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The crosstalk between microbiome and asthma: Exploring associations and challenges

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Abstract

With the advancement of high-throughput DNA/RNA sequencing and computational analysis techniques, commensal bacteria are now considered almost as important as pathological ones. Understanding the interaction between these bacterial microbiota, host and asthma is crucial to reveal their role in asthma pathophysiology. Several airway and/or gut microbiome studies have shown associations between certain bacterial taxa and asthma. However, challenges remain before gained knowledge from these studies can be implemented into clinical practice, such as inconsistency between studies in choosing sampling compartments and/or sequencing approaches, variability of results in asthma studies, and not taking into account medication intake and diet composition especially when investigating gut microbiome. Overcoming those challenges will help to better understand the complex asthma disease process. The therapeutic potential of using pro- and prebiotics to prevent or reduce risk of asthma exacerbations requires further investigation. This review will focus on methodological issues regarding setting up a microbiome study, recent developments in asthma bacterial microbiome studies, challenges and future therapeutic potential.

KEYWORDS

asthma, clinical immunology, omics- and systems biology, regulatory aspects

1 | INTRODUCTION

Asthma is a chronic inflammatory airway disease characterized by reversible airway obstruction and hyperresponsiveness leading to recurrent episodes of exacerbations, breathlessness, chest tightness, and coughing. According to the Global Burden of Disease Study, asthma was the most prevalent chronic respiratory disease world-wide, affecting 358.2 million people and causing 0.4 million deaths in 2015.[1] The increasing prevalence of asthma[1,2] and its heterogeneous nature[3] increases the disease burden and introduces some challenges for the appropriate diagnosis and treatment.

The human body harbours trillions of symbiotic microbiota.[4] The genetic constituent of these microbiota is termed as the "human microbiome".[5,6] World-wide microbiome projects have been conducted to understand the role of the microbiome in health and disease.[7,8] One of the investigated disorders in relation to the microbiome is asthma. In the past, studies have focused on identifying pathogenic micro-organisms, such as bacteria, viruses and fungi that can be potentially associated with the disease process of asthma.[9-12] The link between microbiome and allergic disorders was further elucidated with the introduction of the "hygiene hypothesis",[13] which was based on the observation that children who were more exposed to infectious agents during early childhood were less prone to get

allergic disorders. Studying the role of the microbiome and how it interacts with the host will provide opportunities to better understand the complex disease process of asthma.

The development of micro-organism identification technologies from culture-dependent to DNA/RNA sequencing technologies has made more in-depth microbiome research possible. The focus shifted to a wider prospective, including both commensal and pathogenic micro-organisms and their interaction with the human body and their role in asthma. To date, several cross-sectional and longitudinal microbiome studies have been conducted in asthmatics with different phenotypes, which have revealed many associations between bacterial composition and asthma. However, further research is still needed to reach definitive conclusions.

In this review, we aim to provide an overview of methodological aspects of conducting asthma microbiome studies. In addition, we will explore the associations and role of bacterial microbiota in asthma and discuss the challenges that hinder appropriate interpretation of the results and transfer of knowledge into clinical practice.

2 | METHODOLOGICAL ASPECTS OF ASTHMA MICROBIOME STUDIES

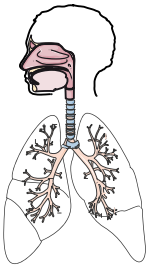
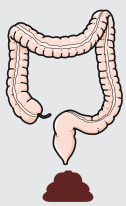
2.1 | Sampling of the microbiome

The first step in the analysis of the microbiome is to obtain samples. Samples for analysis of the microbiome in relation to asthma either originate from the naso-/oropharyngeal route (such as nose, throat), airways or from the large intestinal tract (gut). Table 1 represents commonly used sampling compartments, and their main advantages and disadvantages.

Since many different sampling compartments and methods are currently used,[14-18] obtained results are difficult to compare. For example, a study conducted in 39 asthmatics and 19 healthy controls revealed that statistically significant differences in bacterial α-diversity (diversity in individual site or sample) and β-diversity (intervariability, between sites or samples) were found between bronchial brushings and bronchoalveolar lavage (BAL) fluid within individuals for both asthmatics and control groups.[14] In a study of 30 asthmatic children, significant differences in bacterial α- and β-diversity were found between nasal brushes and nasal washes within individuals.[15] In another study comparing bacterial composition in bronchial aspirates and bronchial brushings in 13 adults with severe asthma, the composition of the microbiota in the bronchial brushings showed a specific pattern that was not represented in bronchial aspirates.[16] Induced sputum was found to be more compositionally similar to bronchial brushings than oral washes or nasal brushings in adult atopic asthmatics (n = 22).[17] A study conducted in 60 individuals comparing stool samples with rectal swabs found that certain bacterial taxa are highly specific to the sample site, while other taxa can be found in both sample types and are specific for the individual.[18]

Although the choice of the sampling compartments in studies depends on several factors such as patients' age, disease state and available facilities, it is of importance to note that microbial communities can change between sampling compartments within the same individual, for example mucosa-associated or luminal bacteria. In

TABLE 1 Microbiome sampling compartments, advantages and disadvantages

Route	Sampling compartment	Advantages	Disadvantages
Naso-/oropharyngeal route and airways 	Nasal swab or wash	Non-invasive, high patient acceptability, easy to sample frequently	Patients may feel slightly uncomfortable As it only represents the upper respiratory tract, it may not be suitable if the study aim was to characterize the lower respiratory microbiome
	Saliva, buccal swab or wash	Non-invasive, high patient acceptability, easy to sample frequently	Differences related to gender, pH and diet intake should be accounted for
	Sputum (spontaneous or induced)	Relatively non-invasive, can represent microbiota from the lower respiratory tract	May be cross-contaminated from bacteria from the saliva or oral cavity, patient's cooperation is required to assure the quality of sample
	Bronchoalveolar lavage and bronchial aspirates	Good representation of the microbiota from the lower respiratory tract	Invasive, less patient acceptability, risk of cross-contamination during aspiration
	Bronchial brushings and biopsies	Good representation of the microbiota from the lower respiratory tract, including mucosa-associated microbiota	Invasive, less patient acceptability
Gut 	Stool	Non-invasive	Patient might feel uncomfortable collecting stool samples
	Rectal swab	Non-invasive, easy to sample frequently	Patients may feel more discomfort than stool samples, patient acceptability may be low, may be cross-contaminated by bacteria from the skin

addition, it is very important to consider the effect introduced by treatment with drugs on the microbiome present in different sampling compartments. For instance, studies have shown that medications such as proton pump inhibitors, antibiotics, antidepressants, statins, antidiabetics and probably others can influence gut microbiota.[19,20]

2.2 | Available microbiome analysis techniques

Various techniques are available to analyse the microbiome (Table S1). In the past, microbial studies in asthma were dependent on the growth of the micro-organisms on laboratory culture media, which was limited to certain pathogenic micro-organisms.

More recent studies have shown that culture-based methods are relatively insensitive to detect certain micro-organisms, such as atypical bacteria.[21,22] Therefore, other techniques have been used such as serologic testing, which depends on identifying antibodies against a pathogen, for example microimmunofluorescence or ELISA.[23,24] Newer methods, which target DNA of a pathogen, such as PCR and reverse transcriptase PCR, have been used in microbiome asthma studies,[25-27] with some commercially available PCR kits for detecting certain pathogens.[28,29]

With the emergence of novel nucleotide sequencing technologies, microbiome research shifted from a single pathogenic micro-organism to a microbial community-oriented approach, studying commensal and pathogenic bacteria and their interactions. Culture-independent or sequencing techniques start with the extraction of the nucleic acid (DNA and/or RNA) of the collected samples and can be subdivided into amplicon sequencing or whole-genome shotgun (WGS) sequencing.

Amplicon sequencing of the 16S ribosomal RNA (rRNA) of the microbiome is one of the most common techniques used recently in microbiome asthma studies. 16S rRNA is found in all bacteria and archaea, and is ideal for classifying micro-organisms. There are nine separate hypervariable regions (V1-V9) within the 16S rRNA gene[30] that are targeted by the sequencing platforms. On the other hand, WGS sequencing depends on fragmentation and random sequencing of the totally extracted microbiome DNA. It has been reported that WGS sequencing has improved accuracy and provides better detection of microbial species when compared to 16S amplicon sequencing.[31] Moreover, WGS sequencing provides the opportunity to assess the functional characteristics of the microbiome because it targets the whole genome instead of particular regions. However, it is more expensive and requires higher computational power than amplicon sequencing, and is therefore less used.

Various sequencing platforms are available which differ based on the read length, sequencing depth and error rates.[32] The most frequently used platforms in the literature are Illumina platforms (eg, MiSeq and HiSeq) and 454 pyrosequencing. Other platforms are also available such as Ion Torrent, PacBio and SOLiD. It is important to note that different sequencing platforms may produce variable results for the same samples.[32,33] For a clear comparison between sequencing approaches and platforms, see recent articles written by Liu *et al*[34] and Tessler *et al*[35]

In addition to the methodological aspects we discussed, we recommend reading a recently published article that highlights the best methodological practices that should be taken into account in the microbiome respiratory research such as quality control, data handling, microbial contamination, low biomass samples and standardized protocols.[36]

3 | LATEST INSIGHTS INTO MICROBIAL DYSBIOSIS AND ASTHMA PHENOTYPES (SEQUENCING STUDIES)

Samples for high-throughput microbiome sequencing studies in asthma patients are either taken at a certain time-point of patients' life (cross-sectional studies) or at two or more time-points (longitudinal studies).

3.1 | Cross-sectional studies

Various cross-sectional studies have studied associations between airway or gut bacterial dysbiosis at single time-points in relation to asthma phenotypes, for example mild-moderate, severe, and uncontrolled asthma or inflammatory phenotypes, such as eosinophilic and neutrophilic asthma. Most of the studies compared the microbial compositions between asthmatics with different severity levels and healthy controls, and sometimes with other airway disorders, such as cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD). Table 2 summarizes the main findings of airway microbiome cross-sectional studies conducted in asthmatic patients.

Regarding airway bacterial composition, various studies have shown that *Proteobacteria*, particularly *Haemophilus* species, are more common in asthmatic patients, while *Bacteroidetes* and *Actinobacteria* are more common in healthy controls.[37-43] However, it is not clear whether this difference in bacterial composition is a feature of asthma itself, related to corticosteroid use, or both. For example, there was an increase in the relative abundance of *Proteobacteria* and decrease in the abundance of *Bacteroidetes* and *Fusobacteria* in endobronchial brushings samples with increasing corticosteroid use.[14] Furthermore, it was reported that there were no differences in microbial compositions at phyla level between healthy controls and corticosteroid-free asthma patients.[44] A possible explanation for the effect of corticosteroids on microbiota is that they suppress or disturb mucosal immune system and therefore lead to overgrowth of bacteria. This overgrowth probably leads to harmful health effects if opportunistic or pathogenic micro-organisms became abundant such as fungal candidiasis associated with the use of inhaled corticosteroids.[45] However, it may still lead to beneficial effects by increasing abundances of beneficial commensal bacteria.[46] The results of other studies may reflect that corticosteroids are not the only influencers of the bacterial compositions.[47-49] For instance, in a study of 42 atopic corticosteroid-naïve asthmatics, 21 atopic non-asthmatics and 21 healthy controls, corticosteroid-naïve asthmatics had a distinct airway bacterial profile when compared to the

TABLE 2 Cross-sectional microbiome asthma studies

Authors	Number and type of participants	Age group	Samples collected	Technique used to assign bacterial taxa	Outcome	Key finding
Pang *et al*[53], 2019	24 mild-to-moderate asthma patients (12 eosinophilic and 12 non-eosinophilic) and 12 healthy controls	Adults	Induced sputum	16S ribosomal RNA-based method (targeting V3-V4 region)	Differences in microbiota composition between eosinophilic, non-eosinophilic asthma patients and healthy controls	Asthmatics especially the non-eosinophilic group showed decreased diversity compared to healthy controls
Pérez-Losada *et al*[57], 2018	163 children with asthma	Children (6-18 y)	Nasal washes	16S ribosomal RNA-based method (targeting V4 region)	Characteristics of nasal microbiota between clusters of paediatric asthma phenotypes	Clustering on patients clinical and biochemical characteristics revealed three distinct asthma phenotypes Bacterial diversity was significantly different between the three asthma phenotypic clusters. At phyla level, relative abundances of *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* differed significantly within the three clusters, while at genera level, *Moraxella*, *Corynebacterium*, *Dolosigranulum* and *Prevotella* were significantly different
Lee *et al*[43], 2018	60 young adults and elderly with asthma and 20 healthy controls	Young adults and elderly	Nasopharyngeal swabs	16S ribosomal RNA-based method and shotgun metagenomics	Differences in microbiota composition between young adults and elderly asthmatic and non-asthmatic patients	No significant differences in bacterial diversity between asthmatics and healthy controls in both young adults and elderly patients In young adults, the relative abundance of *Proteobacteria* phylum was higher in asthmatics compared with healthy controls, while in the elderly, the relative abundance of *Moraxella* was higher in healthy controls compared with asthmatics In young adults, FEV_1 predicted was inversely correlated with *Prevotella, Neisseria* and *Fusobacterium*. In contrast to the elderly, where FEV_1 predicted was positively correlated with *Burkholderia* and *Psychrobacter*
Durack *et al*[17], 2018	22 atopic asthma patients, 12 atopic non-asthma patients and 11 healthy controls	Adults	Protected bronchial epithelial brushes (PBs), induced sputum, oral wash and nasal brushes (NB)	16S ribosomal RNA-based method (targeting V4 region)	Microbiota compositional similarity between different sampling compartments	Bacterial composition similarity was greatest between bronchial brushes and induced sputum samples especially in atopic asthma and atopic non-asthma patients Number of bacterial taxa shared between induced sputum-bronchial brushes and between nasal brushes-bronchial brushes was highest in atopic asthma patients compared with healthy controls
Begley *et al*[143], 2018	24 adults with asthma and 8 healthy controls	Adults	Stool	16S ribosomal RNA-based method (targeting V4 region)	Relationship of microbiota composition to asthma characteristics and phenotypes	Beta diversity indices were inversely correlated with FEV_1 and positive specific IgE to aeroallergen, respectively Clustering on bacterial composition of asthma patients showed three clusters which differed mainly in FEV_1 *Bifidobacterium* and *Lachnospiraceae* bacterial families were more abundant in asthmatics, while *Bacteroides* and *Enterobacteriaceae* families were more abundant in healthy controls

(Continues)

TABLE 2 (Continued)

Authors	Number and type of participants	Age group	Samples collected	Technique used to assign bacterial taxa	Outcome	Key finding
Buendía *et al*[144], 2018	42 asthma patients with fixed airway obstruction, 74 with reversible airway obstruction and 66 with no airway obstruction	Children and adults (8-70 y)	Stool	16S ribosomal RNA-based method (targeting V4 region)	Relationship of gut microbiota composition to airway obstruction in asthma patients living in the tropics	No significant differences in bacterial richness and diversity were found between the three phenotypes Bacterial families *Streptococcaceae*, *Enterobacteriaceae* and *Veillonellaceae* which corresponds to genera *Streptococcus*, *Escherichia*, *Shigella* and *Megasphaera* discriminated between patients with fixed airway obstruction and other groups
Yang *et al*[145], 2018	111 adults with chronic rhinosinusitis (CRS)	Adults	Nasal swabs	16S ribosomal RNA-based method (targeting V4 region)	Differences in microbiota composition between CRS patients with or without asthma	No significant differences in alpha diversity between asthmatic CRS patients and others. In addition, alpha diversity was not related to emergency room visits, ACT or FEV_1 *Streptococcus* genus was significantly higher in relative abundance in asthmatic CRS patients compared to others *Proteobacteria* phylum was significantly higher in asthmatic CRS patients with emergency room visits compared to other asthmatics
Wang *et al* [52], 2018	36 adults with asthma, and 185 healthy controls	Adults	Stool	Shotgun metagenomics	Differences in microbiota composition between asthmatics and healthy controls	Lower bacterial richness and diversity were found in asthmatics compared to healthy controls *Clostridium bolteae*, *Clostridium ramosum*, *Clostridium spiroforme* and *Eggerthella lenta* were more abundant in asthmatic patients, while *Faecalibacterium prausnitzii*, *Sutterella wadsworthensis* and *Bacteroides stercoris* were more abundant in healthy controls Functional analysis shows that modules related to short-chain fatty acid (SCFA) production such as acetate and butyrate are more enriched in healthy controls
Taylor *et al* [54], 2018	167 adults with stable asthma	Adults	Induced sputum	16S ribosomal RNA-based method (targeting V1-V3 region)	Relationship of airway microbiota composition to asthma inflammatory phenotypes	Patients with neutrophilic asthma had lower bacterial diversity and higher dissimilarity compared with eosinophilic asthma patients Neutrophilic asthma patients had higher relative abundances of *Haemophilus* and *Moraxella* and lower relative abundances of *Streptococcus*, *Gemella* and *Porphyromonas* compared with eosinophilic and paucigranulocytic asthma patients
Yang *et al*[55], 2018	20 adults with neutrophilic asthma and 34 with non-neutrophilic asthma	Adults	Induced sputum	16S ribosomal RNA-based method (targeting V3-V4 region)	Differences in microbiota composition between neutrophilic and non-neutrophilic asthma	Neutrophilic asthma is associated with lower bacterial richness and diversity compared with non-neutrophilic asthma *Proteobacteria* phylum especially *Haemophilus* and *Moraxella* were more abundant in neutrophilic asthma patients. In contrast, *Firmicutes*, *Actinobacteria* and *Saccharibacteria* phyla were more abundant in non-neutrophilic asthma patients

(Continues)

TABLE 2 (Continued)

Authors	Number and type of participants	Age group	Samples collected	Technique used to assign bacterial taxa	Outcome	Key finding
Fazlollahi *et al*[60], 2018	31 adults with non-exacerbated asthma, 20 with exacerbated asthma and 21 healthy controls	Adults	Nasal swabs	16S ribosomal RNA-based method (targeting V3-V4 region)	Differences in microbiota composition between exacerbated, non-exacerbated asthma and healthy controls	At phyla level, Bacteroidetes and Proteobacteria were enriched in asthma patients compared to healthy controls At genera level, *Prevotella*, *Alkanindiges* and *Gardnerella* were more abundant in exacerbated asthma patients, while *Dialister* was more abundant in non-exacerbated asthma patients No bacteria were more enriched in healthy controls compared to the asthma groups
Goldman *et al*[146], 2018	15 children with severe asthma, 11 non-asthmatic children and 5 patients with CF	Children (no defined age range)	Bronchoalveolar lavage (BAL)	16S ribosomal RNA-based method (targeting V4 region)	Differences in microbiome profiles between the three groups	Similar bacterial taxa were found in both severe asthma and non-asthmatic children. However, CF patients had lower richness and diversity *Firmicutes*, *Proteobacteria* and *Cateriodetes* were the most predominant bacterial phyla across all groups Severe asthma and non-asthmatic groups differed significantly in 15 bacterial genera. *Bacteroides*, *Faecalibacterium*, *Roseburia* and 10 others were enriched in severe asthma samples, whereas *Proteus* and *Capnocytophaga* were more abundant in non-asthma samples
Boutin *et al*[147], 2017	27 children with asthma, 57 with CF and 62 healthy controls	Children (6-12 y)	Oropharyngeal swabs	16S ribosomal RNA-based method (targeting V4 region)	Differences in microbiome profiles between the three groups.	Children with CF had lower bacterial diversity and total abundance compared to asthmatic children and healthy controls Asthmatics had higher abundance of *Haemophilus* compared to CF and healthy controls
Li *et al*[50], 2017	25 adults with severe asthma, 24 non-severe asthma and 15 healthy controls	Adults	Induced sputum	16S ribosomal RNA-based method (targeting V3-V5 region)	Relationship between airway microbiota, asthma severity and inflammatory type	No significant different in microbial richness and diversity and at phyla level between severe, non-severe asthmatics and healthy controls At the family level, severe asthmatics had higher abundance in *Pseudomonadaceae* and *Enterobacteriaceae* compared to non-severe asthmatics and healthy controls Eosinophilic asthma patients had higher abundance of *Actinomycetaceae* compared to non-eosinophilic asthma patients Healthy subjects had the highest abundance of *Porphyromonadaceae* compared to other groups
Durack *et al*[47], 2017	42 atopic steroid-naïve asthma patients, 21 atopic non-asthma patients and 21 non-atopic healthy controls	Adults	PBs	16S ribosomal RNA-based methods (targeting V4 region)	Differences in airway microbiota in steroid-naïve atopic asthma patients, atopic non-asthma patients and non-atopic healthy controls	Asthmatic patients had higher abundances of *Haemophilus*, *Neisseria*, *Fusobacterium,* and *Porphyromonas*, and *Sphingomonodaceae* family, and decreased abundances of *Mogibacteriaceae* family, and *Lactobacillales* order compared to other groups

(Continues)

TABLE 2 (Continued)

Authors	Number and type of participants	Age group	Samples collected	Technique used to assign bacterial taxa	Outcome	Key finding
Ruokolainen *et al*[59], 2017	196 randomly selected children from; Finnish (n = 98) and Russian (n = 98) Karelia	Children (7-11 y and followed again at 15-20 y)	Skin and nasal swabs	DNA sequencing	Changes in allergy in children from school age until young adulthood from two different regions, Finland and Russian Karelia, with corresponding microbiome changes in skin and nasal cavity	Allergic disorders, such as asthma, atopic eczema, hay fever, rhinitis and atopic sensitization, were three- to ten-fold more common in the Finland when compared to Russian Karelia Microbiota from skin and nasal cavity showed that overall microbial diversity and abundance of *Acinetobacter* genus were higher in Russian Karelia
Millares *et al*[16], 2017	13 patients with severe asthma	Adults	Bronchial biopsies and aspirates	16S ribosomal RNA-based methods	Differences in bronchial bacterial composition and their functional capacities between bronchial biopsies (BB) and bronchial aspirates (BA) in patients with severe IgE-mediated asthma patients	*Bacteriodetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria* were the most abundant phyla in both BB and BA samples, while *prevotella* and *streptococcus* are the most predominant genera *Fusobacteria* showed significantly higher relative abundances in BA contrast to *Proteobacteria* was significantly lower in BA when compared with BB
Sverrild *et al*[48], 2016	23 steroid-free asthma patients and 10 healthy controls	Adults	BAL	16S ribosomal RNA-based methods (V4 region)	Relationship between airway microbiome and airway inflammation in steroid-free asthmatics and healthy controls	Asthmatic patients with low levels of eosinophils had different microbial profiles compared with asthmatic patients with high levels of eosinophils and healthy controls; they had more abundance of *Neisseria*, *Bacteroides and Rothia* species and less abundance of *Sphingomonas*, *Halomonas* and *Aeribacillus* species
Depner *et al*[51], 2016	A total of 327 rural farm and non-farm children; 125 children with asthma and 202 controls	Children (6-12 y)	Nasal and throat swabs	16S ribosomal RNA-based methods (V3-V5 region)	Differences in nasal and throat microbiota between children with asthma and healthy controls in both farm and non-farm children	Children with asthma had lower nasal bacterial diversity compared with healthy controls. No difference was found in throat samples Asthmatic children had increased abundance of *Moraxella* bacterial genus (*Proteobacteria* phylum), but this was only evident in non-farm children
Jung *et al*[44], 2016	89 steroid-naïve asthma patients and 36 healthy subjects	Adults	Induced whole sputum	16S ribosomal RNA gene terminal restriction fragment length polymorphism (T-RFLP)	Differences in nasal microbiota between adult steroid-naïve asthmatics and healthy controls	No significant differences in microbial diversity and composition at phylum level were found between asthmatics and healthy controls Slight significant differences in the OTUs between the two groups were found
Pérez-Losada *et al*[15], 2016	30 asthmatic children	Children (6-17 y)	Nasal brushes (NB) and nasal washes (NW)	16S ribosomal RNA-based methods (V3-V5 region)	Spatial variations in microbiome between NB and NW	The most predominant nasopharyngeal bacterial genera in both NB and NW were *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Haemophilus*, *Fusobacterium*, *Prevotella* and *Dolosigranulum* NB microbiome had higher α-diversity when compared to NW Both samples showed significant differences in abundances and community composition at genera level

(Continues)

TABLE 2 (Continued)

Authors	Number and type of participants	Age group	Samples collected	Technique used to assign bacterial taxa	Outcome	Key finding
Zhang *et al*[42], 2016	26 severe asthma, 18 non-severe asthma and 12 healthy subjects	Adults	Induced sputum	16S ribosomal RNA-based method (targeting V3-V5 region)	Differences in microbiome profile between severe and non-severe asthma patients and healthy controls	Patients with severe and non-severe asthma had a reduced prevalence of *Bacteriodetes* and *Fusobacteria* when compared to healthy controls There was a high increase in the prevalence of *Firmicutes* and a minor increase in *Proteobacteria* in severe asthmatics when compared to healthy controls Also, *Firmicutes* was increased in severe asthmatics when compared to non-severe asthmatics Non-severe asthmatics showed an increase in *Proteobacteria* compared to healthy controls and severe asthmatics
Simpson *et al*[56], 2016	46 patients with poorly controlled asthma	Adults	Induced sputum	16S ribosomal RNA-based method, and PCR	Sputum microbiome profile in adults with poorly controlled asthma	Patients with neutrophilic asthma had lower bacterial diversity and high prevalence of *Haemophilus influenza* compared with non-neutrophilic (eosinophilic) asthma, while patients with eosinophilic asthma had high prevalence of *Tropheryma whipplei* Neutrophilic asthma patients had lower abundance of *Actinobacteria* and *Firmicutes*, and higher abundance of *Proteobacteria* compared with patient with non-neutrophilic asthma
Denner *et al*[14], 2015	39 asthmatic patients and 19 control	Adults	Endobronchial brushings (EB) and BAL	16S ribosomal RNA-based method (targeting V4 region)	Lower airway microbiome profile in relation to clinical characteristic of asthma, corticosteroid medications and airway eosinophilia	Brush samples of asthmatic patients had higher abundance of *Lactobacillus*, *Pseudomonas* and *Rickettsia* species compared to controls, in contrast, healthy controls had higher abundance of *Prevotella*, *Streptococcus and Veillonella* Relative abundances of bacterial taxa were significantly associated with corticosteroid use. There was a decrease in relative abundance of *Bacteriodetes* and *Fusobacteria* and increase in *Proteobacteria* based on oral corticosteroid use FEV_1 levels were found to influence EB microbial diversity and profile

(Continues)

TABLE 2 (Continued)

Authors	Number and type of participants	Age group	Samples collected	Technique used to assign bacterial taxa	Outcome	Key finding
Huang *et al*[41], 2015	40 severe asthma patients A comparison was further made on 41 mild-moderate asthma subjects, and 7 healthy controls	Adults	PBs	16S ribosomal RNA-based methods, followed by in silico predictive metagenomic analysis of bacterial groups of interest	Bronchial bacterial composition and its association with disease-related features, such as BMI, ACQ scores, sputum total leucocytes, bronchial biopsy eosinophils	*Proteobacteria* associated with worsening ACQ and sputum leucocyte count; in contrast, *Actinobacteria* associated with improving ACQ scores *Protecobacteria* and *Firmicutes* were negatively correlated with biopsy eosinophils; in contrast, *Actinobacteria* was positively correlated Greater bacterial burden associated with less variation in asthma control and less eosinophil infiltration in bronchial tissue Severe asthmatics were enriched with *Klebsiella* compared with health controls, and *Actinobacteria*, *Gammaproteobacteria* and *Klebsiella* compared with mild-moderate asthmatics In contrast, several families of *Proteobacteria* were more abundant in mild-moderate asthmatics compared with severe patients, with the exception of *Entrobacteriaceae* which was more enriched in severe asthmatics
Ogorodova *et al*[148], 2015 (Russian article)	50 patients with bronchial asthma (23 with mild-moderate asthma and 27 with severe uncontrolled asthma) and 88 patients with COPD (57 with mild-moderate severity and 31 with severe course of disease)	Adults	Oropharyngeal swabs	16S ribosomal RNA-based method (targeting V3-V4 region)	Differences in oropharyngeal microbiota composition between patients with bronchial asthma and chronic obstructive pulmonary disease with different severity levels	There was a decrease in prevalence of *Prevotella* and increase of species *Bifidobacterium longum*, *Prevotella nanceiensis*, *Neisseria cinerea*, *Aggregatibacter segnis* and *genera Odoribacter*, *Alloiococcus*, *Lactobacillus*, *Megasphaera*, *Parvimonas* and *Sneathia* in severe uncontrolled asthma patients in comparison with patients which have mild persistent asthma In asthma patients compared to COPD patients, there was an increase in prevalence of *Prevotella melaninogenica* and genera *Selenomonas*, *Granulicatella* and *Gemella*, and decrease of *Prevotella nigrescens*, *Haemophilus* influenza and genera *Aggregatibacter*, *Alloiococcus*, *Catonella*, *Mycoplasma*, *Peptoniphilus* and *Sediminibacterium*
Park *et al*[40], 2014	18 asthma patients, 17 COPD patients and 12 healthy controls	Adults	Oropharyngeal swabs	16S ribosomal RNA-based method (targeting V1-V3 region)	Differences in oropharyngeal microbiota composition between asthma, COPD patients and healthy controls	Asthma and COPD patients had higher abundance of *Proteobacteria* and *Firmicutes* (particularly *Pseudomonas* and *Lactobacillus* species) compared to healthy controls; in contrast, healthy controls had higher abundance of *Streptococcus*, *Veillonella*, *Prevotella* and *Neisseria* species
Green *et al*[149], 2014	28 severe treatment-resistant neutrophilic asthma subjects	Adults	Induced sputum	16S rRNA gene T-RFLP	Abundance of bacterial taxa in severe treatment-resistant asthmatics and their association with clinical characteristics and airway inflammatory markers	Airway colonization with *Haemophilus* spp, *Streptococcus* spp or *Moraxella catarrhalis* (members of the phyla *Proteobacteria* and *Firmicutes*) positively correlates with sputum neutrophilia and Il-8, lower FEV_1, and longer disease duration, with *M catarrhalis* the bacterial species most associated with neutrophilic disease

(Continues)

TABLE 2 (Continued)

Authors	Number and type of participants	Age group	Samples collected	Technique used to assign bacterial taxa	Outcome	Key finding
Goleva *et al*[49], 2013	39 subjects with asthma (29 corticosteroid-resistant and 10 corticosteroid-sensitive) and 12 healthy controls	Adults	BAL	16S ribosomal RNA-based method	Differences in airway microbial composition between corticosteroid-resistant, corticosteroid-sensitive asthmatics and normal control subjects	No difference in microbial phyla composition was found between corticosteroid-resistant, corticosteroid-sensitive individuals, and healthy controls in terms of richness, diversity, evenness and community composition. However, significant variations in the percentage of sequences of different bacterial genera were found
Marri *et al*[39], 2013	10 patients with mild asthma and 10 healthy controls	Adults	Induced sputum	16S ribosomal RNA-based method (targeting V6 region)	Differences in airway microbiota between asthmatics and controls	Higher bacterial diversity was found in samples of asthmatic patients compared to controls. *Proteobacteria* were significantly higher in asthmatics compared to controls. In contrast, *Firmicutes* and *Actinobacteria* were non-significantly higher in controls
Haung *et al*[38], 2011	65 sub-optimally controlled asthma patients, and 10 healthy subjects	Adults	PBs	16S ribosomal RNA microarray	Differences in bronchial bacterial composition between suboptimal controlled asthmatics and controls	Bacterial diversity was significantly higher in asthmatic subjects compared to controls Methacholine P20 concentrations were negatively correlated with bacterial diversity, suggesting that bacterial diversity is positively correlated with bronchial hyperresponsiveness. The most predominant bacterial phyla associated with bronchial hyperresponsiveness were *Proteobacteria*
Hilty *et al*[37], 2010	24 adults patients, 11 with asthma, 5 with COPD and 5 healthy controls 20 children, 13 with difficult asthma and 7 controls	Adults, and children (up to 17 y)	Adults: Naso-oropharyngeal swabs, bronchial duplicate cytology brushings Children: BAL	16S ribosomal RNA-based method	Differences in airway microbiota between asthma, COPD patients and controls	Microbial community from the nose was distinct from the oropharyngeal and bronchial brushings *Proteobacteria*, especially *Haemophilus* spp, were more common in diseased patients (asthmatics and COPD) compared to controls; in contrast, *Bacteriodetes*, especially *Prevotella* spp, were more common in controls than asthmatics and COPD patients

Abbreviations: ACQ, Asthma Control Questionnaire; BA, bronchial aspirates; BAL, bronchoalveolar lavage; BB, bronchial biopsies; BMI, body mass index; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; CRS, chronic rhinosinusitis; EB, endobronchial brushings; FEV_1, forced expiratory volume in 1 second; IgE, immunoglobulin E; NB, nasal brushes; NW, nasal washes; OTU, operational taxonomic unit; PBs, protected bronchial epithelial brushes; T-RFLP, terminal restriction fragment length polymorphism.

other groups.[47] In the same study, atopic asthmatic patients were further randomized into fluticasone arm versus placebo which later revealed that corticosteroids may cause changes in certain bacteria.[47] In another study of 23 corticosteroid-free asthma patients and 10 healthy controls, bacterial dysbiosis was found between asthma patients with a low level of eosinophils when compared with asthma patients with high levels of eosinophils and healthy controls.[48] This suggests that asthma disease by itself, different phenotypes, and medications used (including corticosteroids) play a significant role in shaping the microbiome profiles.

Regarding bacterial diversity, some studies have shown that asthma patients had higher bacterial diversity in the airways when compared to healthy controls.[38,39] Again, this might be related to the effect of corticosteroids, which by suppressing immunity would enhance the growth of diverse microbiota. However, in other studies, no significant differences in bacterial diversity were found between asthma patients and healthy controls.[43,44,50] Other studies showed that asthmatic patients had lower airway and gut microbiome diversity when compared to healthy controls.[51-53]

Inflammatory phenotypes of asthma have been shown to be associated with airway bacterial dysbiosis. Three studies have reported that neutrophilic asthma patients had lower sputum bacterial richness and diversity and showed higher abundances of *Proteobacteria* phylum especially *Moraxella* and *Haemophilus* compared with non-neutrophilic, eosinophilic or paucigranulocytic asthma phenotypes.[54-56] In contrast, eosinophilic asthma patients had higher sputum abundances of *Actinobacteria* phylum especially *Actinomycetaceae* family compared with non-eosinophilic or neutrophilic asthma patients.[50,56] The microbial dysbiosis observed between different asthma phenotypes has driven some research groups to phenotype cluster their patients based on asthma clinical and biochemical characteristics before exploring microbial dysbiosis between these phenotypes. Two studies have shown that microbial composition can differ between the identified phenotypic clusters.[57,58] Observed microbial differences between different asthma phenotypes may help later to better diagnose them and provide an opportunity to use the microbiome as a biomarker in precision medicine approaches of asthma.

However, it is important to note that the above-mentioned studies measured microbiota in different sampling compartments which might induce variability of the obtained results and limit the ability to directly apply microbiome results in clinical care. In addition, some variations in results are still observed when comparing studies originating from the same sampling compartment. It should be noted that other factors can play a role in results dissimilarities. For instance, individuals originating from different populations might contribute to this variability because of the exposure to different environments and risk factors. A study shown that children from Russian Karelia had higher overall bacterial diversity and abundance of genus *Acinetobacter* and were less likely to have allergic disorders including asthma, compared to children from Finland Karelia.[59] In addition to different populations and environments, other factors related to multiple asthma phenotypes studied,[50,53,55,60] asthma and non-asthma medications used besides corticosteroids,[61] dietary habits[62] and age groups.[43] Moreover, it should be noted that sample size is a huge determining factors for inconsistencies found in reported microbiome statistical differences which should be considered while designing the microbiome asthma studies.

In conclusion, there is no distinct microbiome profile that can be said to be responsible for or associated with the asthma disease process. Under the umbrella of inconsistencies in reported results, several factors might hold responsible such as different populations, environments, age groups, drugs, diet and study design.

3.2 | Longitudinal studies

In longitudinal microbiome studies, patients have been followed prospectively to study associations between early life microbial dysbiosis and asthma development later in life or changes in the microbiome composition between two time-points (Table 3). Often the gut microbiome in children has been investigated in this type of studies. A recent study showed that an immature gut microbiome profile at 1 year of age in children born to asthmatic mothers was associated with asthma development at 5 years of age.[63] However, another study showed that children who developed asthma at 7 years of age (n = 8) had distinct gut bacterial profiles at one week and one month, but not at one year of age.[64] Similarly, it was shown that during the first three months of life, children with high risk of asthma had lower abundances of certain bacteria genera in their stool compared to others.[65,66] A different study showed that there was an association between gut bacterial diversity at 1 month and 1 year of age and allergic sensitization, but not with asthma development until 6 years of age.[67]

Only limited studies are available studying early life airway bacterial composition and the risk of developing asthma. One study showed that chronic wheeze at 5 years of age was associated with early airway colonization with *Streptococcus* during the first year of life.[68] In another study, cultures from the hypopharyngeal region aspirates of 321 infants at 1 month showed that colonization with *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* increased the risk for recurrent wheeze and asthma at 5 years of age.[69]

Despite that there was inconsistency about the most important time period (whether the first 3 months or the first year of life) to shape asthma risk later in life, it is apparent that gut or airway microbiota disturbances at an early age, especially the first year of life, are risk factors for asthma development. This bacterial dysbiosis may explain why early life antibiotic exposure may increase risk of later asthma development.[70-72]

In conclusion, longitudinal microbiome studies may help to uncover the shifts that occur in microbiome profiles over time. Studying the time-associated changes in the microbiome is essential because the microbiome is dynamic by nature and is influenced by several environmental and biological conditions. In addition to the studies that investigate early life microbial dysbiosis and its relation to asthma risk, more longitudinal investigation should be also conducted in patients

TABLE 3 Longitudinal microbiome asthma studies

Authors	Number and type of participants	Samples	Time of collection	Technique used to assign bacterial taxa	Outcomes	Key finding
Dzidic *et al*[150], 2018	80 children (47 allergic and 33 healthy)	Saliva	3, 6, 12, 24 mo and 7 y of age	16S ribosomal RNA-based method (targeting V1-V5 region)	Allergic symptoms and sensitization at 7 y of age	Allergic children, particularly asthmatics, had lower bacterial diversity compared to healthy children at 7 y of age During early infancy, there was an increase in relative abundances of *Gemella haemolysans* in allergic children, while healthy children showed increased abundances of *Lactobacillus gasseri* and *Lactobacillus crispatus*
Stokholm *et al*[63], 2018	690 children	Stool	1 wk, 1 mo and 1 y of age	16S ribosomal RNA-based method (targeting V4 region)	Asthma at 5 y of age	Children at 1 y of age who have an immature gut microbiome profile have increased risk of asthma at 5 y of age, but this effect is only evident in children who are born to asthmatic mothers
Pérez-Losada *et al*[151], 2017	40 asthmatic children (6-18 y)	Nasopharyngeal washes	2 samples collected 5.5-6.5 mo apart	16S ribosomal RNA-based method (targeting V4 region)	Temporal dynamics of airway microbiome in asthmatic children and their stability over time	No significant differences in α- and β-diversity were found between seasons There were significant differences in relative abundances of *Haemophilus*, *Staphylococcus*, *Moraxella* and *Corynebacterium* between summer and fall season and between age groups
Fujimura *et al*[152], 2016	298 children	Stool	1 and 6 mo	16S ribosomal RNA-based method (targeting V4 region)	Atopy diagnosis at 2 y of age and asthma diagnosis at 4 y of age	Neonates with highest risk of atopy and asthma diagnosis showed reduced relative abundances of *Bifidobacterium*, *Akkermansia* and *Faecalibacterium* bacterial taxa and high relative abundances of *Candida* and *Rhodotorula* fungi They also showed enriched fecal pro-inflammatory metabolites
Stiemsma *et al*[66], 2016	76 children (39 preschool asthmatic children and 37 matched healthy control)	Stool	3 mo and 1 y of age	16S ribosomal RNA-based method (targeting V3 region)	Asthma diagnosis by 4 y of age	Asthma patients at 3 mo of age showed decreased abundance of the genus *Lachnospira* and a trend for increased abundance of the species *Clostridium neonatale* The ratio of these two bacteria (L/C) was negatively associated with asthma risk by 4 y of age
Arrieta *et al*[65], 2015	319 children (136 with wheezing, 87 with atopy, 22 both, and 74 controls)	Stool	3 mo and 1 y of age	16S ribosomal RNA-based method (targeting V3 region)	High risk of asthma development (asthma diagnosis at 3 y of age)	No significant difference was found in microbiome diversity among the four groups During the first 100 d of life, children with high risk of asthma had lower abundance of the bacteria genera; *Lachnospira*, *Veillonella*, *Faecalibacterium* and *Rothia*
Teo *et al*[68], 2015	234 children	Nasopharyngeal aspirates	2, 6 and 12 mo of age during two states; healthy condition and episodes of acute respiratory illness	16S ribosomal RNA-based method	Impact of dynamics of nasopharyngeal microbiome, during the first year of life during both healthy condition and episodes of acute respiratory infections, on allergic sensitization at 2 y and chronic wheeze at 5 and 10 y of age	Children who had atopy by age of 2 y and developed chronic wheeze at 5 y of age were twice likely to have early colonization with *Streptococcus*, and lower respiratory tract infection during the first year of life compared to those who did not develop wheezing

(Continues)

TABLE 3 (Continued)

Authors	Number and type of participants	Samples	Time of collection	Technique used to assign bacterial taxa	Outcomes	Key finding
Abrahamson *et al*[64], 2013	47 children	Stool	1 wk, 1 mo and 1 y of age	16S ribosomal RNA-based method (targeting V3-V4 region)	Allergic diseases at school age (7 y), such as; asthma, eczema and allergic rhinitis	8 children who developed asthma had lower microbial diversity compared to non-asthmatic children at 1 wk and 1 mo of age, but no significant difference at 1 y of age No significant difference in bacterial relative abundances was found between children with asthma, eczema or allergic rhinitis and others at all age groups
Bisgaard *et al*[67], 2011	411 children	Stool	1 mo and 1 y of age	PCR of 16s ribosomal RNA and bacterial cultures	Allergic diseases until age of 6 y	Bacterial diversity at 1 mo and 1 y of age was inversely associated with allergic rhinitis, allergic sensitization (skin prick test and serum specific IgE) and blood eosinophils. No association was found with asthma development or atopic dermatitis

Abbreviations: IgE, immunoglobulin E; PCR, polymerase chain reaction.

with already established asthma disease to uncover the dynamic relation between the microbiome and the asthma disease process.

4 | HOW CAN THE MICROBIOME INFLUENCE ASTHMA DEVELOPMENT?

Various studies have found associations between microbial dysbiosis in the airways and/or intestinal tract and the risk of asthma development; however, the causal relationship remains unclear. In this section, we discuss two different processes, which may explain how the microbiome might influence asthma risk and the evidence supporting them.

4.1 | From hygiene hypothesis to old friends theory

The "hygiene hypothesis" that was first developed by Strachan *et al* assumes that excessive cleanliness, vaccination and antibiotic use can lead to an over-reactive immune system.[73,74] It was suggested that too much hygiene lowers the threshold of immune adaptation, it becomes sensitive and it over-reacts to substances that are normally not recognized by the immune system, like pollen and other allergens. In contrast, more exposure to micro-organisms may lead to immune tolerance and hence less recognition to allergens. Various studies have shown that more exposure to micro-organisms during childhood has protective effects against asthma development, for example; growing up in a farm environment, exposure to livestock animals and dogs, drinking unpasteurized milk and breastfeeding all have protective effects on asthma development.[75-79]

However, the limitation to this hypothesis is that it cannot be generalized to all populations. For instance, Japanese individuals are less prone to develop asthma than individuals living in the United States and Australia,[80] despite that they are exposed to a more hygienic environment.[81] The Japanese people have different dietary habits, eat more fermented products and possibly have a microbiome profile that protects them from allergy. Due to the inconsistencies to the hygiene hypothesis found in various studies, a revision has been suggested by the introduction of the "old friend" theory by Rook et al.[82] This theory proposed that micro-organisms co-evolved with humans (old friends) which have been essential for the development of the immune system.[82] Exposure to industrialized environments diminishes these micro-organisms with subsequent defective immune regulation which results in predisposition of the human to chronic inflammatory diseases, such as asthma. Therefore, exposure to natural environments, such as breastfeeding, natural delivery, outdoor activities, healthy-balanced diets and suitable use of antibiotics can re-establish the healthy microbiome environment and consequently reduces risk of allergic disorders.[83]

4.2 | The gut-lung axis and effects on immunity

Gut microbiota produce different types and amounts of short-chain fatty acids (SCFAs) by fermentation of dietary carbohydrates

depending on the food type.[84] High-fibre diets produce the largest amount of SCFAs. Acetate, proprionate and butyrate are the most abundant SCFAs.[85,86] SCFAs go to the liver via the portal vein where they are metabolized and those who escape metabolism pass into the systemic circulation and consequently reach other organs such as the lungs. A case-control study conducted in 476 children found that high vegetable fibre intake was protective against moderate to severe airway hyperresponsiveness,[87] which may suggest that low SCFAs in the gut may be associated with asthma development. A recent prospective birth cohort study conducted on 301 children has shown that the highest levels of butyrate and propionate (>95th percentile) found in the stool of one-year-old infants were later associated with reduced risk of asthma and other allergic disorders at 3 and 6 years of age which might be linked to dietary intake.[88] In a mice study, a high-fibre diet increased levels of circulating SCFAs which decreased susceptibility to allergic airway inflammation, in contrast to low fibre diet which showed the reverse effect.[89]

Short-chain fatty acids have affinity for G-protein-coupled receptors such as GPR41, GPR43 and GPR109a, which are known to regulate mucosal immunity.[89-92] In preclinical and clinical studies, high-fibre diet and SCFAs were found to reduce inflammatory biomarkers and improve lung function through an action mediated through these receptors.[89,93] In addition to the effects of SCFAs on receptors, butyrate has been shown to act as histone deacetylase inhibitor (HDACi).[94] HDACis have been shown to inhibit airway hyperresponsiveness and have anti-inflammatory effect with therapeutic potential in patients with asthma.[95,96] Figures S1 and S2 show the proposed mechanisms by which SCFAs interacts with airway environment through a gut-lung axis–dependent pathway in both healthy and diseased states.

In addition to the effect of SCFAs, certain bacterial taxa, whether commensal or pathogenic, have an influence on immunity. Mice studies have shown that commensal bacteria, such *as Clostridium*, *Lactobacillus*, *Bifidobacterium*, *Bacteroides* (or their capsular polysaccharides), have been found to induce Treg cell production, and/or reduce T_H1, T_H2 and T_H17 responses and inflammatory process in some tissues including the airways.[97-104] *Bifidobacterium longum* was found in higher prevalence in the gut of healthy children (n = 102) compared to those with high risk of allergic asthma and atopic dermatitis (n = 99).[105] Increased abundance of nasopharyngeal lactobacillus was associated with reduced risk of wheezing at two years of age in 118 infants.[106] In the respiratory tract, nasopharyngeal colonization with *S pneumoniae* and *H influenzae* leads to increased production of CXCL8 and CXCL2 in nasal lavage fluid of mice, and amplification of asthma-like pro-inflammatory responses.[107] A study conducted on the bronchial epithelial brushings of severe asthmatics (n = 40), mild-moderate asthmatics (n = 41) and healthy controls (n = 7) showed that *Actinobacteria* was correlated with gene expression of FK506 binding protein (FKBP5), which is a marker of corticosteroid responsiveness, while *Proteobacteria* was correlated with Th17 gene expression.[41] In a study conducted in 8 asthmatics and 6 healthy controls, asthma patients had higher abundance of *Proteobacteria*, and particularly *M catarrhalis* was associated with increased expression of inflammatory mediators (eg CCL20, IL1A and IRAK2) and apoptosis (eg TNF and C8orf4).[108,109] A mice study confirmed the pathogenic role of *Proteobacteria* inhaled through the nostrils, which induced severe lung inflammation and immunopathology characterized by prevalent airway neutrophilia, and expression of cytokine/chemokine profile.[110] These results are in line with the cross-sectional asthma studies which were discussed previously and imply that increased abundances of certain bacterial taxa, for example *Proteobacteria* and *Firmicutes*, and decreased abundances of others, for example *Bacteroidetes* and *Actinobacteria*, in asthmatics are probably influencers of asthma disease development, risk and severity.

5 | THERAPEUTIC VALUE OF THE MICROBIOME INSIGHTS FOR ASTHMA: PRO-BIOTIC, PREBIOTIC AND SYNBIOTIC INTERVENTIONS

The importance of the microbiota in the asthma disease process has raised interest in the possibility of using dietary supplements influencing the intestinal bacteria, such as pro-biotics[111] and prebiotics[112] and their combinations synbiotics to treat or control asthma. Pro-biotics are "live micro-organisms that, when administered in adequate amounts, confer a health benefit to the host",[111] while prebiotics are "substrates that are selectively utilized by host micro-organisms conferring a health benefit".[112] Synbiotics are combinations of both pro- and prebiotics.

Most evidence of beneficial effects of pro-, pre- and synbiotics comes from animal studies. Mice studies showed that administration of *Bifidobacterium breve* or *adolescentis* strains and *Lactobacillus plantarum* or *rhamnosus* strains significantly decreased allergic airway inflammation in OVA- or birch pollen-induced asthma mice models.[113-116] It is worthy to note that the effect of pro-biotics in mice asthma models is more prominent in neonates than adults.[117]

One of the available therapeutic options is CpG oligodeoxynucleotides (CpG-ODN) which are synthetic single-stranded DNA that have affinity for TLR9. Mice and other animal (eg cats and rhesus monkeys) studies have shown that CpG-ODN can significantly reduce airway inflammation, hyperreactivity and remodelling [118-124] and the effect was more pronounced when it was combined with the antigen or used as nanoparticle formulations.[119,121,122,125,126]

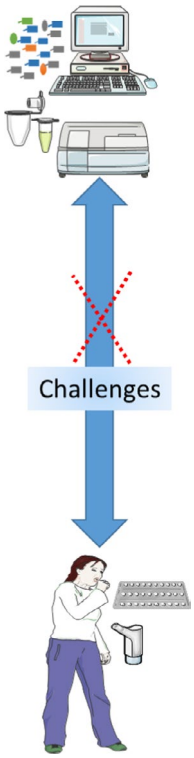
In a house dust mite (HDM)-induced asthma mice model, dietary galacto-oligosaccharides were found to prevent airway hyperresponsiveness, airway eosinophilia and T_H2 response, in a comparable way to budesonide[127] and the effect was eliminated in a Treg depleted model which might suggest that it is a Treg cell–mediated response.[128] Synbiotic combination of *B breve* and non-digestible oligosaccharides was found to have a strong airway anti-inflammatory properties in mice asthma models,[129,130] and the anti-allergic effect was greater than their individual effects.[131]

In contrast to mice studies, little evidence has been found for the beneficial effect of pro-biotics in human asthma. A meta-analysis conducted on 3257 infants from nine randomized clinical trials found that the risk ratio (RR) of asthma in infants received pro-biotics was

0.99 (95% confidence interval [CI] 0.81-1.21) and RR of wheeze was 0.97 (95% CI 0.87-1.09).[132] Another meta-analysis conducted on 25 randomized clinical trials showed that pro-biotics significantly reduced total IgE levels (−7.59 U/mL mean reduction, 95% CI: −14.96 to −0.22); however, they did not significantly reduce risk of asthma or wheeze (relative risk = 0.96, 95% CI: 0.85-1.07).[133] A randomized controlled trial was conducted on 1,302 asthmatic subjects older than 5 years of age to assess whether pro-biotic intake can reduce antibiotic prescribing for winter respiratory tract infections.[134] In this trial, no significant difference between the intervention group (n = 652) and control group (n = 650) was seen regarding the proportion of participants who were prescribed new courses of antibiotics (odds ratio = 1.04; 95% CI, 0.82-1.34).[134] Despite the diminished effects of pro-biotics shown in human controlled clinical trials, a recent study showed that the delayed gut microbial development in infants with high risk of asthma can be altered with daily oral supplementation of *Lactobacillus rhamnosus* GG compared with placebo, and this effect was lost 6 months after stopping the supplementation.[135] By changing gut microbial maturation early in infancy, there is a possibility to modify asthma risk later in life. This study provides a new potential strategy for using pro-biotics as an early preventive measure in high-risk infants.

In an open-label trial of 20 adult human asthmatics allergic to HDM, A-type CpG-ODN given as adjuvant to subcutaneous immunotherapy with HMD allergen extract was found to be safe and produce nearly complete alleviation of allergic symptoms after 10 weeks of immunotherapy.[136] In a double-blind trial, where 63 asthmatic patients were treated with 7 injections of either QbG10 (bacteriophage with CpG-motif G10 inside) or placebo, it was found that two-thirds of the intervention group had their asthma well controlled after 12 weeks of treatment compared to one-third of the placebo group.[137] For the effect of prebiotics on humans, a meta-analysis conducted on 249 infants from 2 trials showed that prebiotics reduce the risk of asthma/wheezing compared to the control

TABLE 4 Challenges that interfere for optimum delivery of gained knowledge and therapeutic potential of microbiome and "-biotics" products from bench to bedside

From bench to bedside	Challenges	Recommendations
	Different sampling compartments and whether they are mucosal or luminal should be considered while studying microbiome in asthma	It is important to investigate the microbiome from various sampling compartments in the same studied population and whether drugs (both asthma and non-asthma medications) are associated with microbial changes in certain compartments. Therefore, it might be wise to collect samples from multiple compartments while conducting microbiome asthma studies and adjust for effects introduced by certain medications
	Different sequencing approaches and platforms might produce variability in reported results	The choice of desired method depends on various factors, such as costs, quality and error rates of the produced sequencing reads. However, investigators should take into account that some of the variability can be introduced by the choice of the sequencing platforms and techniques. Therefore, this is important to consider when comparing results from different studies
	Some inconsistencies in the reported results have been found in microbiome asthma studies, which might influence clinical applicability and relevance	Large multi-centre international microbiome studies, accounting for the effect of multiple possible confounders, should be conducted to reach more definitive conclusions
	Different ways of reporting studies results, in terms of bacterial taxa, whether at phylum, genus or species level and using specialized microbiological terms can make hindrance for non-expert healthcare professional to see clinical relevance and can produce difficulty to directly compare results between studies	An agreement of clinical experts, stakeholders and healthcare organizations on standardized structure or pattern (as possible) for reporting results of microbiome studies will help to facilitate comparison, interpretation and systematic analysis of the combined work of world-wide research groups
	Some of the published microbiome asthma studies have investigated the gut microbiome profile without reference to the diet composition or the type of meal the patients usually consume which is an important determinant for the production of SCFAs	Accounting for diet while investigating the microbiome should be undertaken in the asthma studies
	There is hindrance in the optimum transfer of the therapeutic potential of products targeting microbiota(-biotics) from animal studies to humans	A thorough investigation is required to adequately enclose barriers of transmission, and whether personalizing or tailoring "-biotics" to certain patient/groups of patients will show more beneficial effect

group (RR = 0.37, 95% CI: 0.17-0.8); however, low number of events made the precision questionable.[138]

In summary, mice studies show promising results of using pro-, pre- and synbiotics in asthma models, while evidence in humans is scarce, especially for pro-biotics. One possibility for decreased therapeutic outcomes in infants is that the intended therapeutic outcomes are long term (for years) which can be influenced by different factors, such as environmental exposure, different dietary habits and dynamicity of microbiome over time. Whether the individual should receive high-fibre diet or making certain diet restrictions should be also considered in clinical trials of pro-biotics. New strategies for using products targeting the microbiota to prevent or treat asthma should be further investigated. Moreover, the role of precision medicine is very important to define certain individuals or patients' groups who can benefit most from the potential therapeutic "-biotics" options.

6 | THE ASTHMA MICROBIOME FROM BENCH TO BEDSIDE: CHALLENGES

Results of microbiome studies in patients with asthma convey the importance of the role of microbiome either in the intestinal tract or in the airways in shaping asthma. However, using combined information from different studies to elaborate the asthma-microbiome interaction in humans is hindered by methodological, design and interpretations issues/challenges in microbiome studies (Table 4). We suggest some recommendations to overcome them.

More efforts are required from healthcare professionals and researchers to conduct large-scale microbiome asthma studies, if possible, accounting for and/or minimizing sources of variability, to obtain a clearer overview of the microbiome role in asthma. We still do not clearly know the type of link between reported microbial dysbiosis and different asthma characteristics; whether is it an association or causation? And what is the direction in case of the latter? The finding that early life microbial dysbiosis was associated with increased risk of asthma later in life might suggest that the microbiome is responsible to some extent to asthma disease development. However, this does not negate that the link might be bidirectional. More research is needed to investigate the relationship behind just "associations."

Translating the results of microbiome asthma studies from sequencing reads into applicable clinical guidelines requires a systematic approach and cooperation of multi-disciplinary research groups. One strategy is to use the microbiome to better refine asthma phenotypes (eg neutrophilic asthma) diagnosis, for which certain therapeutics targets might be directed. Another strategy is to detect groups of asthma patients with clear microbial dysbiosis for whom interventions (eg pre-, pro- and synbiotics) to reshape the gut and/or airway microbiome might be more beneficial.

The microbiome is thought to have host genetic background,[139] cause epigenetic modulations,[140] affect host metabolism[141] and be influenced by environmental exposures.[142] This necessitates integrating the microbiome with other omics layers, such as metabolomics, proteomics, transcriptomics, genomics, epigenomics and exposomics (environmental factors), which will help to provide more insight into the mechanisms of complex disease processes such as asthma. Hopefully, this will aid to discover new biomarkers for better diagnosis and/or new therapeutic targets in asthmatic patients.

CONFLICT OF INTEREST

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SUPPORTING INFORMATION

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