# Comparison of Composition and Diversity of Bacterial Microbiome in Human Upper and Lower Respiratory Tract

**Zhi-Hong Feng[1], Qin Li[2], Si-Ran Liu[2], Xiao-Nan Du[2], Chen Wang[3,4], Xiu-Hong Nie[1], Wei Wang[2], Sun Ying[2]**

[1]Department of Respiratory Medicine, Beijing Xuanwu Hospital, Capital Medical University, Beijing 100053, China
[2]Department of Immunology and The Research Centre of Microbiome, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, China
[3]Department of Respiratory Disease, Capital Medical University, Beijing 100069, China
[4]China-Japan Friendship Hospital, National Clinical Research Center for Respiratory Diseases, Beijing 100029, China

Zhi-Hong Feng and Qin Li contributed equally to the study.

**Key words:** 16S rRNA Gene Sequencing; Bronchoalveolar Lavage Fluid; Microbiota; Respiratory Tract; Sputum

## Introduction

Microbiome residing in the airways and parenchymal tissues, as a biological barrier of respiratory tract, plays an important role in maintaining the normal functions of individual's respiratory system and preventing the invasion and colonization of exogenous pathogens.[1] Changes of microbial community may result in the occurrence and progression of infectious pneumonia and acute exacerbation of chronic diseases, through disordering commensal microorganisms and increasing invasive ability of pathogens.[2] Traditional methods for detecting bacteria normally rely on culture technique, which have a number of critical problems, including time-consuming and relatively low positive rate of detection. In the last 10 years, 16S rRNA gene sequencing technique has been thought to be more comprehensive and reliable than those of culture-dependent methods for monitoring bacterial microbiome in skin, organs, and tracts quantitatively and qualitatively.[3] In this case, choosing credible samples are critical to evaluate the community composition of microbiome in the host tracts, including respiratory tract. In the present study, we aimed to compare the composition and diversity of bacterial community structure in different samples collected from the individuals' airways using 16S rRNA gene sequencing technique.

## Methods

### Ethical approval

The study was approved by the Medical Ethics Committee of Xuanwu Hospital, Capital Medical University, China. All individuals signed written informed consent.

Six male individuals (age: 55–78 years old), diagnosed with lung space occupying lesions based on X-ray computed tomography and normal lung function performed by spirometry, were recruited from Xuanwu Hospital, Capital Medical University, China. Individuals with a history of smoking, upper respiratory tract infection within 4 weeks, and antibiotics used within 3 months were excluded from the study.

Three types of samples, oral wash fluid (OWF), induced sputum, and bronchoalveolar lavage fluid (BALF), were obtained from participants according to standardized procedures.[3] The isolation, amplification, and sequencing of bacterial DNA from each sample were performed by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The amplicons were obtained using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), which covered V3-V4 region of the bacterial 16S rRNA genes, and then sequenced by Illumina MiSeq Platform (San Diego,

**Address for correspondence:** Dr. Xiu-Hong Nie, Department of Respiratory Medicine, Beijing Xuanwu Hospital, Capital Medical University, Beijing 100053, China
E-Mail: 13391812138@126.com

**Access this article online**

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.204934



**Received:** 01-01-2017 **Edited by:** Ning-Ning Wang
**How to cite this article:** Feng ZH, Li Q, Liu SR, Du XN, Wang C, Nie XH, Wang W, Ying S. Comparison of Composition and Diversity of Bacterial Microbiome in Human Upper and Lower Respiratory Tract. Chin Med J 2017;130:1122-4.

CA, USA). The resulting sequences were trimmed using trimmomatic and clustered into operational taxonomic units (OTUs) with a 97% similarity threshold by USEARCH. Alpha-diversity analysis estimating community richness (Chao and Ace indices) and diversity (Shannon and Simpson indices) was calculated using Mothur software (http://www.mothur.org/wiki/). Phylum-level taxonomic distribution and Venn graph were performed in R language. Statistical analyses were performed using GraphPad Prism 6 version (GraphPad Software, Inc., San Diego, California, USA).

## Results

After collection of the sequences trimmed and normalized, 33,628 high-quality reads of each sample were obtained. Alpha-diversity analysis showed that community richness (Ace and Chao indices) of BALF was higher than those in oral wash or in sputum ($P < 0.05$) [Figure 1a and 1b]. Furthermore, there was also a significant difference in the community diversity (Shannon and Simpson indices) between BALF and sputum ($P < 0.05$) [Figure 1c and 1d]. The most two abundant bacterial phyla in terms of both sequences and OTUs were *Proteobacteria* and *Firmicutes* in BALF, sputum, and OWF (the proportion of which accounted for all sequences in samples was 77.69%, 65.86%, and 63.96%, respectively). The third abundance of the sequences belonged to *Bacteroidetes*. The sum of relative abundance of *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* was more than 90% [Figure 1e]. The number of OTUs shared among BALF, sputum, and OWF was 571; the number shared between BALF and sputum was 109, while the number of OTUs shared between sputum and OWF was just 21 [Figure 1f].

## Discussion

Changes of bacterial microbiome are associated with pathogenesis of infectious and noninfectious diseases. The credible samples are critical to evaluate the community composition of microbiome. To investigate the composition and diversity of the microbiome of airway, we analyzed and compared the microbiota of BALF, sputum, and OWF using bacterial 16S rRNA gene sequencing technique.

Our data showed that the community richness and diversity of BALF were higher than those in OWF or in sputum. Such observations appeared to be different from other studies.[4] A potential explanation is that microbiome in airways may vary with local environment and individual status, especially in those elders (median age was 64 years) with pulmonary space occupying lesions. In BALF, sputum, and OWF, the most relative abundances of microbiota were *Proteobacteria* and *Firmicutes* phylum. At genus level, *Haemophilus*, normally causing the secondary infection in the respiratory tract, existed in all three types of samples. This suggests that such bacteria might exist in upper and lower airway, even in healthy individuals. Moreover, there was substantial overlap in the microbial composition among the samples, and the percentages of total number of OTUs in sputum consistence with BALF or OWF were highly represented as 85.55% and 79.39%, respectively. This suggested that microbiota in the lower airways might derive from the upper respiratory tract.

It should be noted that although the next generation sequencing provides a great chance to investigate the microbiome of airways, some attentions still need to be considered, such as differentiation between dead and alive bacteria and interpretation of data.[5] In addition, when sampling BALF or sputum, contamination from the upper respiratory tract and lacking of homogeneity of the bacterial community crossing the airway are also inevitable in most instances, even if the strict operational instructions are followed. Such limitations may cause that the many studies of microbiota based on BALF and sputum overestimate bacterial composition and load in lower respiratory tract.

In summary, our data provide direct evidence to evaluate sampling methods for investigating bacterial microbiome in airways, while induced sputum samples represent those in the lower airway in a great extent. Therefore, induced sputum

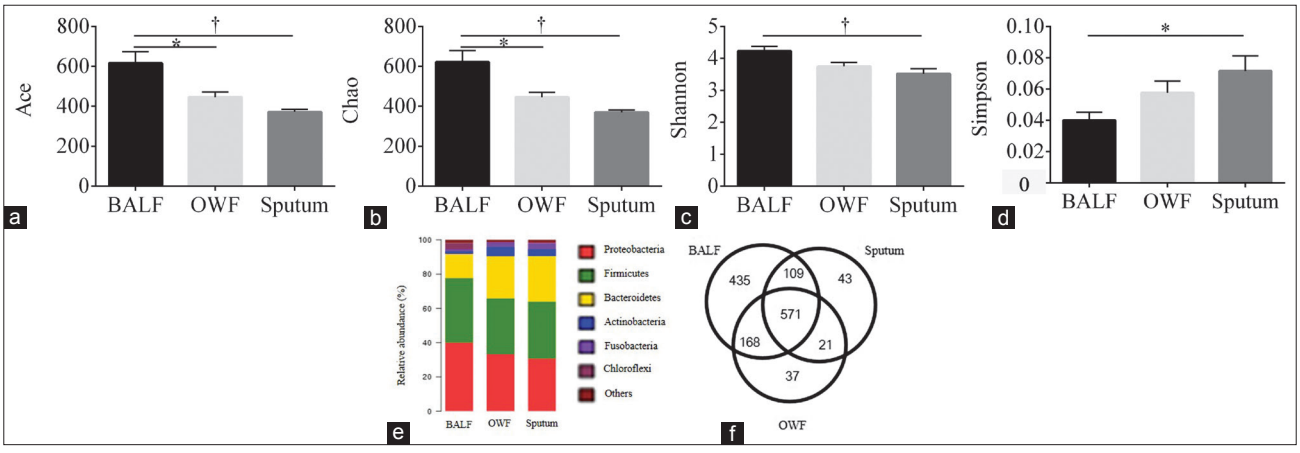


**Figure 1:** Comparison of composition and diversity of bacterial microbiome in human respiratory tract. (a-d) Alpha-diversity analysis in community richness and diversity. OTUs were clustered with a 97% similarity threshold; (e) relative abundance of the bacterial phyla in BALF, sputum, and OWF. The relative abundance of <1% had been classified as others; (f) overlaps of BALF, sputum, and OWF in OTUs composition. *$P < 0.05$; †$P < 0.01$; BALF: Bronchoalveolar lavage fluid; OWF: Oral wash fluid; OTUs: Operational taxonomic units.

could be a potential source for exploring microbiota in the lower airways because it is convenient to be obtained and makes less discomfort of individuals.


## Financial support and sponsorship

This study was supported by the grants from the National Natural Science Foundation of China (No. 81373177, and No. 81471594), the Key Projects in the National Science and Technology pillar program (No. 2013BAI09B10) and the Basic-clinical Research Cooperation Issues of Capital Medical University (No. 17JL90).


## Conflicts of interest

There are no conflicts of interest.

## References


1. Zhang N, He QS. Commensal microbiome promotes resistance to local and systemic infections. Chin Med J 2015;128:2250-5. doi: 10.403/0366-6999.162502.
2. Segal LN, Blaser MJ. A brave new world: The lung microbiota in an era of change. Ann Am Thorac Soc 2014;11 Suppl 1:S21-7. doi: 10.1513/AnnalsATS.201306-189MG.
3. Iwai S, Fei M, Huang D, Fong S, Subramanian A, Grieco K, *et al.* Oral and airway microbiota in HIV-infected pneumonia patients. J Clin Microbiol 2012;50:2995-3002. doi: 10.1128/JCM.00278-12.
4. Pragman AA, Kim HB, Reilly CS, Wendt C, Isaacson RE. The lung microbiome in moderate and severe chronic obstructive pulmonary disease. PLoS One 2012;7:e47305. doi: 10.1371/journal.pone.0047305.
5. Sze MA, Hogg JC, Sin DD. Bacterial microbiome of lungs in COPD. Int J Chron Obstruct Pulmon Dis 2014;9:229-38. doi: 10.2147/COPD.S38932.