

Bacterial Species and Inflammatory Cell Variability in Respiratory Tracts of Patients with Chronic Obstructive Pulmonary Disease Exacerbation: A Multicentric Study

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Background and Aim: Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) has profound effects on disease progression and patients' quality of life. Emerging evidence suggests an association between alterations in the respiratory microbiome flora species and airway inflammation in patients with AECOPD. The present study aimed to describe the inflammatory cells and bacterial microbiome distributions in respiratory tract in Egyptian patients with AECOPD.

Subjects and Methods: The present cross-sectional study included 208 patients with AECOPD. Sputum and broncho-alveolar lavage samples from the studied patients were submitted to microbial cultures using appropriate media. Total and differential leukocytic counts and were done via automated cell counter.

Results: The present study included 208 AECOPD patients. They comprised 167 males (80.3%) and 41 females (19.7%) with an age of 57.9 ± 4.9 years. AECOPD was categorized as mild, moderate and severe in 30.8%, 43.3% and 26%, respectively. Sputum samples had significantly higher TLC, neutrophil percent and eosinophil percent when compared with BAL samples. In contrast, lymphocyte percent was significantly higher in BAL samples. Sputum specimens had significantly lower frequency of positive growths (70.2% versus 86.5%, $p = 0.001$). Among the identified organisms, sputum specimens had significantly lower frequency of *Strept. pneumoniae* (14.4% versus 30.3%, $p = 0.001$), *Klebsiella pneumoniae* (19.7% versus 31.7%, $p = 0.024$), *Haemophilus influenzae* (12.5% versus 26.9%, $p = 0.011$), *Pseudomonas aeruginosa* (2.9% versus 10%, $p = 0.019$) and *Acinetobacter spp.* (1.9% versus 7.2%, $p = 0.012$) growths when compared with BAL samples.

Conclusion: The present study could identify a distinctive pattern of inflammatory cell distribution in sputum and BAL samples of AECOPD patients. The most commonly isolated organisms were *Klebsiella pneumoniae* and *Strept. pneumoniae*.

Keywords: chronic obstructive pulmonary disease, COPD exacerbation, inflammatory cells, bacterial microbiome, sputum culture, bronchoalveolar culture

Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of chronic morbidity. It is one of the top three causes of death worldwide and 90% of these deaths occur in low- and middle-income countries.^{1,2} Airways of almost 50% of stable COPD patients are colonized by potentially pathogenic microorganisms (PPMs). The most commonly isolated microbes are *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, and *P. aeruginosa*.³

Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is an acute and sustained worsening of a patient's condition from a stable state.⁴ Possible risk factors include air pollution, seasonal change, ethnic background, associated comorbidities, poor exercise capacity and obesity.⁵ Management strategy usually includes bronchodilators and corticosteroids as first-line treatments. In patients with severe exacerbations, noninvasive ventilation, magnesium, ketamine, and epinephrine may be considered. Mechanical ventilation may be needed in selected situations.⁶

AECOPD has profound effects on disease progression and patients' quality of life. Prevention of early recurrence and identification of causative agents remains one of the top unmet needs in management of AECOPD.⁷ Remarkably, newly developed vaccines against the most frequent bacteria identified in AECOPD are assessed by undergoing trials.⁸ Emerging evidence suggests an association between alterations in the respiratory microbial flora species and airway inflammation in patients with AECOPD.⁹ Identification of respiratory microbiome has been increasingly recognized as a cornerstone in a modern management direction of AECOPD.^{10,11}

Interestingly, it has been shown that clinical features of AECOPD can significantly vary between various populations. This is probably related to genetic and environmental factors. In addition, variations in the distributions of the respiratory microbiota between various populations may be a contributing factor.¹² For example, the predominant isolated organism in one South Korean study¹³ and another Ethiopian one¹⁴ was *Pseudomonas aeruginosa*, while *Klebsiella pneumoniae* prevailed in an Indian study.¹⁵ It was also noted that isolated organisms may vary between upper and lower respiratory tracts in patients with stable COPD and AECOPD.¹⁶ In addition, exaggerated inflammatory response in AECOPD patients was related to poor outcome.¹⁷ Distribution of inflammatory cells in respiratory tracts of AECOPD patients may be related to disease severity and treatment response.^{18,19}

The present study aimed to describe the isolated bacterial species and inflammatory cell distributions in upper and lower respiratory tracts in Egyptian patients with AECOPD.

Patients and Methods

This cross-sectional study was conducted at chest diseases and clinical pathology departments of Al-Zahraa, Al-Hussein, Zagazig and Benha university hospitals during the period from January 2022 to August 2022. An informed written consent was gotten from every patient before enrollment into the study in accordance with Declaration of Helsinki guidelines. The study was approved by the ethical committee of Al-Azhar University Faculty of Medicine for Girls.

The study included 208 patients with AECOPD. COPD was diagnosed, and its severity was graded according to the modified criteria defined in the Global Initiative for Chronic Obstructive Lung Disease guidelines.²⁰ Clinically, an exacerbation was defined as a worsening of respiratory symptoms that led the patient to contact health-care facilities and assessed using the Anthonisen criteria.²¹ AECOPD was staged as mild if there was worsening of symptoms that were self-managed (eg increase in salbutamol use) and improved without systemic corticosteroids or antibiotics. Moderate AECOPD was defined if treatment with systemic corticosteroids or antibiotics or both was required. Severe AECOPD was defined if hospitalization was required.²²

Spirometry was performed according to the standard recommendations.²³ Fixed airways obstruction was diagnosed if there was post-bronchodilator $FEV_1/FVC < 0.7$. Patients were excluded if they refused to perform or did not complete fiberoptic bronchoscopy (FOB) or if they had other chest diseases.

Sputum Sampling

Sputum samples were collected in the day of admission and before starting antibiotic or steroid therapy according to Shepherd.²⁴ Samples were placed in a sterile container and delivered immediately to the microbiology laboratory where they were rapidly processed in less than two hours.

Bronchoalveolar Lavage Sampling

Bronchoalveolar lavage (BAL) sampling was performed in the same day of admission before starting antibiotic or steroid therapy after detailed explanation of the technique to the patients. Oxygen was administered by a nasal cannula, and flows were adjusted upward from 2 L/minute to keep oxygen saturation $> 90\%$. With the patient placed in a semi-supine position under midazolam (2.5- mg) sedation and topical anesthesia, the fiberoptic bronchoscope (FOB) was introduced

through the nose, passed through the vocal cords, and a complete airway inspection was performed. FOB was gently impacted or “wedged” through both middle lobe and lingual bronchi. BAL was obtained by aspiration of any secretion and instillation of 80 mL of sterile isotonic saline solution followed by immediate aspiration by suction into a clean and sterile container (polypropylene). The BAL fluid was transported to the microbiology laboratory immediately and processed in less than two hours.²⁵

Examination of Sputum and BAL Gram-Stained Smear

Before counting the inflammatory cells or culturing sputum sample, a Gram-stained smear was done directly from the specimen to assess the quality of the sample using Bartlett’s grading system (Q score). Sputum samples harboring ≥ 10 leucocytes with < 25 squamous epithelial cells per low-power field ($< 10/\text{LPF}$) were accepted and cultured.²⁶ If the sputum or BAL samples were considered of good quality (lower respiratory tract specimen), examination of the slide under oil immersion (1000X) magnification for bacteria was done and culturing the specimen was performed.

Cultivation, Quantification and Identification of Sputum and BAL Specimens

Sputum and BAL cultures were done on routine media used for the isolation and identification of respiratory pathogens including (blood agar, chocolate agar, and MacConkey agar). Semi-quantitative cultures were done using the calibrated loop method. About 0.1 mL of specimen was plated onto solid media, and CFU were counted. CFU/mL was calculated using the formula: $\text{cfu/mL} = (\text{no. of colonies} \times \text{dilution factor})/\text{volume of culture plate}$.²⁷

Blood agar and chocolate agar plates were incubated in 5–7% CO_2 , while MacConkey agar plates were incubated in aerobic conditions. All plates were incubated at $36.0 \pm 1.0^\circ\text{C}$ for 24 hours.²⁸ To avoid overestimation of airway colonization with bacterial microbiome, specimens with $\text{CFU} < 10^4/\text{mL}$ were considered colonization and excluded from the study, whereas specimens with $\text{CFU} \geq 10^4/\text{mL}$ were considered infection and further processed for the identification of bacteria using biochemical reactions.²⁹ For gram-positive bacteria, catalase, mannitol fermentation, DNase, and optochin sensitivity tests were used while for gram-negative bacteria, TSI, LIA, MIO, Indole, Citrate utilization, Urease and Oxidase tests were used.

Total Leukocytic and Differential Leukocytic Count in Sputum and BAL

After good processing of sputum and BAL samples, small amounts of liquefied sputum and BAL fluid specimens were centrifuged at an appropriate speed, resuspended, and analyzed for TLC and differential count. TLC and differential count were done via automated cell counter using a hematological analyzer (SysmexXE-21N, Kobe, Japan). The TLC/mL and the percentage of neutrophils, lymphocytes and eosinophils were recorded.

Statistical Analysis

Data were statistically analyzed by the Statistical Package for Social Science (SPSS) program version 17.0 (SPSS Inc., Chicago, USA). The Shapiro–Wilk test was used for testing normality of the studied variables. Descriptive analysis was done for each item, and the results were expressed mean \pm SD for parametric data, and as percentages for nominal data. Comparisons to assess the difference between the groups were done using the Chi-square χ^2 test for qualitative data and by independent t -test for parametric data. A linear correlation coefficient was used for the detection of a correlation between inflammatory cells in sputum and its corresponding one in BAL. Statistical significance was considered at a p value < 0.05 .

Results

The present study included 208 AECOPD patients. They comprised 167 males (80.3%) and 41 females (19.7%) with an age of 57.9 ± 4.9 years. AECOPD was categorized as mild, moderate and severe in 30.8%, 43.3% and 26%, respectively. Other clinical findings of the studied patients are shown in Table 1. Total and differential leukocytic counts in sputum and BAL are shown in Table 2. Sputum samples had significantly higher TLC (6.5 ± 1.2 versus 5.6 ± 1.5 cells/mL, $p = 0.001$) neutrophil percent (83.4 ± 7.4 versus 38.1 ± 18.6 , $p = 0.001$) and eosinophil percent (2.8 ± 1.6 versus 0.7 ± 0.7 , $p = 0.001$)

Table 1 Characteristics of Studied Patients (n = 208)

Age (Years) Mean \pm SD	57.9 \pm 4.9
Male/female n	167/41
FEV ₁ /FVC ratio mean \pm SD	62.4 \pm 4.8
FEV1% mean \pm SD	54.2 \pm 12.7
FVC % mean \pm SD	65.2 \pm 10.7
FEF 25–75% mean \pm SD	45.3 \pm 11.1
COPD severity n (%)	
Mild	4 (1.9)
Moderate	113 (54.3)
Severe	91 (43.8)
AECOPD severity n (%)	
Mild	64 (30.8)
Moderate	90 (43.3)
Severe	54 (26.0)

Abbreviations: AECOPD, Acute exacerbation of chronic obstructive pulmonary disease; COPD, Chronic obstructive pulmonary disease; FEV₁, Forced expiratory volume 1; FEF, Forced expiratory flow; FVC, Forced vital capacity.

Table 2 Comparison of Inflammatory Cells Between Sputum and BAL in Patients with AECOPD

		Sputum (n = 208)	BAL (n = 208)	P-value
TLC/ mL	Mean \pm SD	6.5 \pm 1.2	5.6 \pm 1.5	0.001
	Range	4.18–9	1.8–7.9	
Neutrophils %	Mean \pm SD	83.4 \pm 7.4	38.1 \pm 18.6	0.001
	Range	13.0–91.6	15.0–73.3	
Lymphocytes %	Mean \pm SD	2.5 \pm 1.2	14.1 \pm 6.6	0.001
	Range	0.81–5.1	3.5–28.9	
Eosinophils %	Mean \pm SD	2.8 \pm 1.6	0.7 \pm 0.7	0.001
	Range	1.32–6.82	0.1–3.8	

Abbreviation: TLC, Total leucocytic count.

when compared with BAL samples. In contrast, lymphocyte percent was significantly higher in BAL samples (2.5 ± 1.2 versus 14.1 ± 6.6 , $p = 0.001$) (Table 2). Correlation analysis identifies significant correlation between TLC and differential count elements in sputum and BAL samples (Table 3).

Sputum specimens had significantly higher frequency of positive growths (70.2% versus 86.5%, $p = 0.001$). Among the identified organisms, sputum specimens had significantly lower frequency of *Strept. pneumoniae* (14.4% versus 30.3%, $p = 0.001$), *Klebsiella pneumoniae* (19.7% versus 31.7%, $p = 0.024$), *Haemophilus influenzae* (12.5% versus

Table 3 Correlation Between Sputum and BAL Cellular Findings

Sputum	BAL							
	TLC		Neutrophil Count		Lymphocyte Count		Eosinophil Count	
	r	p	r	p	r	p	r	p
TLC	0.5	<0.001	–	–	–	–	–	–
Neutrophil count	–	–	0.51	<0.001	–	–	–	–
Lymphocyte count	–	–	–	–	0.79	<0.001	–	–
Eosinophil count	–	–	–	–	–	–	0.51	<0.001

Abbreviation: TLC, Total leucocytic count.

Table 4 Comparison of Bacterial Microbiome Between Sputum and BAL in Patients with AECOPD

	Sputum (n = 208)	BAL (n = 208)	p-value
No growth n (%)	62 (29.8%)	28 (13.5%)	0.001
Isolated organisms n (%)			
Gram +ve			
<i>Strept. pneumoniae</i>	30 (14.4)	63 (30.3)	0.001
<i>Staphylococcus aureus</i>	41 (19.7)	45 (21.6)	0.151
Gram -ve			
<i>Klebsiella pneumoniae</i>	41 (19.7)	66 (31.7)	0.024
<i>Haemophilus influenzae</i>	26 (12.5)	56 (26.9)	0.011
<i>E. coli</i>	22 (10.6)	27 (13)	0.083
<i>Pseudomonas aeruginosa</i>	6 (2.9)	21 (10)	0.019
<i>Acinetobacter spp.</i>	4 (1.9)	15 (7.2)	0.012
<i>Enterobacter</i>	1 (0.5)	1 (0.5)	1.0

26.9%, $p = 0.011$), *Pseudomonas aeruginosa* (2.9% versus 10%, $p=0.019$), and *Acinetobacter spp.* (1.9% versus 7.2%, $p = 0.012$) growths when compared with BAL samples (Table 4, Figures 1 and 2).

Distribution of bacterial microbiome combinations is shown in Table 5. BAL samples retrieved significantly higher frequency of two (31.5% versus 17.1%) and three (15.5% versus 0.0%) organisms as compared to sputum samples.

Discussion

In the present study, we could identify a distinctive pattern of inflammatory cell distribution in sputum and BAL samples of AECOPD patients. Our findings are in line with Maestrelli et al³⁰ who found that the relative proportion of inflammatory cells was different in sputum, BAL, and bronchial mucosa. Accumulation of neutrophils in the airways is recognized as a prominent feature of COPD, with the extent of neutrophilic infiltration both in the airways and tissues correlating with disease severity.³¹ The higher neutrophil percentage in sputum may be attributed to the fact that sputum originates from the upper and mid airways that are well known for their neutrophil-rich secretions compared with BAL.³²

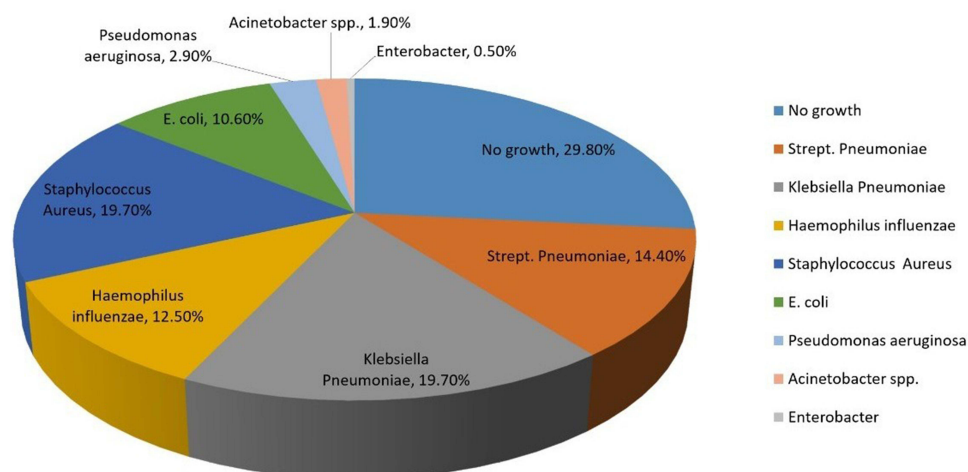


Figure 1 Distribution of bacterial organisms in large airways in patients with COPD exacerbation.

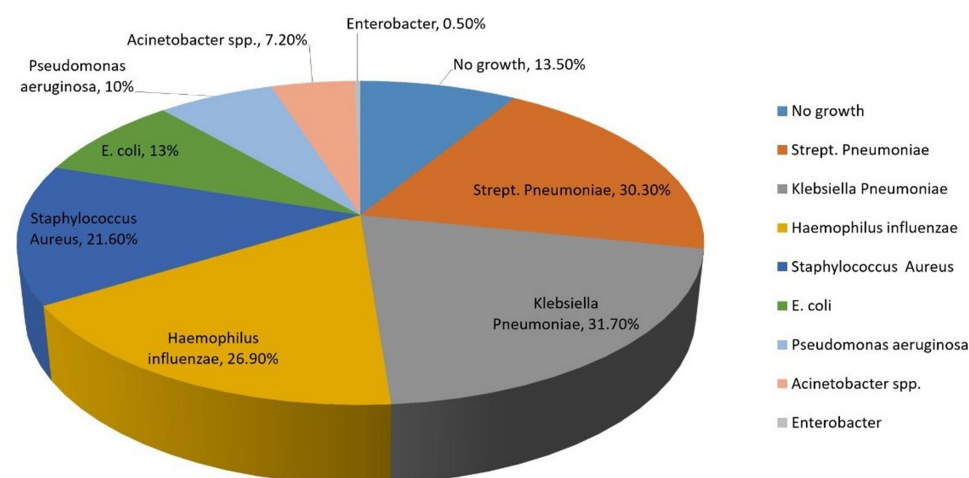


Figure 2 Distribution of bacterial organisms in small airways in patients with COPD exacerbation.

In current study, we found that only the level of lymphocytes in sputum was significantly lower than in BAL. We speculated that the low level of lymphocytes in the sputum better reflected the immunosuppression of COPD and further information is required to evaluate the correlation between lymphocytes and COPD. In COPD, sputum consists mainly of neutrophils, while it has less macrophage and even less lymphocytes. BAL, on the other hand, consists mainly of macrophages, with less neutrophils and lymphocytes.³³

In the present study, there were higher rates of isolation of *Klebsiella pneumoniae*, *Strept. pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Acinetobacter spp.* from BAL than sputum. These findings indicate that in patients with AECOPD, the bronchiolar and alveolar compartments are harboring more bacterial microbiome than large airways.

In comparison, De Serres et al³⁴ found that the most frequently isolated organism from sputum of 108 patients with AECOPD was *S. aureus*. Sharan³⁵ found that the commonest isolate was *Klebsiella pneumoniae* followed by *Staphylococcus aureus* from sputum of 107 patients with AECOPD. Monsó et al³⁶ found that the most prevalent microorganism in sputum of COPD exacerbation patients was *H. influenzae* followed by *M. catarrhalis* and *S. pneumoniae*.

Table 5 Distribution of Bacterial Microbiome Combinations in Sputum and BAL in Patients with AECOPD

	Sputum (n = 208)	BAL (n = 208)	p-value
Isolated organisms count n (%)			
• One organism	121 (82.9)	96 (53)	0.002
• Two organisms	25 (17.1)	57 (31.5)	
• Three organisms	-	28 (15.5)	
Combinations of bacterial microbiome n (%)			
<i>Haemophilus influenzae</i> and <i>Staphylococcus aureus</i>	3 (1.4)	3 (1.4)	1.0
<i>Haemophilus influenzae</i> and <i>Strept. pneumoniae</i>	10 (4.8)	29 (13.9)	0.001
<i>Pseudomonas aeruginosa</i> and <i>Klebsiella spp.</i>	4 (1.9)	6 (2.9%)	0.156
<i>Klebsiella</i> and <i>Staphylococcus aureus</i>	5 (2.4)	11 (5.3%)	0.126
<i>Klebsiella</i> and <i>E. coli</i>	3 (1.4)	-	0.082
<i>E. coli</i> and <i>Acinetobacter spp.</i>	-	4 (1.9)	0.082
<i>Klebsiella pneumoniae</i> and <i>Acinetobacter spp.</i>	-	3 (1.4)	0.317
<i>Haemophilus influenzae</i>, <i>Klebsiella</i> and <i>Staphylococcus aureus</i>	-	9 (4.3)	0.044
<i>E. coli</i>, <i>Pseudomonas</i>, and <i>Klebsiella pneumoniae</i>	-	14 (6.7)	0.001
<i>Acinetobacter spp.</i>, <i>Klebsiella pneumoniae</i>, and <i>Strept. pneumoniae</i>	-	6 (2.9)	0.014

In another study, *Streptococcus* was the most commonly isolated organism in sputum of 36 Chinese patients with AECOPD.³⁷ Another work from Norway³⁸ found that *Streptococcus*, *Veillonella*, *Prevotella* and *Gemella* were the most abundant genera in patients with moderate and severe COPD exacerbations, while the study of Goolam Mahomed et al³⁹ identified *Haemophilus* and *Streptococcus* as the most common genera.

This disagreement reflects the diverse respiratory microbiome components among different populations with AECOPD which may be attributed to genetic, immunological and environmental factors.

Altered microbial profile in the respiratory tract may predispose to AECOPD through multiple mechanisms. Recently, Zhu et al,⁴⁰ suggested that changes of microbiota can modify interleukin-17a (IL-17a) expression by downregulating microRNAs (miR-122 and miR-30a) expression. Findings of the present study may have significant therapeutic implications. Prevention of AECOPD by modifying the lower respiratory tract using different medications was suggested.⁴¹

In conclusion, the present multicentric study documented the microbial profile of the upper and lower respiratory tract of Egyptian patients with AECOPD. We could identify a distinctive pattern of inflammatory cell distribution in sputum and BAL samples of AECOPD patients. The most commonly isolated organisms were *Klebsiella Pneumoniae* and *Strept. pneumoniae*.

Conclusions of the present study may have some limitations. Microbial findings during exacerbations were not compared to corresponding results in the stable state which are strongly recommended in a subsequent study. Also, the microbial isolation and identification methods used by the present study have known limitations regarding specificity, sensitivity and reliability, which may differ from that used by other studies.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the ethical committee of Al-Azhar University Faculty of Medicine for Girls (Approval No. 886), and all patients provided informed consent before enrolment.

Informed Consent

Informed consent was obtained from all patients.

Consent for Publication

All authors reviewed the manuscript and approved its submission.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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