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Editorial

Lung microbiome studies – new insights to respiratory diseases

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Can lung microbiome knowledge be exploited to promote health and to potentially diagnose, prevent, and treat lung disease? Before finding an answer to this we need to understand the meaning of the two words microbiota and microbiome. Primarily a microbiota is an ecological community of commensal, symbiotic or pathogenic living microorganisms found in and on all multicellular organisms studied to date from plants to animals as well as humans. Microbiota includes bacteria, archaea, protists, fungi and viruses. The term microbiome refers to the genome of microorganisms' entire population, including their metabolites. That is, all of the genes inside these microbial cells, are what constitute the microbiome.

Recognizing the importance of the microbiome in human health, in 2008 the National Institute for Health (NIH) launched the Human Microbiome Project (HMP) to characterize the communities of bacteria on and within the human body and to explore their effects on health and disease. However due to historic belief that lungs were considered sterile in health, lung microbiome was not included initially in the HMP. But with the aid of novel culture-independent techniques researchers have demonstrated the presence of diverse communities of microbes in the lower respiratory tract paying the way to a project named "Lung HIV Microbiome", aiming to outline lung microbiome and shed light to changes observed in lung disease.

According to a review the microbiome of the lung has relatively less bacterial biomass when compared to the lower gastrointestinal tract yet displays considerable diversity. The composition of the lung microbiome is determined by elimination, immigration and relative growth within its communities. Chronic lung disease alters these factors. Bacterial loads from bronchoalveolar lavage have reported ranges from 4.5 to 8.25 log copies per/ml. Further analysis of lung tissue samples demonstrates some 10 - 100 bacterial cells per 1000 human cells. Considering as the gold standard, culture-based techniques have been widely used for microorganism identification from biological fluids [sputum, bronchoalveolar lavage (BAL)] and tissues. Considerable number of microbial species still cannot be grown in most defined culture conditions. As around 0-20% microorganisms are culturable in laboratory growth media, the need for novel methods in pathogen identification became vital and advances made in molecular biological techniques such as PCR, microarrays and metagenomics led to the greater understanding of microbiota characteristics in healthy persons and patients suffering from

diverse lung disorders. The gene coding for 16S rRNA consists of highly conserved regions alternating with nine hypervariable regions (V1-V9). Small sequences within these hypervariable regions were shown to be distinct among bacterial species. Sequence analysis and comparison of accurate sequences with those available in verified databases have allowed identification of both slow and rapidly growing mycobacteria, and unculturable bacteria, such as *Treponema pallidum* in lungs both in healthy as in diseased. Most widely used technique for the organism identification was the Sanger sequencing which is based on elongation of the primer of interest to accurately determine DNA sequences. The major disadvantage of conventional Sanger method, is the poor throughput. But the answer for that was the next generation sequencing (NGS). NGS, is able to carry out multiple sequencing reactions, thereby increasing the output. Additionally mutations in small cells subpopulations are also recognized and gives the chance to examine differences in microbiota among separate lung regions.

An argument against molecular techniques is that 16S rRNA gene sequencing, along with other DNA-based methods, overestimate the lung bacterial load, as they cannot differentiate between viable and non-viable microorganisms and hence these novel techniques should be viewed as a complement to, not a replacement for, traditional methods of microbial identification. Validity of this statement has been opposed by one researcher who was able to cultivate 61% of species identified with 16s rRNA gene sequencing methods in healthy human BAL samples.

The human microbiota inhabits several organs and is primarily colonized by six phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria and Cyanobacteria. Bacteroides, Firmicutes and Proteobacteria have been systematically identified in healthy lungs using culture independent techniques and *Streptococcus, Pseudomonas, Prevotella* and *Veillonella* were the genera identified. Research have also shown that lung microbiota significantly differs from mouth bacteria. Differences were also identified between BAL samples and oropharynx samples from same individuals and also between healthy smokers and non-smokers lung microbiota. In comparison to gastrointestinal tract, it appears that respiratory tract has largely homogenous microbiota. Considering lung transplants, a greater variety of different bacteria had been identified, compared to healthy controls. These were in the majority Proteobacteria, whereas in healthy lungs Proteobacteria (class Gammaproteobacteria) and Firmicutes were predominant.

Although geographical differences have been reported in gut microbiota in healthy controls the expectations were that lung microbiome would be altered according to climate. Patients diagnosed with cystic fibrosis from two centers from U.K. and U.S had revealed significant heterogeneousness between the groups in bacterial populations inhabiting lower respiratory tract. Studies have shown that there is no significant spatial variation in healthy individual's lung microbiota, thus proving that BAL results coming from a discrete lung segment can be representative of the individual's microbiota, if healthy. However lung microbiota was shown to differentiate among segments in severe COPD and CF patients. Culture-independent techniques, revealed that COPD patients lower airways exhibited a statistically important decrease in Bacteroidetes (specifically *Prevotella* spp.) and a reverse increase in Proteobacteria phylum (particularly *Haemophilus* spp.), thus proving for the first time an alteration in COPD lung microbiota. No important differences were observed in terms of quantitative results, though bacterial diversity was reported to be significantly diminished in patients diagnosed with moderate to severe COPD. Their BAL samples were highly abundant in *Prevotella*, *Pseudomonas*, *Streptococcus* and *Heamophilus*, genera which were also present in healthy controls. A study had listed phylum Firmicutes, in severe COPD patients while another study showed the presence of *Moraxella*, *Curvibacter* and *Corynobacterium* and correlated the presence of *P. Aeruginosa* with a significant decrease in microbiome diversity identified in COPD patients BAL samples. The conclusion arrived was that COPD patients clustered not according to disease stage but according to the use of inhaled corticosteroids and other bronchodialators, a fact attributed to their interference with immune response to lung microbiota.

External stimuli (allergens) or microbial communities causing acute infections trigger an asthma exacerbation. Studies have shown that allergies may develop early in life and may even occur before birth Research reveals that during exacerbations lower respiratory tract of asthmatic young children is inhabited by *Moraxella catarrhalis, Haemophilus-influenzae,* or *Streptococcus pneumoniae*. Studies based on sequence analysis by PCR revealed that nasal colonization by *Moraxella catarrhalis* and *Streptococcus pneumoniae* correlates highly with severe asthma exacerbations caused by rhinovirus. Most adult asthmatics sputum samples show greater variety of bacteria and high abundance of Proteobacteria than the healthy. To the extent that idiopathic pulmonary fibrosis (IPF) is concerned, it was shown that specific genera, including *Staphylococcus* and *Streptococcus*, were more abundant in progressive disease rather than stable IPF state. IPF patients had double the bacterial burden of healthy controls and specifically *Haemophilus, Streptococcus, Neisseria,* and *Veillonella* species. Also this bacterial abundance

was shown to be predictive of progressive lung dysfunction and death, inferring the pathogenesis of the disease. Researchers are currently studying lung microbiota in patients diagnosed with lung cancer and the impact of changes in lower respiratory tract microbiome in carcinogenesis. Female patients with no smoking history who were being diagnosed with lung cancer, had shown a significant microbiome disturbance in sputum that was not seen in oral washes samples. Their sputum samples showed higher abundance in *Streptococcus*, *Granulicatella* and *Abiotrophia* genera when compared to healthy controls. According to literature as these bacteria have the potential to cause infections of nervous system, higher and lower respiratory tract and chronic vascular inflammation, and suggest a new role of microbiota in lung cancer pathogenesis. The researchers are also looking at the role of the pulmonary microbial communities in diverse lung diseases such as rheumatoid arthritis (RA). According to experts as this research still in its early stages, lung microbiome research has focused only on the identification of bacterial phyla, but as in other body sites, the interactions between fungi and bacteria may occur in the lungs at physical and chemical levels which needs to be investigated. In addition eukaryotes and viruses have been largely neglected, mainly due to technical difficulties, such as their significantly lower biomass and the lag in the development of databases such as the Ribosomal Database Project. No studies to date have examined the dynamic changes that may occur in the lower respiratory tract microbiota as childhood progresses.

The growing literature has demonstrated that a distinct microbiota of the respiratory tract is present both in health and in various respiratory diseases, though the biological and clinical significance of these findings yet to be explored and according to many the value of lung microbiome studies has been undervalued.

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