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ORIGINAL ARTICLE

Pattern of Bacterial Infection in Acute Exacerbation of Chronic Obstructive Pulmonary Disease (COPD) in a Tertiary Care Hospital

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Abstract

Background: *Acute exacerbation of COPD is one of the important problems during management of COPD & has considerable impact on morbidity, mortality and quality of life. Acute exacerbation can occur due to bacterial or viral infection & non-infectious causes. Sputum culture & real-time PCR can identify the pattern of bacterial infection. Rational use of antibiotic can reduce the cost of treatment & possibility of antibiotic resistance.*

Material and Methods: *This cross sectional study was conducted on hospital admitted & outdoor attended acute exacerbation of COPD patients using sputum Gram stain, culture & Real time PCR.*

Results: *The mean age of the respondents was 60.02±10.55 years. Maximum patients 49(79.0%) age above 50 years followed by 13(21.0%) below 50 years. Most patients had dyspnea 54(87.1%) followed by increased sputum volume 31(50.0%), increased wheez or cough 23(37.1%), increased sputum purulence 19(30.6%). Considering sputum culture & RT-PCR, 29(46.8%) patients was found microbiologically positive. Klebsiella was isolated most frequently 10(34.5%) followed by Haemophilus 7(24.1%). Type 1 & type 2 AECOPD showed more bacteriological positivity than type 3 (85.7%, 80.8% & 6.9% respectively, p=<0.001). Bacteria isolation was significantly associated with increased sputum purulence & volume (89.5% & 67.7% respectively, p=<0.001) but not in case of dyspnea (48.1%, p=0.573).*

Conclusion: *In conclusion, bacterial isolation was more common in type 1 & type 2 AECOPD than type 3, Klebsiella infection was most common. Increased sputum purulence & volume significantly indicates bacterial infection in AECOPD.*

Keywords: *COPD, Acute exacerbation, Sputum culture, Real-time PCR.*

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Introduction

COPD is a common, preventable & treatable disease that is characterized by persistent symptoms & airflow limitation that is due to airway &/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases, according to GOLD 2019[1]. The chronic airflow limitation that is characteristic of COPD is caused by a mixture of small airways obstruction & parenchymal disease. Chronic inflammation causes structural changes, narrowing of the small airways & destruction of the lung parenchyma that leads to the loss of alveolar attachments to the small airways & decreases lung elastic recoil. In turn, these changes diminish the ability of the airways to remain open during expiration. A loss of small airways may also contribute airflow limitation & mucocilliary dysfunction is a characteristic feature of the disease[2]. COPD constitutes a major health problem[3]. COPD is currently the 4th leading cause of death in the world[4]. Prevalence of COPD in the total population ranged from a low of 7.8% in Mexico City, Mexico, to a high of 19.7% in Montevideo, Uruguay. The prevalence of COPD among Dhaka City population in Bangladesh is 11.4%[5]. Using operational severity criteria adopted from Global Initiative for Obstructive Lung Disease (GOLD) mild, moderate, severe and very severe COPD were found in 42.7%, 27.2%, 20.4% and 9.7% respectively[5]. COPD exacerbations are very common, affecting about 20% of COPD patients[6]. The estimated annual costs of COPD are $24 billion, and 70% are related to exacerbations requiring hospitalization[7]. Acute exacerbations of COPD (AECOPD) have considerable impact on morbidity, mortality and quality of life[8].

Common triggers for AECOPD include viral and/or bacterial infection of the tracheobronchial tree and air pollution, but the cause of approximately one-third of severe exacerbations cannot be identified[9]. 78% of patients with severe COPD exacerbations admitted to the hospital are associated with respiratory virus and/or bacterial infection. The presence of infection with viruses (48.4%) and/or bacteria (54.7%) at exacerbation was associated with both clinical and lung function indicators of increased exacerbation severity[10].

Importantly, the presence of co infection with both viruses and bacteria was found in 25% of exacerbations and these patients had more severe functional impairment and longer hospitalization[10]. A survey including 69,820 patients who had been hospitalized for exacerbations of COPD in 360 hospitals throughout the United States showed that 85% of all patients were given antibiotics, but not all patients equally experienced benefit from antibiotics, patients with signs of bacterial infection and more severe exacerbations seem to be benefitted from antibiotics, prescribing antibiotics for viral infections or noninfectious causes of AECOPD is ineffective and increases the risk of toxicity and the development of bacterial resistance[11]. In patients with COPD, the clinical manifestations of systemic inflammation due to infectious and non-infectious causes are similar. The differential diagnosis of these two conditions is very important in order to administer the correct treatment regimen and avoid unnecessary antibiotic use, thus reducing the morbidity, mortality and care-related costs. Nevertheless, antibiotic use in AECOPD is undoubtedly still excessive, exposing patients to considerable cost and potential adverse effects, and driving antibiotic resistance. In this context, a rapid, specific test to identify lower respiratory bacterial infections would be a major advancement, limiting the inappropriate use of antibiotics which is considered to be a main cause of the spread of antibiotic- resistant bacteria[12].

Culture from sputum yields pathogenic bacteria in 32.2% of AECOPD cases[13]. Sputum Gram stain & culture showed Gram negative bacteria caused exacerbation in 63.33% of the patients, Gram positive bacteria in 23.33% of them and atypical bacteria in 13.34% of the patients. *Haemophilus influenza* (30%) was the most common organism followed by *Streptococcus pneumoniae* (23.33%), *Pseudomonas aeruginosa* (23.33%), *Moraxella catarrhalis* (10%), *Mycoplasma pneumoniae* (6.67%), & *Chlamydia pneumoniae* (6.67%)[6].

Materials and Methods

This was a cross sectional study conducted between January 2019 to August 2019 in Department of Respiratory Medicine of Bangladesh Medical University (BMU).

COPD patients attended in Respiratory Medicine Department of BMU in inpatient & outpatient for acute exacerbation was selected. Ethical approval was taken from Institutional Review Board, BMU. The patients were informed about the purpose of the study and ethical issues. The following eligibility criteria were employed to select the study population.

Inclusion criteria:

All patients attended in Respiratory Medicine Department with acute exacerbation of COPD.

Exclusion criteria:

1. Patient with any type of immunosuppression-malignancy, organ transplantation, cytotoxic drugs.

2. Preexisting lung disease other than COPD.
3. Pulmonary tuberculosis.

Consecutive sampling was applied. Total 153 patients were screened. Among them 9 were excluded for pleural effusion, 12 for bronchiectasis, 6 for consolidation, 7 for bronchial carcinoma, 24 for below standard sputum, 13 for not giving consent & 20 for lost to follow up & 62 patients were selected as study sample.

Operational definitions were set according to GOLD 2019[1]. Acute exacerbation of COPD was defined & classified as Type 1: Increased dyspnea, sputum volume & sputum purulence, Type 2: When two of these symptoms were present, Type 3: When one of the three symptoms was present in addition to at least one of the following

- Upper respiratory infection within the past 5 days
- Fever without other cause
- Increased wheezing or cough
- Increase in respiratory rate or heart rate by 20% as compare with baseline[14]

Spontaneous expectorated sputum was collected in early morning in front of investigator & stained with Gram stain & cultured in appropriate media (Chocolate agar, Blood agar & McConkey's agar). Sputum was cultured when there was <10 epithelial cells & >25 leukocytes per low power field. ≥ 1000c.f.u./ml considered as significant growth. Real-time PCR for respiratory bacteria done by Seegene Allplex respiratory panel 4 & FTD Bacterial pneumonia_CAP which detects *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila, Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus, Moraxella catarrhalis, Bordetella pertussis.* Compatible instrument was CF×96tm Real-time PCR (Bio-Rad). After collection, the data was checked and rechecked for omission, inconsistencies and improbabilities. Means and standard deviations (SD) was used to summarize continuous variables, while percentages were used for categorical variables. Data analysis was performed by Statistical Package for Social Science (SPSS), version-22.

Results

This table shows Acute exacerbation type -3 was found in maximum cases (46.8%) followed by 41.9% patients' type 2 and 11.3% patients had type 1.

Table-I

Distribution of the study subjects by acute exacerbation type (N=62)

Acute exacerbation type	Frequency	Percentage (%)
Type-1	7	11.3
Type-2	26	41.9
Type-3	29	46.8
Total	62	100.0

Table-2 shows among 29 positive cases, gram positive 4 (13.8%) cases, gram negative 24(82.8%) cases, 1(3.4%) case was atypical and mixed 1 case.

Table-II

Distribution of the study subjects by type of isolated bacteria (RT-PCR and culture) (N=29)

Bacteria type	Organism	Frequency	Percentage (%)
Gram positive	*Streptococcus pneumoniae*	3	10.3
	Staphylococcus aureus	1	3.4
Total		4	13.8
Gram negative	*Klebsiella pneumonae*	10	34.5
	Haemophilus influenzae	7	24.1
	Moraxella catarrhalis	3	10.3
	Legionella pneumophila	2	6.9
	Pseudomonas aeruginosa	2	6.9
Total		24	82.8
Atypical	*Mycoplasma pneumoniae*	1	3.4
Total		29	100.0
Mixed	*Haemophilus influenzae +*		
	Streptococcus pneumoniae	1	3.4

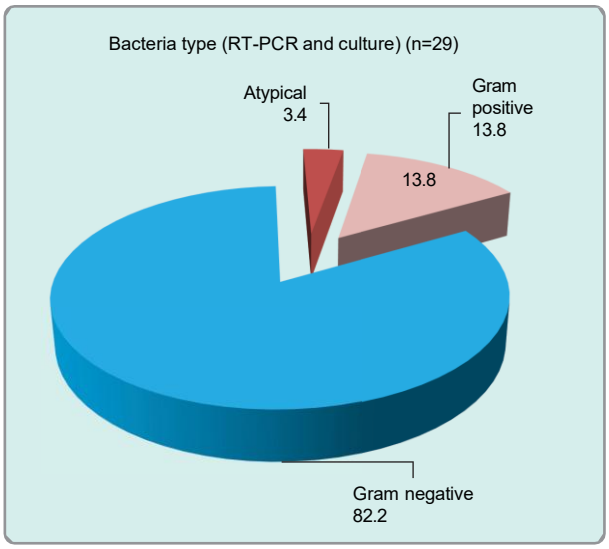

Figure-1: *Bacteria type*

Table-3 shows *Pseudomonas* was only isolated in type-1 exacerbation, *Klebsiella* was more frequent in type-1 & type-2 (33.3% in both) & *Haemophilus* in type-2 (28.6%).

Table-6 shows that dyspnea, increased sputum volume and increased sputum purulence relatively more in *Klebsiella pneumoniae*

Table-III

Distribution of the organism on the basis of sputum culture and RT-PCR by acute exacerbation type (N=29)

Acute exacerbation type	n	Gram negative					Gram positive		Atypical
		Klebsiella pneumonae	*Pseudomonas aeruginosa*	*Haemophilus influenzae*	*Moraxella catarrhalis*	*Legionella pneumophila*	*Streptococcus sp*	*Staphylococcus aureus*	*Mycoplasma pnemoniae*
Type-1	6	2(33.3%)	2(33.3%)	0(0.0%)	0(0.0%)	0(0.0%)	2(33.3%)	0(0.0%)	0(0.0%)
Type-2	21	7(33.3%)	0(0.0%)	6(28.6%)	3(14.3%)	2(9.5%)	1(4.8%)	1(4.8%)	1(4.8%)
Type-3	2	1(50.0%)	0(0.0%)	1(50.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

This table shows that bacteria isolation was significantly more in type-1 AECOPD compared to type-2 and type-3.

Table-IV

Association of sputum RT-PCR and culture positivity with type of acute exacerbation of COPD (N=62)

Type of acute exacerbation of COPD	n	Sputum RT-PCR and culture positivity		Chi-square test
		Negative	Positive	
Type I df=2 p= <0.001*	7	1(14.3%)	6(85.7%)	χ^2 =34.86
Type 2	26	5(19.2%)	21(80.8%)	
Type 3	29	27(93.1%)	2(6.9%)	
Total	62	33(53.2%)	29(46.8%)	

*significant

This table shows that bacteria isolation was significantly more in increased sputum volume and purulence.

Table-V

Association of sputum RT-PCR and culture positivity with symptoms of acute exacerbation of COPD (N=62)

Type of acute exacerbation of COPD	n	Sputum RT-PCR and culture positivity		Chi-square test
		Negative	Positive	
Dyspnea	54	28(51.9%)	26(48.1%)	χ^2 =0.317, df=1 p= 0.573^{ns}
Increased sputum volume	31	10(32.3%)	21(67.7%)	χ^2 =10.95, df=1 p=< 0.001*
Increased sputum purulence	19	2(10.5%)	17(89.5%)	χ^2 =20.06, df=1 p=<0.001*

*significant, ns= not significant

Table-VI

Distribution of the organism on the basis of symptoms

Symptoms	n	Gram negative				Gram positive			Atypical
		Klebsiella pneumonae	*Pseudomonas aeruginosa*	*Haemophilus influenzae*	*Moraxella catarrhalis*	*Legionella pneumophila*	*Streptococcus sp*	*Staphylococcus aureus*	*Mycoplasma pnemoniae*
Dyspnea	26	8(30.8%)	2(7.7%)	6(23.1%)	3(11.5%)	2(7.7%)	3(11.5%)	1(3.8%)	1(3.8%)
Increased sputum volume	21	8(38.1%)	2(9.5%)	3(14.3%)	3(14.3%)	2(9.5%)	2(9.5%)	0(0.0%)	1(4.8%)
Increased sputum purulence	17	7(41.2%)	2(11.8%)	4(23.5%)	0(0.0%)	0(0.0%)	3(17.6%)	1(5.9%)	0(0.0%)

Discussion

This cross sectional study conducted in the Department of Respiratory Medicine, Bangladesh Medical University, Dhaka including 62 acute exacerbation of COPD patients to assess the pattern of bacterial infection.

In the present study we found type-3 AECOPD is most common among study samples (46.8%) because most of AECOPD usually mild[15]. Bacterial infection causes inflammation & recruitment of neutrophil in airway. In current study sputum purulence is associated with more bacterial isolation (89.5%). In present study, predominant Gram negative infection may indicate micro aspiration of oral secretions. El-Halim and Sayed (2015) [6] reported in their study Gram negative bacteria caused COPD exacerbation in 66.33% of the patients, Gram positive bacteria in 23.33% of them and atypical bacteria in 13.34% of the patients. Eller et al. (1998) [16] reported on patients with acute exacerbations of COPD who require mechanical ventilation, 64% of the bacteria isolated by a protected specimen brush were Gram-negative bacteria. Miravitlles et al. (1999) [17] indicated higher prevalence of infection in severe AECOPD. Current study also found higher incidence of bacterial isolation (85.7%) from type-1 or severe AECOPD.

The common organisms in gram positive bacteria were *Streptococcus* (10.3%), *Staphylococcus* (3.4%), in gram negative pathogen common organisms were *Klebsiella* (34.5%), *Haemophilus* (24.2%), *Moraxella* (10.3%) & *Pseudomonas* (6.9%) in present study. Nair et al. (2019) [18] observed predominantly isolates of *Pseudomonas* in 10.1% of patients. Chawla et al. (2008)[19] also showed *Pseudomonas* in 25.95% cases. These findings are similar to current study because they found *Pseudomonas* predominantly in severe exacerbations & present study showed isolation of *Pseudomonas* only in type-1 AECOPD. In an Indian study done by Patel et al. (2015) [20] in Gujarat in 2011-2012, *Streptococcus pneumonia* was found to be the commonest pathogen. Difference in bacterial isolation may represent the geographical variation of microorganisms.

Klebsiella pneumonia was found as the predominant isolate in study by Madhvi et al. (2012)[21]. This study also found *Klebsiella* as the commonest pathogen.

Kuwal et al. (2018)[22] demonstrated bacterial pathogens (PPMs) were identified in 47% of all hospitalized patients, based on sputum culture, *Pseudomonas* and *Klebsiella* were the most frequently isolated organisms. Boixeda et al. (2012) [23] reported *Pseudomonas* in 38.23% of cases and Eller et al. (1998)[16] reported *Pseudomonas* sand Enterobacteriaceae spp in 48.2% patients of their study. Thus, this is in line with the previous studies. The higher proportion (34.5%) of isolation of *Klebsiella* in the present study also deserves special mention. Kuwal et al. (2018) [22] reported *Klebsiella* in 29.41% cases. Lin et al. (2007)[24] reported high prevalence of infection with K. pneumoniae (19.6%) and P. aeruginosa (16.8%) among hospitalized patients of AECOPD. However, the isolation of *Klebsiella* was predominated in mild COPD.

Conclusion

Bacterial isolation was more common in more severe AECOPD (type-1 & type-2 than type-3), Klebsiella infection was most common. Increased sputum purulence & volume significantly indicates bacterial infection in AECOPD.

References

1. GOLD science committee report 2019. Eur Respir J. 2019 May 18;53(5):1900164. doi: 10.1183/13993003.00164-2019. PMID: 30846476
2. Kim, V., Crapo, J., Zhao, H., Jones, P.W., Silverman, E.K., Comellas, A., Make, B.J. and Criner, G.J., 2015. Comparison between an alternative and the classic definition of chronic bronchitis in COPDGene. *Annals of The American Thoracic Society*, *12*(3), pp.332-339.
3. Murray, C.J., Lopez, A.D. and World Health Organization, 1996. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020: summary.
4. Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y. and AlMazroa, M.A., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, *380*(9859), pp.2095-2128.
5. Mosharraf-Hossain, K.M., Islam, S., Kalam, A.A., Pasha, M.M., Sultana, F., Hossain, R.Z., Amin, A. and Murshed, K.M., 2009. Detection of chronic obstructive pulmonary disease using spirometric screening. *Mymensingh Medical Journal: 18*(1 Suppl), pp.S108-112.
6. El Halim, A.A. and Sayed, M., 2015. The value of serum procalcitonin among exacerbated COPD patients. *Egyptian Journal of Chest Diseases and Tuberculosis*, *64*(4), pp.821-827.
7. Sullivan, S.D., Ramsey, S.D. and Lee, T.A., 2000. The economic burden of COPD. *Chest*, *117*(2), pp.5S-9S.
8. Seemungal, T.A., Donaldson, G.C., Bhowmik, A., Jeffries, D.J. and Wedzicha, J.A., 2000. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, *161*(5), pp.1608-1613.
9. White, A.J., Gompertz, S. and Stockley, R.A., 2003. Chronic obstructive pulmonary disease• 6: the aetiology of exacerbations of chronic obstructive pulmonary disease. *Thorax*, *58*(1), pp.73-80.
10. Papi, A., Bellettato, C.M., Braccioni, F., Romagnoli, M., Casolari, P., Caramori, G., Fabbri, L.M. and Johnston, S.L., 2006. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *American journal of respiratory and critical care medicine*, *173*(10), pp.1114-1121.
11. Ram, F.S., Rodriguez-Roisin, R., Granados-Navarrete, A., Garcia-Aymerich, J. and Barnes, N.C., 2006. Antibiotics for exacerbations of chronic obstructive pulmonary disease.*Cochrane Database Systemic Review*, *2*(2), p.CD004403.
12. Wenzel, R.P. and Wong, M.T., 1999. Editorial response: managing antibiotic use—impact of infection control. *Clinical Infectious Diseases*, *28*(5), pp.1126-1127.
13. Stolz, D., Christ-Crain, M., Morgenthaler, N.G., Leuppi, J., Miedinger, D., Bingisser, R., Müller, C., Struck, J., Müller, B. and Tamm, M., 2007. Copeptin, C-reactive protein, and procalcitonin as prognostic biomarkers in

acute exacerbation of COPD. *Chest*, *131*(4), pp.1058-1067.

14. Anthonisen, N.R., Manfreda, J., Warren, C.P.W., Hershfield, E.S., Harding, G.K.M. and Nelson, N.A., 1987. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Annals of Internal Medicine*, *106*(2), pp.196-204.
15. MacIntyre, N. and Huang, Y.C., 2008. Acute exacerbations and respiratory failure in chronic obstructive pulmonary disease. *Proceedings of the American Thoracic Society*, *5*(4), pp.530-535.
16. Eller, J., Ede, A., Schaberg, T., Niederman, M.S., Mauch, H. and Lode, H., 1998. Infective exacerbations of chronic bronchitis: relation between bacteriologic etiology and lung function. *Chest*, *113*(6), pp.1542-1548.
17. Miravitlles, M., Espinosa, C., Fernandez-Laso, E. Martos, J.A., Maldonado, J.A., and Gallego, M., 1999. Relationship between bacterial flora in sputum and functional impairment in patients with acute exacerbations of COPD. *Chest, 116*(1), pp.40-46.
18. Nair, R.S., 2019. Microbial Pattern in Acute Exacerbation of COPD and its Relevance to COPD Severity. *The Journal of the Association of Physicians of India*, *67*(1), p.44.
19. Chawla, K., Mukhopadhyay, C., Majumdar, M. and Bairy, I., 2008. Bacteriological profile and their antibiogram from cases of acute exacerbations of chronic obstructive pulmonary disease: A hospital based study. *Journal of clinical and diagnostic research*, *2*(1), pp.612-616.
20. Patel, A.K., Luhadia, A.S. and Luhadia, S.K., 2015. Sputum bacteriology and antibiotic sensitivity pattern of patients having acute exacerbation of COPD in India–A preliminary study. ***J*** *Pulm Respir Med*, *5*(238), p.2.
21. Madhavi S, Ramarao MV, Janardhanrao R 2012. Bacterial etiology of acute exacerbations of chronic obstructive pulmonary disease. Journal of Microbiology and Biotechnology Research; 2: pp. 440-44.
22. Kuwal, A., Joshi, V., Dutt, N., Singh, S., Agarwal, K.C. and Purohit, G., 2018. A prospective study of bacteriological etiology in hospitalized acute exacerbation of COPD patients: Relationship with lung function and respiratory failure. *Turkish thoracic journal*, *19*(1), p.19.
23. Boixeda, R., Rabella, N., Sauca, G., Delgado, M., Martínez-Costa, X., Mauri, M., Vicente, V., Palomera, E., Serra-Prat, M. and Capdevila, J.A., 2012. Microbiological study of patients hospitalized for acute exacerbation of chronic obstructive pulmonary disease (AE-COPD) and the usefulness of analytical and clinical parameters in its identification (VIRAE study). *International journal of chronic obstructive pulmonary disease*, *7*, p.327.
24. Lin, S.H., Kuo, P.H., Hsueh, P.R., Yang, P.C. and Kuo, S.H., 2007. Sputum bacteriology in hospitalized patients with acute exacerbation of chronic obstructive pulmonary disease in Taiwan with an emphasis on *Klebsiella* pneumoniae and *Pseudomonas aeruginosa*. *Respirology*, *12*(1), pp.81-87.