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Microbiome Analysis Using Bronchoscopy for Diagnosis and Prognosis of Lung Disease: A Systematic Review

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Abstract

With their ability to produce antibiotics, influence drug transport, and serve as vehicles or adjuvants for drug delivery, microbial signatures may provide new information on the pathophysiology of different lung illnesses. Most investigations of lung microbiome signatures were previously conducted using bronchoalveolar lavage (BAL) fluid and usually required bronchoscopy, a technique that involves passing an optical device through the airways to visualize the tracheobronchial tree. In the context of lung illnesses, this method is a multipurpose modality with diagnostic and therapeutic potential. To diagnose lung illness using bronchoscopy samples, we conducted a comprehensive literature search to identify clinical trials that evaluated the use of microbial signature analysis using polymerase chain reaction (PCR). Only 17 of the 1,784 studies met the inclusion criteria. The effect of pulmonary microbiota on the outcome of lung disease has been the subject of few studies. The data and results indicated that microbial signatures are significantly associated with lung disease. Despite conflicting findings, bronchoscopy-based analysis of lung microbiome signatures for lung disease diagnosis and prognosis remains a promising new area of treatment. Analysis of lung microbial signatures opens the door to the possibility of restoring native microorganisms and treating dysbiosis by manipulating the composition of the lung microenvironment.

Keyword: Microbiota, Lung Disease, Bronchoscopy, Diagnosis, Prognosis

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Abbreviations: Acute respiratory distress syndrome (ARDS); bronchoalveolar lavage (BAL); Coronavirus disease 2019 (COVID-19); chronic obstructive pulmonary disease (COPD); idiopathic pulmonary fibrosis (IPF); Newcastle-Ottawa Scale (NOS); polymerase chain reaction (PCR); population, intervention, comparison, and outcome (PICO); transbronchial needle aspiration (TBNA).

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INTRODUCTION

The microbiome, also known as the microbial signature, is an exhaustive inventory of all microbes that inhabit the human body and impact health. The multipurpose microbial signature may produce antibiotics, alter the rate and direction of drug transport, serve as a drug delivery method, or act as an adjuvant to other drugs.[1] Probiotics and prebiotics are examples of microbial signatures that are also thought of as medications. Probiotics are essential for preserving the microbial equilibrium in the respiratory system, and there has been a recent increase in interest in the link between lung microbiota and respiratory disorders.[2] Probiotics are living microorganisms that, when consumed in adequate amounts, have beneficial effects on host health. While probiotics are widely recognized for their role in maintaining gut health, emerging research suggests that they also play a crucial role in preserving the microbial balance within the respiratory system. By supporting a healthy microbiota in the upper respiratory tract, probiotics help reduce the risk of infections, such as upper respiratory tract infections (URIs), acting as a defense against viral and bacterial invasions. Certain probiotic strains have demonstrated the ability to inhibit the growth of harmful pathogens, produce antimicrobial substances, and enhance the integrity of the epithelial cell barrier in the respiratory tract. These functions are vital for maintaining a balanced and resilient respiratory microbiota. Moreover, probiotics regulate immune system activity, influencing both innate and adaptive immune responses. This regulatory effect helps the host immune system recognize and combat potential threats more effectively, contributing to the prevention and management of respiratory diseases.[3] The role of microbial signatures in the pathophysiology of human illnesses, especially lung diseases, has been extensively studied.[4] Researchers have shown that the shape and content of lung microbial signatures may predict the outcomes of chronic respiratory disorders.[5] Previous studies have shown that the lung microbiome influences immunological modulation and disease progression and prognosis.[6] Growing evidence suggests that lung microorganisms play a crucial role in the development of lung diseases.[7]

Lung illnesses are better understood through research on lung microbial signatures. Currently, the 16S rRNA gene is used in molecular biochemical procedures, such as polymerase chain reaction (PCR), for bacterial identification in microbial signature analysis.[8] Bacterial species and genera can be identified using these small, conserved regions of the genome. Until recently, bronchoscopy was the gold standard for analyzing bronchoalveolar lavage (BAL) fluid for microbial signatures in the lungs.[9]

A bronchoscope is an optical device inserted into the airways to visually inspect the tracheobronchial tree. This method has several diagnostic applications in medical fields. Sampling procedures may include bronchial brushing, bronchial cleaning, transbronchial needle aspiration (TBNA), and BAL.[10]

The primary objective of this study was to evaluate the potential of microbial profiling as a diagnostic and prognostic tool for the management of lung diseases.

MATERIALS AND METHODS

This systematic literature review aimed to identify and evaluate studies that met predefined inclusion and exclusion criteria, using a qualitative descriptive analysis approach. The purpose of this methodology was to support the development of robust clinical inquiries through comprehensive evidence synthesis. This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and methodological rigor.[11] The initial step was to identify and merge research papers from all search sources. In the second step, we used the criteria to filter the titles and abstracts of the papers and chose the ones for inclusion. The final step was to determine whether all research publications met the inclusion criteria. Finally, in the fourth step, the pertinent material was extracted and processed according to the title and subject.[12] The systematic review protocol was formally registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42024579893.

Literature search

A systematic literature search was performed across three major databases (PubMed, ProQuest, and Science Direct). For each database, tailored search strategies were applied to account for variations in indexing and search functionalities. Boolean operators (AND, OR, NOT) were adjusted accordingly and not generalized across all platforms to maintain the specificity and sensitivity of each search. A literature search was conducted electronically in December 2022.

Using a combination of Boolean operators (AND and OR) and the medical subject headings (MeSH) equivalent, we used terms relevant to the study issue for Pubmed: ("bronchoscopy" OR "bronchoscopic" OR "bronchoscopies" OR "BAL" OR "bronchial washing" OR "bronchial lavage" OR "bronchoalveolar lavage" OR "lung lavage" OR "bronchopulmonary lavage") AND ("microbiome" OR "microbiota" OR "microbial"). No filters or constraints were implemented during the search.

For Proquest: TI, AB, SU ("bronchoscopy" OR "bronchoscopic" OR bronchoscopies" OR "BAL" OR "bronchial washing" OR "bronchial lavage" OR "bronchoalveolar lavage" OR "lung lavage" OR "bronchopulmonary lavage") AND ("microbiome" OR "microbiota" OR "microbial").

For Science Direct: ("bronchoscopy" OR "bronchoscopic" OR "bronchoscopies" OR "BAL" OR "bronchial washing" OR "bronchial lavage" OR "bronchoalveolar lavage" OR "lung lavage" OR "bronchopulmonary lavage") AND ("microbiome" OR "microbiota" OR "microbial").

Selection criteria

The following eight factors were considered for inclusion in this literature review: (1) Research designs that included randomized control trials, cross-sectional studies, case-control studies, or cohort studies. (2) Pulmonology's most common diseases should be discussed. (3) Bronchoscopy should be used as the sampling tool. (4) The study population consisted of adults. (5) The microbial signature should be identified as a diagnostic and prognostic factor. (6) PCR can be used to analyze microbial signatures. (7) Significant probability values ($p < 0.05$) and diagnostic and prognostic values should be reported. (8) The research should be written in English. Four criteria were used as exclusion criteria in this systematic literature review: (1) Use of non-pulmonary samples for microbial signature analysis. (2) Microbiological signature analysis using microbial culture. (3) Redundant literature. (4) Case reports, literature reviews, case series, meta-analyses, and systematic review research methods.

Data selection and extraction

All records retrieved from the databases were compiled, and duplicates were removed using Rayyan software. The total number of articles retrieved from each database, along with the date and time of each search, was recorded for reproducibility using the Rayyan website. Rayyan facilitates systematic literature reviews, allowing us to choose and retrieve the necessary data. After the data were extracted from a predefined database, unnecessary materials were removed. Data were retrieved using a pre-designed table once the appropriate literature was collected.

Literature quality assessment

The quality of observational studies was evaluated separately using the Newcastle-Ottawa Scale (NOS). Selection, comparability, and exposure/outcome were the three criteria used to evaluate the bias. While cross-sectional studies could only obtain a maximum score of 8, case-control and cohort studies achieved a maximum score of 9. Excellent quality research was defined as a total score of at least 7 for cohort and case-control studies and at least 6 for cross-sectional studies.[13]

RESULTS

Figure shows a flow diagram of the book selection process. By searching the aforementioned databases for relevant terms, 1,784 articles were identified; however, 842 were deemed unnecessary and were eliminated. Additionally, 881 articles were deemed irrelevant based on their titles and abstracts. Subsequently, 59 full-text publications were evaluated to determine their suitability. The qualitative synthesis for the systematic review ultimately included 17 publications.

Table 1 lists the features reported in the literature. Nine studies used case-control research design, six used cohort study design, and two relied on cross-sectional study design. The USA, Italy,

Table 1. Summary of the literature search

Authors	Country	Study Design	Number of Cases	Type of Disease	Control
Bello et al.[14]	Spain	Case control	24 cases, 10 controls	Lung cancer	Patients with no history of lung cancer
Boesch et al.[15]	Switzerland	Case control	35 cases, 10 controls	Immunotherapy in advanced-stage NSCLC	NSCLC undergoing surgery
Cheng et al.[16]	China	Case control	32 cases, 22 controls	Lung cancer	Benign lung disease
An et al.[17]	China	Prospective cohort	80 cases	Immunotherapy in stage 4 NSCLC	N/A
Denner et al.[18]	U.S.A.	Cross sectional retrospective	39 cases, 19 controls	Bronchial asthma	Healthy patients
Of et al.[19]	China	Prospective cohort	67 cases	Severe pneumonia	N/A
Gaibani et al.[20]	Italy	Case control	24 cases, 24 controls	COVID-19	Non-COVID-19 pneumonia
Jang et al.[21]	South Korea	Prospective cohort	84 cases	NSCLC	N/A
Kyo et al.[22]	Japan	Case control	40 cases, 7 controls	Intubated ARDS	Intubated non-ARDS
Lee et al.[23]	South Korea	Prospective cohort	28 cases	Lung cancer	Benign mass
Liu et al.[24]	China	Case control	24 cases, 18 controls	Lung cancer with unilateral lobar mass	Healthy patients
Liu et al.[25]	China	Prospective cohort	9 cases	Lung cancer	N/A
Molyneaux et al.[26]	U.K.	Case control	20 cases, 15 controls	Acute exacerbation of IPF	Stable IPF
Molyneaux et al.[27]	U.K.	Case control	65 cases, 44 controls	IPF	Healthy patients
Ramsheh et al.[28]	U.K., Germany, Italy, Poland, and Hungary	Case control	339 cases, 207 controls	COPD	Healthy patients
Tsay et al.[29]	U.S.A	Cross sectional prospective	85 cases	Lung cancer	N/A
Zhuo et al.[30]	China	Prospective cohort	50 cases	One-side lung cancer and metastases	N/A

*NSCLC = Non-Small Cell Lung Cancer, N/A = Not Applicable or Not Available, COVID-19 = Coronavirus Disease 2019, ARDS = Acute Respiratory Distress Syndrome, IPF = Idiopathic Pulmonary Fibrosis, COPD = Chronic Obstructive Pulmonary Disease

Table 2. Analysis methods and outcomes of microbiota determined by 16S rRNA gene based on literature

Authors	Sampling Method	Type of Microbial Signature	Significant Outcome	NOS Score
Bello et al.[14]	TBLB	*Streptococcus* genus	*Streptococcus* served as a diagnostic factor in lung cancer patients (sensitivity = 93%, specificity = 83.3%).	7
Boesch et al.[15]	TBLB	*Gammaproteobacteria* class	The *Gammaproteobacteria* class was associated with low expression of PD-L1 (p = 0.006) and low progression-free survival (p = 0.003).	8
Cheng et al.[16]	BAL	*TM7-3* class, *TM7* phylum, *Pseudomonadaceae* phylum, *Capnocytophaga, Sediminibacterium, Gemmiger, Blautia, Oscillospira, Stenotrophomonas, Microbacterium, Blautia,* and *Lautropia* genera	The *TM7* phylum, *TM7-3* class, as well as *Capnocytophaga*, *Gemmiger, Sediminibacterium, Blautia,* and *Oscillospira* genera were more common in lung cancer than in benign lung disease (p < 0.05).	7
An et al.[17]	BAL	*Fusobacterium* genus	*Fusobacterium* was associated with poor response to anti-PD-L1 treatment (p < 0.001).	7
Denner et al.[31]	BAL	*Lactobacillus, Pseudomonas,* and *Rickettsia* genera	*Lactobacillus, Pseudomonas*, and *Rickettsia*. were more common in patients with bronchial asthma (p < 0.01).	6
Of et al.[32]	BAL	*Prevotellaceae* and *Actinomycetaceae* families	The *Prevotellaceae* and *Actinomycetaceae* families increased clinical improvement by 14% and 10% in severe pneumonia (95% CI [1.04, 1.25]), p = 0.006; 95% CI (1.02, 1.18, p = 0.01).	9
Gaibani et al.[20]	BAL	*Pseudomonas* spp. species	*Pseudomonas* spp. were more commonly found in COVID-19 than non-COVID-19 pneumonia (p = 0.021)	8
Jang et al.[21]	BAL then TBLB	*Neisseria* genus, *Veillonella dispar*, and *Haemophilus influenzae* species	*Neisseria* bacteria were more commonly found in NSCLC with low PD-L1 levels than in NSCLC with high PD-L1 levels (p = 0.037).[21] In addition, *Veillonella dispar* was more commonly found in NSCLC with high PD-L1 levels (p = 0.028) and in the NSCLC group in comparison with the non-responder group (p = 0.041). Finally, *Haemophilus influenzae* & *Neisseria perflava* highly found in the non-responder NSCLC group (p = 0.041).	8
Kyo et al.[22]	BAL	*Betaproteobacteria* class, *Enterobacteriaceae* family, *Staphylococcus*, and *Streptococcus* genera	The *Betaproteobacteria* class was less common in ARDS patients who did not survive than in ARDS patients who survive (p = 0.012). *Staphylococcus, Enterobacteriaceae,* and *Streptococcus* were significantly associated with the increase of IL-6 levels in ARDS patients who did not survive (p < 0.05).	8

Table 2. Cont...

Authors	Sampling Method	Type of Microbial Signature	Significant Outcome	NOS Score
Lee et al.[23]	BAL	*Firmicutes* phylum, *TM7*, *Veillonella,* and *Megasphaera* genera	The *Firmicutes* and *TM7* phyla were more commonly found in lung cancer than in benign mass ($p = 0.037$ and $p = 0.035$, respectively). *Megasphaera* and *Veillonella* were more common in lung cancer than in benign mass ($p = 0.003$ and $p = 0.022$, respectively; AUC = 0.888; sensitivity = 95.0%, specificity = 75.0% and sensitivity = 70.0%, specificity = 100.0%; $p = 0.002$).	7
Liu et al.[24]	PSB	*Streptococcus* genus	*Streptococcus* could predict the presence of lung cancer (AUC = 0.693, sensitivity = 87.5%, specificity = 55.6%).	8
Liu et al.[25]	BAL	*Oscillospirales* order, *Christensenellaceae* family, *Lactobacillus, Marseilles*, and *Lactococcus* genera	The *Oscillospirales* order, *Christensenellaceae* family, as well as *Lactobacillus, Marseille*, and *Lactococcus* genera were more common in lung cancer ($p < 0.05$).	8
Molyneaux et al.[26]	BAL	*Campylobacter* sp., *Stenotrophomonas* sp., and *Veillonella* sp. species	*Campylobacter* sp. and *Stenotrophomonas* sp. were commonly found in acute exacerbation IPF than stable IPF ($p = 0.02$ and $p = 0.03$), while *Veillonella* sp. were commonly found in stable than IPF acute exacerbation IPF ($p < 0.01$)	7
Molyneaux et al.[27]	BAL	*Haemophilus, Streptococcus*, and *Neisseria* genera and *Veillonella* spp. species	The *Haemophilus, Streptococcus*, and *Neisseria* genera as well as *Veillonella* spp. species were more common in IPF than healthy patients ($p < 0.001$, $p < 0.01$, $p < 0.05$, $p < 0.001$)	8
Ramsheh et al.[28]	Brushing	*Prevotella, Streptococcus*, and *Moraxella* genera	*Prevotella* was found to be lower in COPD patients than in healthy patients ($p < 0.0001$). Meanwhile, *Streptococcus* was more prevalent among COPD patients compared to healthy individuals. ($p < 0.0001$). In addition, *Moxarella* was more common in COPD patients than in healthy patients ($p < 0.0001$). *Prevotella* was more commonly found in COPD patients without inhaled steroid therapy compared to COPD patients with inhaled steroid therapy ($p = 0.021$).	8
Tsay et al.[29]	Brushing	*Streptococcus* and *Veillonella* genera	*Streptococcus* and *Veillonella* were more common in lung cancer ($p = 0.026$).	7
Zhuo et al.[30]	BAL	*Spiroplasma* and *Weissella* genera	*Spiroplasma* and *Weissella* were more common in lung cancer in cancerous lesions than in noncancerous lesions ($p = 0.003$ and $p = 0.009$, respectively).	7
Jang et al.[21]	BAL then TBLB	*Neisseria* genus, *Veillonella dispar*, and *Haemophilus influenzae* species	*Neisseria* bacteria were more commonly found in NSCLC with low PD-L1 levels than in NSCLC with high PD-L1 levels ($p = 0.037$).[21] In addition, the *Veillonella dispar* species was more commonly found in NSCLC with high PD-L1 levels ($p = 0.028$) and in the NSCLC responder group in comparison with the non-responder group ($p = 0.041$). Finally, *Haemophilus influenzae* and *Neisseria perflava* were more commonly found in the NSCLC non-responder group ($p = 0.041$).	8

Acute Respiratory Distress Syndrome (ARDS), Phosphate Solubilizing Bacteria (PSB), Idiopathic Pulmonary Fibrosis (IPF), Chronic Obstructive Pulmonary Disease (COPD), Bronchoalveolar Lavage (BAL), Programmed Death-Ligand 1 (PD-L1), Coronavirus Disease 2019 (COVID-19), Area Under the Curve (AUC), PSB, IPF, NSCLC, ARDS, IPF, and COPD are acronyms

South Korea, Japan, and the UK are among the many nations that hosted these research projects. Five nations- the UK, Italy, Poland, Germany, and Hungary- conducted joint multicenter research for one study. The publications of the papers ranged from 2014 to 2022. Out of the 1,421 cases, 376 were derived from case-control studies. Ten articles mostly dealt with lung cancer, which is the most frequent type of lung illness. Idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), bronchial asthma, and infectious lung disorders such as bacterial and Coronavirus disease 2019 (COVID-19) pneumonia were also discussed in other articles.

Table 2 shows the inclusion criteria for the literature evaluation, including the use of bronchoscopy to collect lung samples for PCR-based microbial signature analysis. The majority of the samples collected using bronchoscopy were from BAL fluid; this method was described in 10

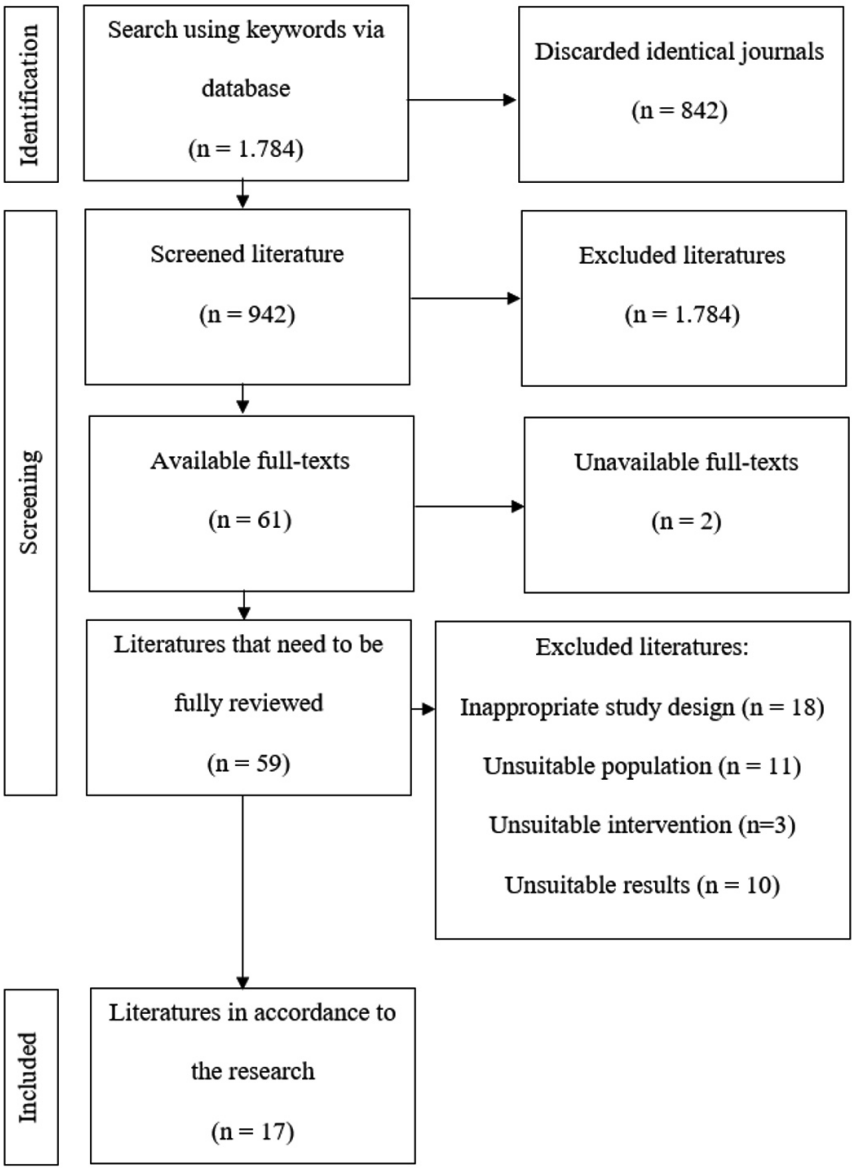

Figure. PRISMA diagram

papers. Alternatively, other studies used brushing or tissue biopsies to collect samples.

Literature quality assessment results

The NOS was used to evaluate the potential for bias in case-control, cross-sectional, and cohort studies. The NOS ratings for each study are shown in Table 2. A score of 8 indicated good quality and little risk of bias, which was achieved in most studies.

Outcome results and microbial signature analysis Lung Diseases in Oncology

Microbial signature analysis has a dual use in diagnosing and predicting outcomes in patients with lung cancer. Streptococcus bacteria showed promising diagnostic results in a study by Bello et al. in patients with lung cancer (sensitivity, 93%; specificity, 83.3%).[14] Liu et al. found that Streptococcus is a good predictor of lung cancer (AUC = 0.693, sensitivity = 87.5%, specificity = 55.6%), lending credence to a previous claim.[24] Nonetheless, a study conducted by Lee et al. revealed that lung cancer had higher levels of *Veillonella* and *sphaera* bacteria than benign masses (p = 0.003 and p = 0.022, respectively; AUC = 0.888).[23] Nonetheless, the findings from microbial signature analyses of immunotherapy-treated lung cancer were contradictory. Low PD-L1 expression (p = 0.006) and progression-free survival (p = 0.003) were related to the Gammaproteobacteria class, according to Boesch et al.[15] Chu et al. found a strong association (p < 0.001) between *Fusobacterium* and a subpar reaction to anti-PD-L1 medication.[17] Jang et al. also showed that compared to Non-Small Cell Lung Cancer (NSCLC) with high PD-L1 levels, NSCLC with low PD-L1 levels had a much higher prevalence of Neisseria bacteria (p = 0.037). According to other findings, the non-responder group had a lower frequency of *Veillonella dispar* than the NSCLC responder group (p = 0.041) and the patients with NSCLC and elevated PD-L1 levels (p = 0.028). *Neisseria perflava* and *Haemophilus influenzae* were more common in the NSCLC non-responder group than in the other groups (p = 0.041 and p = 0.041, respectively).[21]

This study revealed a wide range of microbial signature communities. The TM7 phylum was more common in lung cancer cases than in benign masses, according to Cheng et al. and Lee et al. (p = 0.035 and p < 0.05, respectively).[16,23] Moreover, according to Cheng et al., there was a greater incidence of the TM7-3 class in instances of lung cancer in comparison with cases of benign lung illness (p < 0.05), along with the genera *Capnocytophaga*, *Sediminibacterium*, *Gemmiger*, *Blautia*, and *Oscillospira*.[13] Conversely, Liu et al. revealed that there was an increased prevalence of the *Oscillospirales* order, *Christensenellaceae* family, *Lactobacillus*, *Marseille*, and *Lactococcus* genera in lung cancer (p < 0.05).[25] Tsay et al. found that lung cancer was most often caused by bacteria from the genera *Veillonella* and *Streptococcus* (P = 0.026).[29] A study conducted by Zhuo et al. revealed an interesting finding: *Spiroplasma* and *Weissella* genera were more abundant in malignant lung lesions than in noncancerous lesions (p = 0.003 and p = 0.009, respectively).[30]

Infectious lung disease

Microbial signature analysis has the potential to be a useful predictor of clinical improvement in infectious lung diseases. Patients with severe pneumonia had 14% and 10% better prognoses (p = 0.006 and p = 0.001, respectively) when exposed to microbial signatures from the *Prevotellaceae* and *Actinomycetaceae* families, respectively.[32] *Pseudomonas* spp. was more often detected in COVID-19, according to Gaibani et al. (p = 0.021).[20] Acute respiratory distress syndrome (ARDS) may arise as a result of serious infectious illnesses. The *Betaproteobacteria* class was found to be less prevalent in patients with ARDS who did not survive than in those who did (p = 0.012), according to research conducted by Kyo et al. In the group that did not make it, members of *Staphylococcus, Streptococcus*, and *Enterobacteriaceae* families were associated with higher levels of IL-6. This may be a possible indicator of the severity of inflammation-induced illness in this group (p < 0.005).[22]

Obstructive lung disease

Patients suffering from asthma and chronic obstructive pulmonary disease (COPD) were shown to have higher prevalence of certain microbial signatures when in comparison with

healthy individuals. Individuals with asthma bronchiale had considerably higher levels of *Lactobacillus*, *Pseudomonas*, and *Rickettsia* compared to healthy individuals, according to a study by Denner et al. ($p < 0.01$).[31] According to Ramsheh et al., a significant difference was observed ($p < 0.0001$) in the prevalence of the *Streptococcus* and *Moxarella* genera between healthy individuals and patients with COPD. At the same time, a considerably smaller number of members of the *Prevotella* genus was found in patients with COPD compared to healthy individuals ($p < 0.0001$). Individuals with COPD who did not use inhaled steroids had a greater prevalence of *Prevotella* infection than those who did ($p = 0.021$). The severity of COPD symptoms was inversely associated with *Prevotella* prevalence, but lung function and physical activity were favorably associated.[28]

Idiopathic pulmonary fibrosis

A significant increase in the abundance of *Haemophilus, Streptococcus, Neisseria,* and *Veillonella* species was observed in patients with IPF compared to healthy individuals ($p < 0.001$, $p < 0.01$, $p < 0.05$, and $p < 0.001$, respectively).[27] *Campylobacter* sp. and *Stenotrophomonas* sp. were more prevalent in patients with acute IPF exacerbation than in those with stable IPF. Additionally, compared with stable IPF, acute exacerbation of IPF was less likely to have *Veillonella* sp. ($p < 0.01$).[26]

DISCUSSION

Researchers in the field of microbiology have long assumed that the lungs are completely germ-free.[33] Culture samples obtained from patients with acute or chronic illnesses are the gold standard for identifying and detecting microorganisms in the human body. However, modern technology allows the detection and identification of many bacteria through molecular biochemical analyses, bypassing the need for culture procedures. Researchers can detect and categorize various microorganisms in ecological communities using technologies, such as genomic techniques for molecular biochemical analyses. To reproduce bacterial DNA sequences, this approach employs quantitative PCR to identify 16S rRNA.[4] Crucially, the molecular methods for bacterial identification described earlier can only detect DNA in the material under study and cannot distinguish between live and dead bacteria. Culture methods and other more conventional approaches, on the other hand, need the presence of actual live organisms.

According to the findings of these studies, the lungs may not be completely sterile. According to Dickson et al., the state of microbial signatures in the lungs is affected by three factors: (1) the entry of microbes into the airways, (2) the expulsion of microbes from the respiratory system, and (3) the development of microbes in certain habitats.[6] Oxygen tension, pH, and immunological state are only a few of the lung microenvironmental factors that might change the microbial spectrum.[9] Thus, changes in the dynamic state of lung microbes may lead to the development of lung illness.[6,31]

Lung microbial fingerprints were substantially linked to lung disorders, including asthma, cystic fibrosis (CF), COPD, IPF, and respiratory infections, according to this meta-analysis that gathered data from many investigations. We identified these disorders by collecting samples that included microbes and analyzing them using PCR, which entails sequencing the genomes of the microbes. Prior to classification using the current taxonomy database, the sequences are aligned based on predefined degrees of homology.[9,34,35]

Several studies have compared the lung microbial signatures in healthy individuals with those in illness states, and the findings show that the two groups vary significantly in composition.[27,33,36,37] Lower bacterial diversity, or dominance by a single or small group of taxa, is linked with disease conditions, according to the research.[38] Information gained from genetic and clinical studies has improved our understanding of disease causation within the complex microbiome milieu of healthy individuals and patients with specific lung illnesses.[28,39-43] Nowadays, most people agree that a diverse community of bacteria called the lung microbiota is fundamental for maintaining lung health.[44] Several lung disorders have been linked to dysbiosis, which is characterized by alterations in lung microbiota composition.[34,45,46]

Based on sputum samples, Taylor and Simpson et al. postulated that airway microbial makeup is related to the asthma phenotype. In contrast, patients with eosinophilic asthma show a greater diversity in bacterial load, with relative enrichment in *Moraxella* and *Haemophilus* spp., and a relative decrease in the presence of *Streptococcus*, *Gemella*, and *Porphyromonas*, when treated with high doses of inhaled corticosteroids (ICSs).[47,48] Acute exacerbation of COPD can be prevented by keeping the lung and gut microbial signatures intact, as the gut-lung axis may influence the severity of COPD. Research has shown that, during an acute exacerbation episode, *Bacteroidetes* and *Proteobacteria* are more abundant in the fecal microbial profile, whereas *Firmicutes* and *Actinobacteria* are less abundant, lending credence to this notion.[42,49] Huang et al. found that the lung microbial profile is related to histology and risk of disease progression.[50] Metastatic adenocarcinoma had far lower *Streptococcus* levels than non-metastatic adenocarcinoma, according to bronchial washing fluid samples. Metastatic SCC, on the other hand, had higher levels of *Veillonella* and *Rothia*.[51] Because this could affect the microbial signature composition, it may be important to consider the types of samples that are tested. Durack et al. showed notable differences between sputum and bronchoalveolar lavage fluid (BALF) samples.[15]

Sputum analysis of lung microbial fingerprints remains the gold standard for studying healthy individuals. Given the combination of substances originating from the upper, lower, and oral tracts, the function of sputum as a lung representation is still up for dispute. Because of its exceptional capacity to record the topographical distribution of microbes, the lung tissue is, in theory, the best material for microbial signature analysis of the airway and lungs. Only patients who undergo lung resections, cancer surgeries, or biopsies have been able to benefit from it because of the difficulty in obtaining lung tissue in most therapeutic settings.[51]

Another option for collecting lung disease samples for microbial signature analysis is non-invasive techniques such as bronchoscopy. Currently, BAL fluid is used for most lung microbial signature analyses. Another option is to employ bronchoscopy for bronchial cleaning, biopsies, transbronchial needle aspiration (TBNA), and bronchial brushing.[10] It is possible to introduce oral microbial signatures into the sputum and saliva. As it may reduce the impact of oral contamination, some scientists believe that BAL fluid is a good choice for studying lung microbiomes.[16]

Finally, bronchoscopy-based microbial signature analysis of the lungs to diagnose and predict the prognosis of lung illnesses has yielded inconsistent findings. Therefore, to understand their possible function in lung illness and to characterize the prognosis and reaction of individuals to immunomodulatory treatments, microbial signatures must be understood. Local microenvironments are formed by microbes and/or their metabolism, which affect the immune response and cancer assault mechanisms. According to Bello et al., microbes may control the equilibrium between tumor-induced inflammation and antitumor immunity in different microenvironments.[14]

Microbial signature analysis is a potential method for identifying novel therapeutic targets among lung microbes. The analysis of lung microbial signatures opens the door to the possibility of treating dysbiosis and restoring native bacteria by manipulating the composition of the lung microenvironment. This objective may be improved through the use of antibiotics, quorum-sensing inhibitory compounds, probiotics (health-promoting extrinsic microorganisms), and prebiotics (specific bacterial growth-promoting, non-absorbable chemicals). In addition, treatment interventions based on lung microbial signature analysis may target the most pathogenic microbes, while avoiding other potentially harmful microbes.[9] Some studies have used systemic antibiotics to control respiratory microbiomes. The impact of oral ciprofloxacin on clinical pulmonary endpoints in patients with IPF was first unclear,[52,53] although one trial indicated a possible benefit in terms of mortality.[50] Lung microbial signature results have been observed in several studies of systemic antibiotic use, which has improved our knowledge of the processes driving clinical findings.[54,55] According to the BLESS study, all patients with bronchiectasis who did not have CF showed a decrease in exacerbation rates and changes in the sputum microbiota after receiving long-term erythromycin therapy.[19] Notably, the clinical and

microbiological effects of erythromycin treatment differed depending on whether *P. aeruginosa* was the predominant species in the airway secretions.[19] Studies have shown that erythromycin amplifies resistance genes[56] and may reduce the disease-causing potential of *P. aeruginosa* by disrupting cell-to-cell communication.[57]

As a new sampling method for diagnosing and predicting the prognosis of lung disorders, bronchoscopy offers alternatives and references for microbial signature analysis. However, this comprehensive literature review has a few limitations. One limitation of this study is that the function of microbial signatures in diagnostic and prognostic statistics was not examined using a meta-analysis method. Second, no analysis has been conducted on the impact of microbial diversity on diagnosis and prognosis. Finally, this study only considered publications written in English; papers written in other languages that fulfilled the study requirements were not reviewed.

CONCLUSION

Through the use of PCR for quantitative microbial signature analysis, scientists can detect and categorize a wide range of microbes in ecological communities by focusing on the 16S rRNA gene. Patients with lung disorders may also have samples taken for microbial signature analysis using non-invasive techniques such as bronchoscopy. Therefore, microbial detection is a promising avenue for future treatment strategies. The analysis of lung microbial signatures opens the door to the possibility of treating dysbiosis and restoring native bacteria by manipulating the composition of the lung microenvironment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

IS and IAM conceptualized the study. IS, RYS, and SW wrote the original draft. IS, RYS, SW, IAM, and MM wrote, reviewed, and edited the manuscript. IAM and MM supervised the study. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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