic Susceptibility Pattern in Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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Abstract:

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is characterized by airflow obstruction that is not completely reversible. COPD is usually progressive in nature, associated with an abnormal inflammatory response of the lungs to chronic inhalational exposure to smoke, dust and other air pollutants. It encompasses two broad categories which include Chronic Bronchitis and Emphysema. Over three million people died as a result of COPD in 2005 corresponding to 5% of deaths worldwide. So it would be the third leading cause of death by 2030.Thus that being the aim of this study to identify the aerobic and facultative anaerobic bacteria from the sputum culture of patients admitted with Acute Exacerbation of COPD and to determine the antimicrobial susceptibility pattern of the bacterial isolates.

Material and Method: A total of 100 sputum samples were collected from patients with acute exacerbation of COPD & were processed at Bacteriology lab of the Department of Microbiology, SMS Medical College, Jaipur.

Results: Among 100 clinically diagnosed AECOPD cases, 51 (51%) had growth of pathogenic flora. Among all the positive cases, the majority of the pathogens were gram-negative bacilli 45(76.3%) followed by gram-positive cocci 14 (23.7%).

Conclusion: Empirical treatment should be based on the presumptive etiologic diagnosis developed from all existing epidemiologic, clinical and laboratory records. Once the culture report is accessible, the treatment should be based on the drug to which the organism is most susceptible. If this approach is followed it will definitely help us in curbing the dearth of Antimicrobial resistance.

Keywords: COPD, Sputum culture, Bacteriological profile, Antimicrobial susceptibility, Antimicrobial resistance.

Introduction

COPD encompasses two broad categories which include Chronic Bronchitis and Emphysema. By definition, chronic bronchitis is characterized by chronic cough with expectoration for 3 months in a year for at least two consecutive years.

Emphysema is defined as abnormal distension of the air spaces which is distal to the terminal bronchioles with the destruction of their walls without any obvious fibrosis. According to World Health Organization estimates around 65 million people suffer from moderate to severe COPD.[1] In India, a common lung disorder next to pulmonary tuberculosis is COPD most commonly affecting people in their fourth decade. The incidence is reported as equal among people living in both rural and urban areas. There are around 30 million COPD cases in India. India contributes a significant percentage of COPD mortality worldwide.[2] COPD manifestations rarely occur below the age of 35 years. The national burden was estimated to be around 14.84 million. [3]

Increased COPD cases are said to be associated with demographic and socioeconomic factors such as advancing age, low socioeconomic status and urban residents with lower socioeconomic status. Air pollution and smoking are the highest risk factors in India, which are the main causes of COPD, leading to an increase in microbial infection. [4] The majority of exacerbations are infectious in etiology.[2] Chronic inflammation causes structural changes, narrowing of the small airways and destruction of the lung parenchyma

that leads to the loss of alveolar attachments to the small airways and decreased elastic lung recoil. These changes decrease the airways ability to remain open during expiration.[5]

Acute exacerbation of COPD is defined as sustained declination of the condition of the patients, from the stable state and beyond the normal day-to-day variations, which is acute in onset and requires a change in regular medication in a patient with underlying COPD.[6] Patients with frequent exacerbations present as decreased lung function at an accelerated rate and exacerbations of COPD increase the rate of hospitalization, mortality and decrease the quality of life and increase the economic burden.[1] Studies of sputum and bronchoscopy samples using standard culture and molecular techniques have demonstrated that COPD exacerbations are associated with a markedly increased prevalence of bacteria.[7]

The different bacteriological profiles in patients with poor lung function support that they are involved in the progression of the disease. [8] Various bacteria have been isolated from the sputum in approximately 60% of exacerbations of COPD. Most virulent organisms in the airways of severe chronic bronchitis patients with acute exacerbations include Staphylococcus aureus , Pseudomonas species, and members of the Enterobacteriaceae family.[1]

The increasing antimicrobial resistance is of major concern thus to reduce the emergence of antibiotic resistance it is essential to know the effective and economical antibiotic regimen. The antibiotics selection should be as per the patient's affordability, the severity of exacerbation, bacterial spectrum, and the knowledge of the local bacteriological profile. [8] The rise in bacterial resistance to antibiotics has concentrated our attention on the benefit of early identification of causative agents along with their antibiotic susceptibility pattern in the treatment of COPD cases.[7] Knowledge of the local microbiological profile and antibiotic susceptibility pattern of COPD cases would help in the better selection of antibiotics for empirical therapy.[9] Hence the present study is carried out to know the bacterial etiology of AECOPD with the antibiotic susceptibility pattern of the bacterial isolates.

Material and Methods

The present study was carried out in the Bacteriology Laboratory of the Department of Microbiology SMS Medical College & Attached Hospital Jaipur, Rajasthan from June 2020 to September 2021. A total of 100 sputum samples were collected. Within 24-h post-admission, samples were collected in a sterile wide-mouthed container with a screw cap, under aseptic precautions from clinically diagnosed cases of acute exacerbation of COPD.

Based on the standard guideline, an early morning deep coughed sputum sample was collected. Prior to sputum collection, to avoid oral contamination of the sample, patients were asked to rinse their mouths twice with water and an antiseptic solution. Sputum samples collected aseptically were labelled and transported to the laboratory with properly filled structured proforma within 2 hours of collection, for gram staining and bacterial culture. Samples from patients who had Bronchial asthma/lung abscesses/lung cancer, known cases of pulmonary tuberculosis, cases with ischemic heart disease, and subjects who were recently started on antibiotic therapy either systemically or locally were excluded.

Gram staining was done for all clinical samples which were examined under a light microscope to note the presence or absence of microorganisms, pus cells, and epithelial cells. The specimens were inoculated on Macconkey agar, sheep blood agar, chocolate agar and Thioglycolate broth (TG) for bacterial culture. All plates were incubated aerobically at 37°C and evaluated at 18-24 hours and the plates were discarded if there was no growth. Bacterial isolates were identified by conventional methods according to the standard laboratory protocol, including colony morphology, staining characteristics, and biochemical properties. For antibiotic susceptibility testing, the Kirby-Bauer method was used. The preferable antibiotics and interpretation were done based on Clinical Laboratory Standards Institute Guidelines (CLSI). Ethical clearance was obtained from Institutional Research Review Board before the initiation of the study.

Results

Out of a total of 100 sputum most of the COPD cases were in the 50-70 years of age group (77%). (Table 1)

Table 1: Age distribution of AECOPD cases

Age group (years)	Number	%
30-40	02	02
41-50	08	08
51-60	39	39
61-70	38	38
71-80	09	09
80 & above	04	04
Total	**100**	**100**

Among the 100 samples, 49 (49%) samples had growth of normal commensal flora while 51 (51%) samples were showing growth of pathogenic flora, out of which 43 (84.3%) were pure bacterial isolates and 8 (15.7%) were double growth. The total bacterial isolates were 59. Out of 59 bacterial isolates, 14 isolates were gram-positive cocci and 45 were gram-negative bacilli. (Table 2)

Table 2: Micro-organisms isolated from the culture of sputum samples of AECOPD cases

Isolates				**No. of samples**
Normal commensal flora				49
Pathogenic flora	Gram Positive cocci	14	23.7%	51
	Gram Negative bacilli	45	76.3%	
	Total isolates	59	100%	
Total Samples				**100**

The most common organism isolated was Klebsiella species 19 (32.2%), Pseudomonas spp 12 (20.3%) followed by Coagulase-positive Staphylococcus spp 09 (15.3%). (Table 3)

Table 3: Bacterial isolates among Culture positive cases

Bacterial Isolates	**No.**	**%**
Klebsiella species	19	32.2%
Pseudomonas species	12	20.3%
Coagulase Positive Staphylococcus spp	9	15.3%
Escherichia coli	7	11.9%
Acinetobacter species	5	8.5%
Streptococcus pneumoniae	3	5.1%
Enterobacter cloacae	2	3.4%
Coagulase Negative Staphylococcus spp	2	3.4%
Total	**59**	**100%**

Gram-negative organisms showed the highest susceptibility to Polymyxin B (100%). They showed the least susceptibility to Cefotaxime (21%) and Ampicillin (18%) (Figure 1)

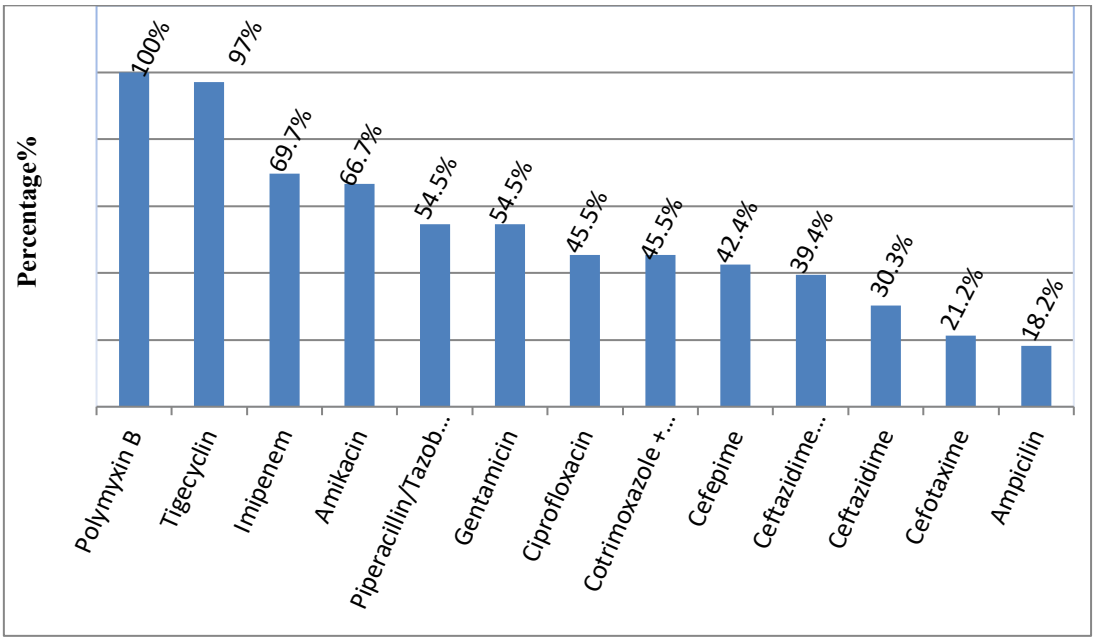

Figure 1: Antibiotic susceptibility pattern of Gram-negative bacilli

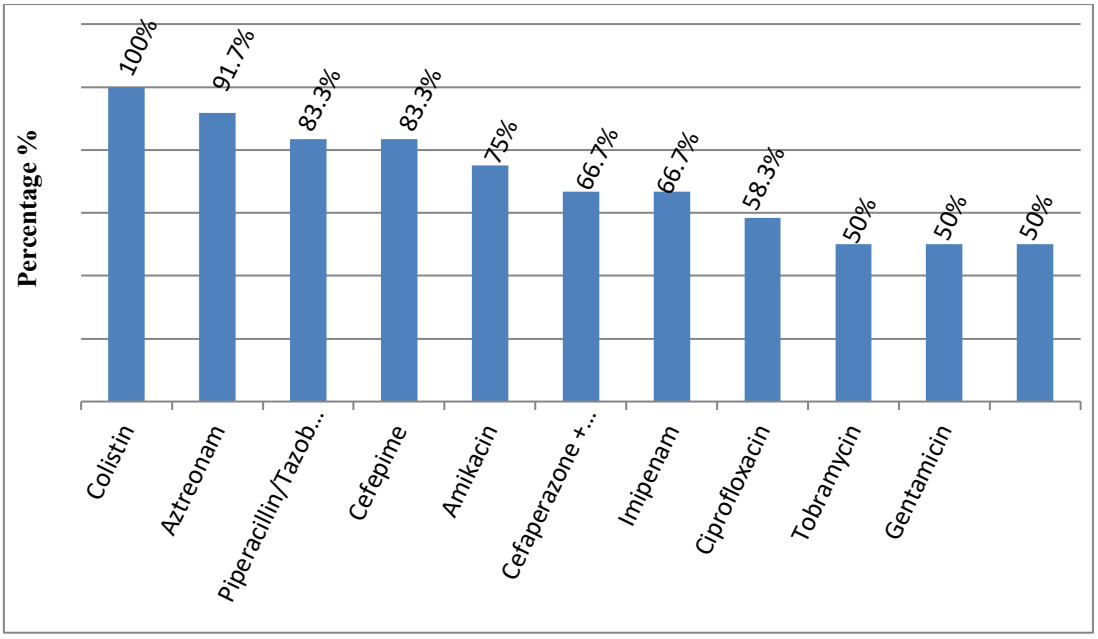

Figure 2: Antibiotic susceptibility pattern of Pseudomonas spp

Pseudomonas spp showed the highest susceptibility to Colistin (100%) and least susceptibility to Ceftazidime (50%) (Figure 2) Coagulase Positive Staphylococci, Coagulase Negative Staphylococci and Streptococcus pneumoniae showed the highest susceptibility (100%) for Vancomycin and Linezolid. (Table 4)

Table 4: Antibiotic susceptibility pattern of CoPS, CoNS, and Streptococcus pneumoniae

Antibiotics	CoPS (9) (%)	CoNS (2) (%)	Streptococcus pneumonia (3) (%)
Linezolid	100	100	100
Vancomycin	100	100	100
Teicoplanin	88.9	100	66.7
Doxycycline	77.8	100	-
Gentamicin	66.7	100	66.7
Clindamycin	66.7	50	-
Cefoxitin	55.6	50	-
Piperacilin Tazobactum	55.6	-	-
Cefepime	55.6	50	33.3
Ampicillin	55.6	100	-
Cotrimoxazle	55.6	100	66.7
Ciprofloxacin	55.6	50	66.7
Erythromycin	33.3	100	66.7

Discussion

Chronic obstructive pulmonary disease accounts for major causes of morbidity and mortality in adults globally. In the next two to three decades this scenario has been predicted to worsen. Episodes of exacerbation add to the burden of the disease and are a major cause of health care utilization including hospitalizations and intensive care admissions.

Most of the exacerbations are associated with infective causes like viruses or bacteria, although non-infective triggers like air pollution are important.[10] Over the years, bacterial organism isolated from acute exacerbations have changed from routine organisms to Enterobacteriaceae family and Pseudomonas species with increased severity of the disease.[11,12] These organisms further worsen the situation by their multidrug resistance and thus limit the therapeutic option.

In the present study, it was observed that majority of AECOPD is prevalent among 51-70 years age group. Due to impairment of immunological defense mechanism, associated co-morbid illness, increased duration of seasonal variation & tobacco smoking, AECOPD was common in the advanced age group as the respiratory tract is more susceptible. This correlates with the study by Roshni et al[13] and the study by A B Dey et al.[14]

Out of 100 cases in the present study, 79 (79%) were males and 21 (21%) females. Males were affected more than females because they were more

involved in smoking & start it in the younger age group, therefore increasing the chances of inhalation and increased environmental exposure or temperature variation.[15] In non-smokers, especially among women, exposure to indoor air pollution was important.[16] A similar observation was made by Miravitlles et al[17] who had 81 % males and 19% females in their study.

Culture positivity depends on sputum nature, transportation time, prior use of antibiotics by the patient and the number of organisms present in the sample. Bacterial pathogens were isolated in 51% of patients with AECOPD. Similarly, Erkan et al [18] observed bacterial pathogens in 55% of AECOPD cases. It is eminent that the incidence 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 the long cold winters, small houses and high levels of indoor pollution.[19]

In our study, the prevalence of Gram-negative isolates was 76.3% as compared to 23.7% of Gram-positive out of a total of 59 bacterial isolates. The greater frequency of isolation of Gram-negative bacteria has also been reported in other studies – Madhvi et al(75%)[20], Basu et al(71.42%)[21]. In the present study, single and multiple organisms were isolated in 84.31% and 15.69% of the study population respectively which was similar to the study conducted by Saxena et al[22] and Narayanagowda et al.[23] The double bacterial growth pattern in the present study was as follows: Klebsiella species with Pseudomonas species in 4 cases, Klebsiella species with Acinetobacter species in 2 cases, Klebsiella species with Staphylococcus aureus in 1 case, E. coli with Pseudomonas species in 1 case. The cases in our study were hospitalized patients of AECOPD, who were mostly suffering from moderate to severe exacerbations and most of them were frequent exacerbators, hence Gram-negative pathogens such as Pseudomonas and Klebsiella were more prevalent and thus can explain the lower numbers of Gram-positive bacteria isolation. [24]

In the present study among pathogenic bacteria, Klebsiella spp was the predominant organism isolated (32.2 %), followed by Pseudomonas spp. (20.3%), Staphylococcus aureus (15.3%), E. coli (11.9%), Acinetobacter spp (8.5%), Streptococcus pneumoniae (5.1%), Coagulase negative staphylococcus spp (3.4%), Enterobacter cloacae (3.4%) which were in accordance with the study conducted by Erkan et al.[18

The antimicrobial susceptibility (AST) pattern differs in different studies as well as at different times in the same hospital in Indian and overseas studies because of the wide availability of over-the-counter antibiotics and different hospital-based antibiotic policies. In our study, AST revealed that the majority of Gram-negative bacteria showed good susceptibility to Polymyxin-B (100%), Tigecycline (96.9%), and Imipenem (69.7%), Amikacin (66.7%) and resistance was found to aminopenicillins and extended-spectrum cephalosporins. The majority of Gram-positive bacteria showed good susceptibility to Linezolid (100%), Vancomycin (100%), Teicoplanin (85.7%), Gentamycin (71.4%), and poor susceptibility to Ciprofloxacin (57.2%), Cefepime (50%), and Erythromycin (50%). The majority of Pseudomonas spp were more commonly susceptible to Colistin (100%), Aztreonam (91.7%), Piperacillin + tazobactam, and Cefepime (83.3%) and least susceptible to Ciprofloxacin (50%) and Gentamicin (50%). Klebsiella pneumoniae which was the most common isolate showed good susceptibility to Polymyxin B, Amikacin, & Imipenem. Staphylococcus aureus which was the prevalent Gram-positive isolate was susceptible to Linezolid, Vancomycin, Teicoplanin, and Doxycycline. 44.4% (4/9) of Staphylococcus aureus were Methicillin resistant (MRSA). Streptococcus sp were susceptible to Linezolid, Vancomycin, and Teicoplanin. Pseudomonas spp were mainly susceptible to Colistin, Aztreonam, Piperacillin + Tazobactam, Cefepime, and Amikacin. E. coli were susceptible to Polymyxin B, Tigecyclin, and Imipenem. The study shows that fourth-generation Cephalosporins, Fluoroquinolones, and Aminoglycoside antibiotics have good susceptibility but Polymyxin-B, Tigecycline, Colistin, Linezolid, Vancomycin, Teicoplanin, Piperacillin-Tazobactam, and Imipenem have the best susceptibility. Similar antibiotic susceptibility patterns were also seen in the study by Aleemullah et al.[25] It was seen that Klebsiella pneumoniae was highly susceptible to Imipenem (100%), Piperacillin-Tazobactam (100%) and Amikacin (81%) while Pseudomonas aeruginosa was susceptible to Imipenem (84.21%), Piperacillin-Tazobactam (84.21%), and Amikacin (78.95%), Staphylococcus aureus was highly susceptible to Vancomycin (100%), Linezolid (100%), and also to Piperacillin Tazobactam (87.5%) and Amikacin (75%), Streptococcus pneumoniae was found to have the highest susceptibility to Vancomycin (100%) and Linezolid (100%) and also to Amikacin (84%), Piperacillin-Tazobactam (88%), and Gentamycin (76%)[25]

Conclusions

The present study concluded that Gram-negative organisms like Klebsiella pneumoniae and Pseudomonas spp were responsible for the majority of the acute exacerbation. The bacteriological profile of the sputum samples suggests the role of commonly encountered pathogens in the

precipitation of AECOPD and highlights the differences in causative agents with respect to other studies. The present study shows that most of the isolates are resistant to commonly used antibiotics. Resistance to antibiotics is a worldwide problem that causes the ineffectiveness of empirical treatment. However, regular surveillance to follow changes in trends of causative organisms and antibiotic susceptibility patterns would be of great help. Such information would be useful for the appropriate selection of empiric antibiotic therapy and to act rapidly in case of major changes in susceptibility patterns. Appropriate use of antibiotics and the importance of completing the full course of antibiotic treatment should be strictly emphasized.[26] The higher antibiotics such as Polymyxin B, Colistin, and Carbapenems should be reserved for multidrug-resistant Gram-negative bacteria, whereas Vancomycin and Linezolid be reserved for drug-resistant Gram-positive bacteria. Microbiological studies of this type should be done regularly in all hospitals to formulate policies on the use of antibiotics and to know the changing spectrum of microorganisms responsible for AECOPD. Furthermore, studies are needed for a better understanding of etiology of AECOPD and its association with respiratory failure as there is limited evidence regarding this.

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