



## Original article

## Variations in the respiratory microbiota amongst asthmatic and non-asthmatic subjects in Jordan

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## ABSTRACT

In this work, variation in microbiota in the lower respiratory tract (LRT) among asthmatic and non-asthmatic subjects is identified. All participants (27 asthmatic patients and 27 non-asthmatic subjects) were asked to expectorate a sputum sample in special sterile tubes after rinsing the mouth with a sterilizing solution. The expectorated sputum specimen was immediately homogenized and stored in the deep freezer for DNA extraction for microbial gene sequencing and sequence analyses. For sequencing the V4 region of the 16S rRNA gene was sequenced using Illumina MiSeq, followed by an analysis of alpha and beta diversity. It was found that asthmatic patients had greater bacterial diversity than non-asthmatic subjects. Bacteria associated to the phyla (Bacteroidetes, Proteobacteria, and Firmicutes) accounted for 90 % of all sequences. The relative abundance of Proteobacteria in the asthmatic patients was higher than that of non-asthmatic (30 % vs 17 %; P-value = 0.044), along with a high abundance of the pathogen *Haemophilus influenzae*. In contrast, Firmicutes (41 %) and Bacteroidetes (31 %) showed higher relative abundances in the non-asthmatic subjects. No significant link was found between the type of asthma drug or the method of drug usage (orally or via inhalation) and the respiratory microbiota. Therefore, the variations in LRS microbiota are not caused by the drugs taken by the asthmatic patients, rather they might be connected to the etiology of asthma. Since the asthmatic patients had higher proportions of *Haemophilus influenzae*, these organisms could be a causative factor in the pathophysiology of asthma.

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## 1. Introduction

In our respiratory system, a complex community of various microorganisms can be found on the mucosal layers of the lower and upper respiratory tracts (LRT and URT, respectively) (Dickson and Huffnagle, 2015). The impact of these microorganisms on our respiratory health remains a matter of debate in terms of it being

beneficial or having a pathological association (Dickson and Huffnagle, 2015). Previously, it was commonly believed that our LRT is a sterile environment that is free of microorganisms (Dickson and Huffnagle, 2015). However, accumulating evidence is showing that a wide range of microorganisms, although fewer in number than those found in the other sites, can reside in this area of a healthy respiratory system (Hilty et al., 2010; Dickson and Huffnagle, 2015; Chung, 2017). In neonates, the presence of such symbiotic microorganisms ensure the maturation of the immune system within these organs and help with allergen toleration (Gollwitzer et al., 2014). However, under certain stimuli, a modification and transient change can convert these symbiotic microorganisms into more pathogenic phenotype microorganisms, which may influence the development and the progression of some disorders such as chronic obstructive lung disorders and asthma (Hilty et al., 2010; Dickson and Huffnagle, 2015; Dickson et al., 2017).

Asthma is a commonly heterogeneous chronic inflammatory disease that is projected to influence almost 300 million patients

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globally (Accordini et al., 2012). It is well documented that bacterial establishment in the respiratory system may play a major role in chronic inflammation. Since asthma is classified as a chronic inflammatory disease of the respiratory system, bacteria may act as the instigator of chronic inflammation (Martin et al., 2001; Sutherland and Martin, 2007; Marri et al., 2013). Recently, with a greater understanding of microbial communities in the LRT, more studies are concentrating on the relationship between the colonization of microbial communities and the efficiency of the immune system, along with the impact of lower airway microbiota on chronic airway diseases, such as asthma (Hilty et al., 2010; Marri et al., 2013; Chung, 2017). With respect to asthma development and progression, the currently available data implicate that bacterial colonization and infection in the bronchial mucosa of the LRT contribute towards the development of the associated chronic inflammatory process (Marri et al., 2013). Accordingly, studies conducted over the last decade have shown variations in the composition of the LRT microbiome between asthmatic patients and healthy people (Hilty et al., 2010; Huang et al., 2015; Durack et al., 2016). In-depth investigations into the reasons behind these variations in the composition of the microbiome have shown that they might be connected to the degree of obstruction of the airway, the use of corticosteroids, and the presence of airway eosinophilia (Denner et al., 2016).

This study thus aims to identify the common microorganisms in the LRT among adult asthmatic and non-asthmatic subjects in Jordan. Moreover, it seeks to identify a common microbial bioindicator that could be used as a diagnostic tool for an early diagnosis of asthma using 16 s ribosomal RNA gene sequencing from sputum samples from the study population.

## 2. Materials and method

### 2.1. Clinical setting

The study was conducted over 10 months, from March to December 2019, with patients recruited from Al Bashir public hospital, located in Amman, the capital city of Jordan.

### 2.2. Ethic al approval

This study was approved ethically by the Jordanian Ministry of Health and by the Institutional Review Board (IRB) at the Applied Science Private University (Approval number 1800164).

### 2.3. Inclusion and exclusion criteria

Asthmatic patients and healthy subjects aged 14 years old or over were included in the study. This was due to patients over 14 being treated as adults at Al Bashir Hospital. Smokers, patients reported to have taken antibiotics for at least two months before study enrolment, and patients who had other respiratory diseases or infections were excluded from the study.

### 2.4. Participant's recruitment

A convenience sample of 54 participants were approached (27 asthmatic patients and 27 healthy subjects).

All the asthmatic patients were approached and identified from an outpatient clinic at Al Bashir Hospital. Study participants who met the inclusion criteria were informed about the study and the nature of their involvement. Once they showed their interest in taking a part in the study, they were asked to provide their verbal consent. Sociodemographic characteristics including age, gender,

marital status, smoking status, medication history were collected from all study participants.

Sputum specimens were collected according to the CDC guidelines for collecting respiratory diseases' samples (CDC, 2020). All participants were asked to expectorate a sputum sample in special sterile tubes indicated for sputum collection. Particular care was taken to prevent the contamination of the sputum sample with the post-nasal drip and saliva by asking the participants to rinse their mouth with sterile water before inducing the sputum sample. Then, the expectorated sputum specimens were immediately homogenized and stored in the deep freezer ( $-80^{\circ}\text{C}$ ) for DNA extraction and the following sequencing steps.

### 2.5. DNA extraction and 16S rRNA sequencing

DNA extraction was done using QIAamp<sup>®</sup> DNA mini kit (QIAGEN) under aseptic techniques in a sterile room for all samples according to the producer instructions. The extracted DNA for the 54 samples collected (27 from asthmatic patients and 27 from healthy subjects) were then stored at  $-80^{\circ}\text{C}$  in a sterile Eppendorf tube until they were shipped to the sequencing facility (Molecular Research LP, Shallowater, TX, USA) for sequencing. Briefly, the V4 variable region of the 16 s rRNA gene was amplified using the following primers: ill27Fmod (AGRGTTCGATCMTGGCTCAG) /ill519R-mod (GTNTTACNGCGGCKGCTG) with barcode on the forward primer. The HotStarTaq Plus Master Mix Kit (Qiagen, USA) was used and the PCR was run for 30 cycles. MiSeq was used for the sequencing according to the manufacturer's guidelines. The sequences were then joined after removal of the barcodes and the sequences with a length  $< 150$  bp and those with ambiguous base calls were removed. Following this, the resulted sequences were denoised, and the Operational Taxonomic Units (OTUs) were generated. The OTUs were classified by clustering at 97 % similarity base. Final OTUs were taxonomically defined using BLASTn against a curated database derived from RDP II and NCBI (<https://www.ncbi.nlm.nih.gov>, <https://rdp.cme.msu.edu>).

For alpha diversity index, namely, Shannon index H, this was carried out in "Past Program" for data analysis version 4.02, after filtering out the reading with relative abundance of  $< 1\%$  (DeJong, 1975).

### 2.6. Data analysis

Demographic data collected about the participants were recorded and analyzed using the SPSS software version 21 (Chicago, IL, USA) after being coded. A comparison of the categorical data between asthmatic and non-asthmatic groups was conducted using Chi-Square test. Then, an independent sample *t*-test was used to compare the continues data, which was used to compare bacterial diversity between the asthmatic and non-asthmatic subjects. One-way ANCOVA was conducted to test for the effect of the variables that showed significant differences between the two groups participating in the study (asthmatic and non-asthmatic); age and gender. The results for the analyses tests was considered statistically significant when the probability value was  $< 0.05$ .

## 3. Results

### 3.1. Demographics characteristics

Most of the study participants were single and lived in Amman. The majority of participants had a high school educational level and most of them were students. When the subjects were compared regarding their demographic parameters, a statistically significant difference between the studied two groups, asthmatic

and non-asthmatic (P value < 0.001), was shown with regards to the mean age of the studied population (Table 1). The difference in gender was also found to be significant between the two groups (P value were < 0.001), with higher rates of females 17 (63 %) in the asthmatic group. The non-asthmatic group was significantly younger than the asthmatic group. Also, a higher number of males (n = 33, representing 61.1 % of the sample size) than females (n = 21, 38.9 %) were enrolled into the study.

Among the asthmatic patients, inhalers were the major treatment used for asthma. The minimum number of asthma treatment used by patients was two medications and the maximum was four medications. The most frequent inhalers used were ventolin® (96 %), followed by symbicort® (58 %), and seretide® (22 %). Some patients (23 %) were taking oral corticosteroid (prednisone), and 25 % of them were taking theophylline.

### 3.2. Airway bacterial diversity and community composition

Bacterial phylogenetic analysis identified 751 different species (detected in at least one subject) in both asthmatic and non-asthmatic subjects, representing 136 bacterial families, 31 class, and 21 phyla. Regarding species relative abundance, only 54 species (28 species from asthmatic patients' samples and 26 species from the non-asthmatic subjects) had a relative abundance of greater than 1.

Bacterial diversity based on the bacterial genera of the samples collected from the asthmatic and non-asthmatic subjects was calculated by the Shannon Index (Fig. 1). In our study, the bacterial diversity of the samples collected from the asthmatic patients is

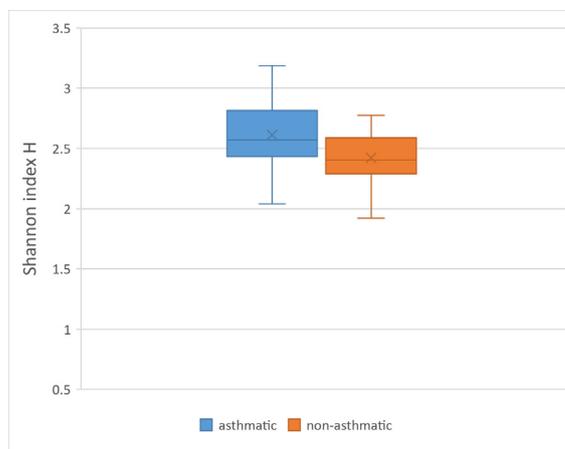


Fig. 1. Bacterial diversity in samples from the asthmatic (n = 27) and non-asthmatic (n = 27) subjects using Shannon Index (P value = 0.009).

significantly greater when compared with the samples from non-asthmatic subjects (P value = 0.009, Independent sample t-test).

All sputum samples had all five of the following bacterial phyla: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Fusobacterium. Relative abundances for the members belonging to Proteobacteria, Firmicutes, and Bacteroidetes phyla together accounted for 90 % of the bacterial community composition in both asthmatic and non-asthmatic subjects.

Variations in the relative abundances of phylum were found between the two studied groups (Fig. 2). The relative abundances

Table 1 Demographic characteristics of the study subjects (n = 54).

|   | Asthmatic n = 27 (50 %) | Non-asthmatic n = 27 (50 %) | Total         | P-value <sup>a</sup> |
|---|-------------------------|-----------------------------|---------------|----------------------|
| <b>Age mean ± SD</b>                      | 44.85 ± 17.04           | 22.66 ± 8.29                | 33.75 ± 17.36 | <0.001 <sup>b†</sup> |
| <b>Gender N (%)</b>                       |                         |                             |               |                      |
| Male                                      | 10 (37 %)               | 23 (85.2 %)                 | 33 (61.1 %)   | <0.001 <sup>a†</sup> |
| Female                                    | 17 (63 %)               | 4 (14.8 %)                  | 21 (38.9 %)   |                      |
| <b>Marital Status N (%)</b>               |                         |                             |               |                      |
| Married                                   | 17 (63 %)               | 4 (14.8 %)                  | 21 (38.9 %)   |                      |
| Widowed                                   | 2 (7.4 %)               | 0                           | 2 (3.7 %)     | <0.001 <sup>a†</sup> |
| Divorced                                  | 2 (7.4 %)               | 1 (3.7 %)                   | 3 (5.6 %)     |                      |
| Single                                    | 6 (22.2 %)              | 22 (81.5 %)                 | 28 (51.9 %)   |                      |
| <b>Living place: N (%)</b>                |                         |                             |               |                      |
| Amman                                     | 26 (96.3 %)             | 27 (100 %)                  | 53 (98.1 %)   | 0.313 <sup>a</sup>   |
| Zarqa                                     | 1 (3.7 %)               |                             | 1 (1.9 %)     |                      |
| <b>Education level N (%)</b>              |                         |                             |               |                      |
| Elementary                                |                         |                             |               |                      |
| High school                               | 5 (18.5 %)              | 0                           | 5 (9.3 %)     | 0.001 <sup>a†</sup>  |
| College                                   | 17 (63.0 %)             | 16 (59.3 %)                 | 33 (61.1 %)   |                      |
| University                                | 4 (14.8 %)              | 0                           | 4 (7.4 %)     |                      |
|   | 1 (3.7 %)               | 11 (40.7 %)                 | 12 (22.2 %)   |                      |
| <b>Number of Family members mean ± SD</b> | 4.88 ± 1.64             | 4.92 ± 1.59                 | 4.90 ± 1.60   | 0.933 <sup>b</sup>   |
| <b>Employment: N (%)</b>                  |                         |                             |               |                      |
| Employed                                  | 6 (22.2 %)              | 7 (25.9 %)                  | 13 (24.1 %)   | <0.001 <sup>a†</sup> |
| student                                   | 2 (7.4 %)               | 20 (74.1 %)                 | 22 (40.7 %)   |                      |
| unemployed                                | 17 (63.0 %)             | 0                           | 17 (31.5 %)   |                      |
| Retired                                   | 2 (7.4 %)               | 0                           | 2 (3.7 %)     |                      |
| <b>Nationality N (%)</b>                  |                         |                             |               |                      |
| Jordanian                                 | 26 (96.3 %)             | 27 (100 %)                  | 53 (98.1 %)   | 0.313 <sup>a</sup>   |
| Syrian                                    | 1 (3.7 %)               | 0                           | 1 (1.9 %)     |                      |
| <b>Origin N (%)</b>                       |                         |                             |               |                      |
| Palestine                                 | 22 (81.5 %)             | 19 (68 %)                   | 41 (75.9 %)   | 0.279 <sup>a</sup>   |
| Jordan                                    | 4 (14.8 %)              | 8 (25 %)                    | 12 (22.2 %)   |                      |
| Syria                                     | 1 (3.7 %)               | 0                           | 1 (1.9 %)     |                      |

<sup>a</sup>Chi-square test, Fisher's test

<sup>b</sup>t-test Independent sample

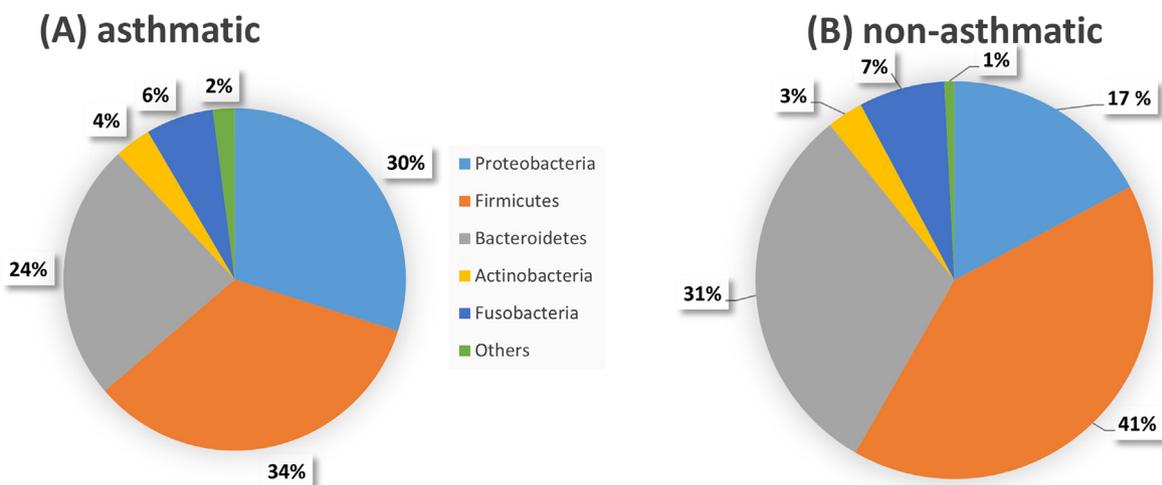


Fig. 2. Relative abundance for bacterial phyla in samples from asthmatic (A) and non-asthmatic subjects (B).

of the members belonging to Proteobacteria were found to be greater in asthmatic patients (30 %) than in the non-asthmatic subjects (17 %), with significant difference (P value = 0.04). Taking into account the significant variations found between the two groups with regards to age and gender showed that both had no significant effect on the relative abundance of the Proteobacteria (P value = 0.592 and 0.849, respectively; one-way ANCOVA). On the other hand, the opposite was the case for members belonging to Firmicutes and Bacteroidetes. The relative abundance of Firmicutes for asthmatic patients was lower than that of the non-asthmatic patients (34 % vs 41 %) with no significant difference (P value = 0.073). Similarly, the relative abundance of Bacteroidetes from the asthmatic patients was less than that of the non-asthmatic subjects (24 % and 31 %, respectively), which also had a non-significant difference (P value = 0.054). Additionally, results from the one-way ANCOVA analysis showed that age and gender had no significant effect on the relative abundance of Firmicutes and Bacteroidetes (P value for age = 0.354, 0.449 respectively), and the P value for gender was 0.251 and 0.355 respectively.

The bacterial sequences representing the five major phyla were dispersed into 118 families. Eleven of these families differed in their relative abundance between the samples from asthmatic

patients and non-asthmatic subjects (Fig. 3). Pasteurellaceae (21 %) and Streptococcaceae (24 %) were more abundant in the asthmatic patients than those who were non-asthmatic subjects. On the other hand, Prevotellaceae (25 %) and Veillonellaceae (10 %) were more common in the samples of non-asthmatic subjects than in those of the asthmatic patients. At the genus level, the results showed that the five most frequent genera were *Haemophilus*, *Streptococcus*, *Prevotella*, *Veillonella*, and *Prophyromonas* (Fig. 4). The asthmatic patients were characterized by higher relative abundance of *Haemophilus* species compared with the non-asthmatic, and higher relative abundance of *Streptococcus* species, whereas *Prevotella*, *Veillonella*, and *Prophyromonas* species were more abundant in the non-asthmatic subjects.

Regarding the differences between the samples at the species level, as defined by the identity of particular operational taxonomic units (OTUs), the heat map of principal OTUs (Fig. 5) showed that the asthmatic samples have more OTUs than non-asthmatics. It was striking that *Haemophilus influenzae* was found in the asthmatic airways with no presence in the healthy LRT. Similarly, a higher abundance of *Prevotella melaninogenica* was found in the asthmatic airways than that of the non-asthmatic; whereas, *Prevotella spp.* in general, *Villionella dispar*, and *Porphyromonas spp.* were

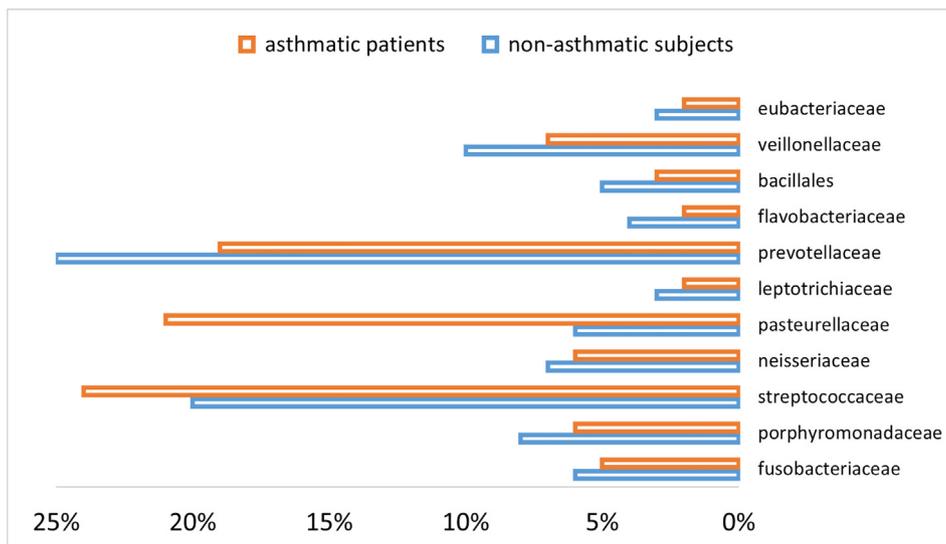


Fig. 3. Relative abundance of bacterial families in samples from asthmatic patients (n = 27) and healthy controls (n = 27).

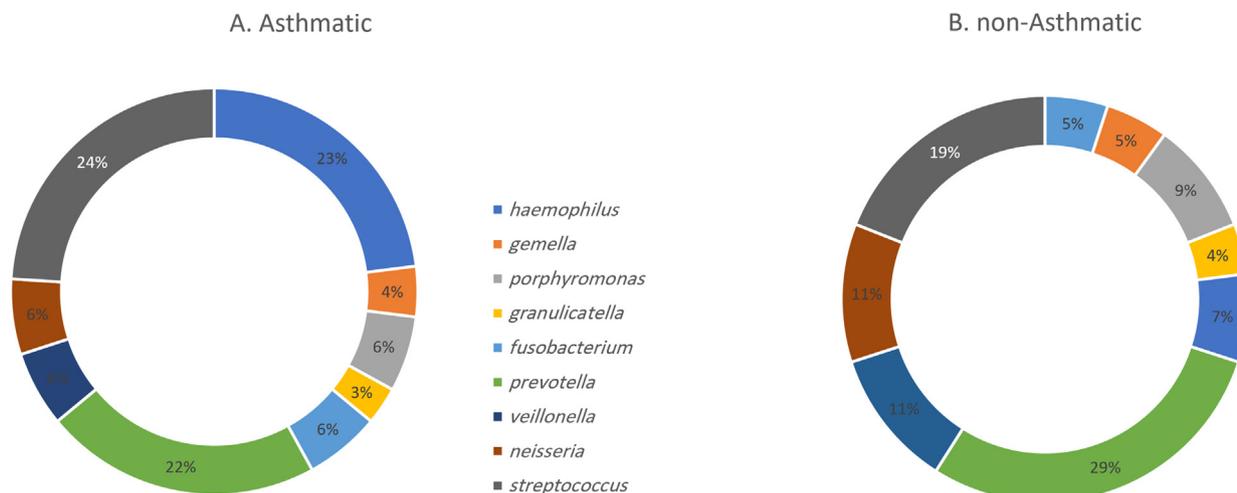


Fig. 4. Relative abundance of different genera found in the asthmatic (A) and non-asthmatic subjects (B).

in higher relative abundance in the non-asthmatic samples. *Streptococcus mitis* was found with high abundance in both asthmatic and non-asthmatic airways.

### 3.3. The effect of the corticosteroid on the microbiota

When conducting the study, all asthmatic patients were taking either inhaled or oral corticosteroid therapy (8 out of 27 patients were taking the oral corticosteroid (Prednisone tablets) daily. Patients on oral or inhaler corticosteroid therapy did not show any increase of bacterial phylum or genus. There was no significant link between any bacterial phylum with corticosteroid therapy (Proteobacteria (P value = 0.492), Firmicutes (P value = 0.206), Bacteroidetes (P value = 0.936)). Also, at a genus level, there was no significant link between bacterial genus with corticosteroid therapy (*Haemophilus*, *Gemella*, *Porphyromonas*, *Granulicatella*, *Fusobacterium*, *Prevotella*, *Veillonella*, *Neisseria*, *Streptococcus*; P value = 0.314, 0.347, 0.986, 0.260, 0.481, 0.786, 0.166, 0.871, 0.173 respectively).

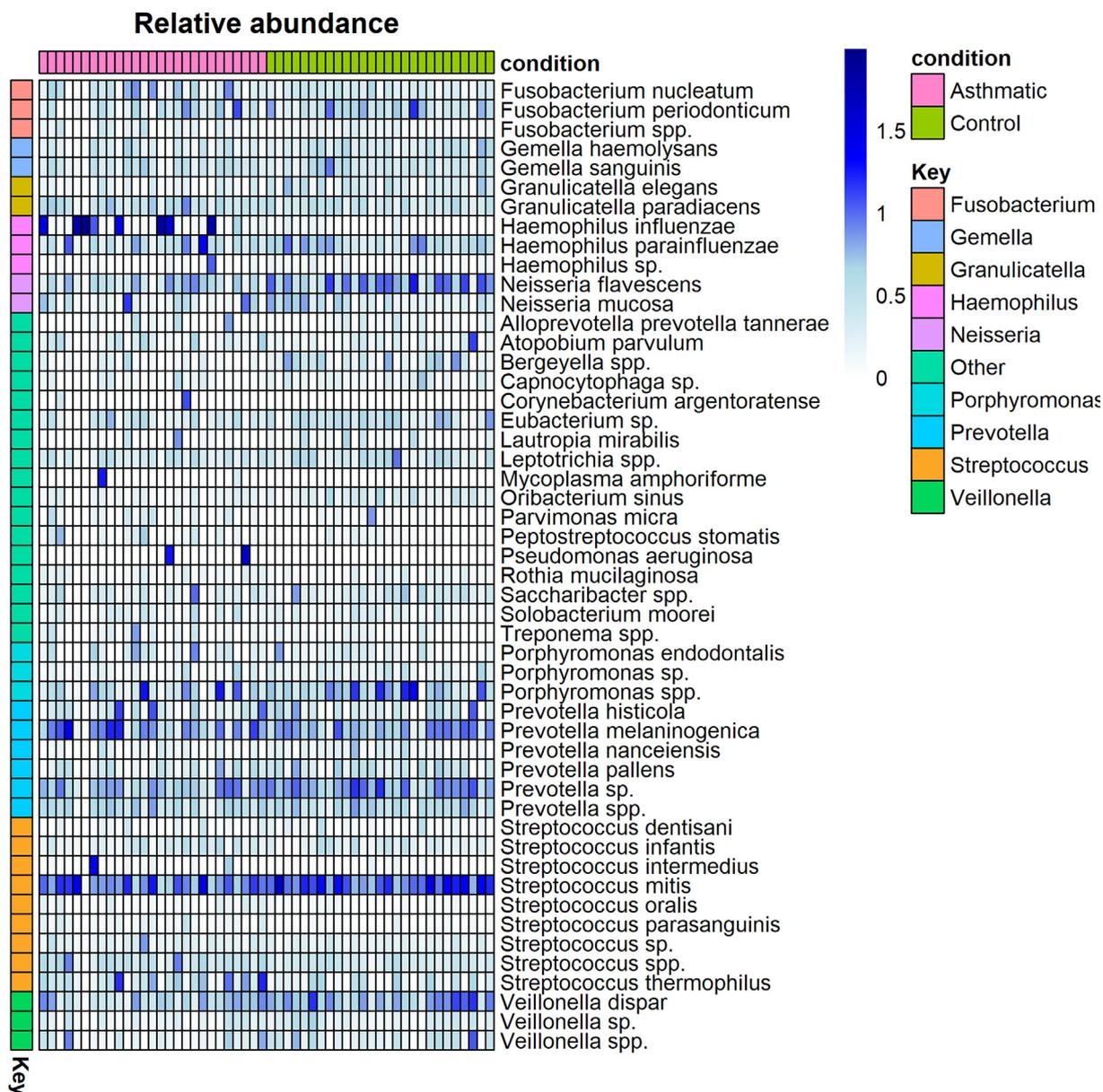
## 4. Discussion

The present study investigated variations in the common bacteria present in the LRT of asthmatic patients and non-asthmatic subjects in Jordan, and identified common bacterial bioindicator(s) that could be used as a potential diagnostic tool for asthma. This was done by taking into consideration the effect of different drug types used by asthmatic subjects, as well as the effect of age and gender on the respiratory microbiota. Regarding bacterial composition, the five major bacterial phyla, namely Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacterium, were detected in all sputum samples taken from asthmatic and non-asthmatic samples, albeit with different proportions. Members belonging to Proteobacteria were more abundant in the samples collected from asthmatic patients than those from non-asthmatic subjects with an obviously higher abundance of the potential pathogen *Haemophilus influenzae*. As the current analysis showed no significant link between variation in the respiratory microbiota and drug type, age, and gender, we propose that *Haemophilus influenzae* may be used as an indicator for the occurrence of asthma. We suggest that if the relative abundance of this pathogen is more than 20 % in the sputum sample retrieved from a subject, it could be a potential indicator that the subject has asthma.

Shannon index H showed that the bacterial diversity of the asthmatic patients is significantly greater compared with the samples from the non-asthmatic subjects. This finding is in agreement with earlier studies that reported increased bacterial diversity and bacteria load in samples from asthmatic patients compared with non-asthmatics (Huang et al., 2011; Marri et al., 2013). However, a more recent study indicated no significant difference in alpha diversity between asthmatic patients and healthy people (Zhang et al., 2016). Also, compared to earlier studies, we noticed a marginally higher number of bacterial families from samples taken from asthmatic patients (Huang et al., 2011; Marri et al., 2013). The extra diversity in our study could have resulted either from using different or more sensitive sequencing technologies. Another possibility behind the raised number of microbial communities could be due to the oral cavity bacteria, which might have resulted from the difficulty of taking the samples from older subjects.

In this study, a higher proportion of the phylum Proteobacteria was detected in the sputum samples from asthmatic patients compared with the samples from non-asthmatic subjects. Our finding is generally concurrent with many previous studies, despite the differences in the type of the samples and the identification technologies used. Other studies also reported a higher relative abundance of Proteobacteria in the LRT of asthmatic patients compared with healthy subjects regardless of the inhaler they used (Hilty et al., 2010; Huang et al., 2011; Marri et al., 2013; Zhang et al., 2016).

Our results of the increased relative abundance of members belonging to Pasteurellaceae (such as *Haemophilus influenzae*) in asthmatic patients' samples support the results of earlier studies. It was shown that for asthmatic patients who were using inhaled corticosteroid treatment, the relative abundance of *Haemophilus* spp. was specifically seen to be increase (Hilty et al., 2010; Huang et al., 2011; Marri et al., 2013; Durack et al., 2017). *Haemophilus influenzae* has consistently been connected with the advancement of asthma and asthma exacerbations (Kraft, 2000; Bisgaard et al., 2007). Therefore, the presence of this pathogen in elevated relative abundance may have a possible influence on chronic airway inflammation. In addition, the presence of an even lower number of the pathogens may provide the inoculum for initiating a secondary bacterial infection following other viral infections and pandemic influenza (Didierlaurent et al., 2007). In a previous study that was conducted using specific probes in a real-time PCR, they found that 80 % of the patients with poorly controlled asthma had higher *H. influenzae* (Simpson et al., 2016). Even



**Fig. 5.** heat map of operational taxonomic units (OTUs) found in the sputum of asthmatics (n = 27) and non-asthmatic healthy subjects (n = 27). On the figure, increased depth of the colour indicates relative abundance of the OTU in an individual sample.

though the PCR does not differentiate between dead and live microorganisms, even dead or degraded bacteria can stimulate an immune response. Simpson and her colleagues (2016) were able to link the variations in the airway microbiome to host inflammatory response in asthma, and showed that there is a reduced bacterial diversity connected to a high relative abundance of *H. influenzae*.

Macrolides have been shown to be effective in reducing exacerbations in patients with severe neutrophilic asthma who have poorly controlled symptoms (Wong et al., 2014). Attempts to use antibiotics in the management of asthma other than using them in the controlling of acute exacerbations have not been tested (Blasi and Johnston, 2007). Thus, searching for efficient vaccines against *Haemophilus influenzae* might also be a novel method in the management of asthmatic patients whose airways are characterized by the permanent presence of this bacteria.

However, our data suggests that Firmicutes, particularly *Veillonella*, have a higher relative abundance in samples from non-

asthmatic subjects, which is supported by the results of Marri et al. (Marri et al., 2013). We also observed a raised relative abundance of Bacteroidetes in samples from non-asthmatic subjects, which comes to an agreement, on the one hand with the result of Hilty et al. (Hilty et al., 2010), and contrasts on the other with the results of Marri et al. (Marri et al., 2013). Firmicutes are Gram-positive bacteria that have some noteworthy pathogenic bacteria, such as *Streptococcus*, *Bacillus*, and *Staphylococcus* species (Schleifer, 2009). Bacteroidetes, on the other hand, are gram-negative bacteria that are widely dispersed in the environment, as well as in the gut, such as *Porphyromonas* and *Prevotella* species (Thomas et al., 2011).

In this study, Bacteroidetes accounted for 25 % to 30 % of the total bacterial phyla found in the studied samples. However, in the study by Marri et al. (2013), Bacteroidetes represented only 1–2 % of the total bacterial community composition. This discrepancy could be due to the differences between the studied populations, since the human microbiome varies between subjects and

populations. Additionally, the method of obtaining the sample and the sequencing method are very important causes of this inconsistency in the relative abundance of members belonging to Bacteroidetes. Samples in the current study were collected from the Jordanian population, while the samples in the Marri et al. (2013) study were from the United States.

Also, the management of asthma has an important effect on variation in the microbial communities between patients from different parts of the world. For example, our asthmatic patients had been given medium to high dose of inhaled corticosteroid, and in some cases they were taking non-continuous oral corticosteroid, while the asthmatic patients in the study of Marri et al. (2013) were not taking oral or inhaled corticosteroids. Although the effect of corticosteroids on the lower airway microbiota is still unclear, studies have demonstrated that they likely alter the lung microbiome and have an extensive immunosuppressive effect (Gedalia and Shetty, 2004). Additionally, certain patients might take antibiotics from time to time, which affect the microbiota; therefore, one of the exclusion criteria in our study was the participant taking antibiotics. Hence, there is a possibility that the reported differences in the airway microbiota from other studies could be attributed to the therapy that the patients were receiving. Also, in our study, no significant link was found between corticosteroid therapy usage (orally or via inhalation) and the bacterial phyla found in the LRT of asthmatic patients. However, our results contrast with the results of Zhang et al., whose findings indicate that severe asthmatic patients with continuous oral corticosteroid therapy was associated with increased Firmicutes (Zhang et al., 2016).

Bacteroidetes, mainly *Prevotella* spp. were more abundant in non-asthmatic subjects than in asthmatic patients. *Prevotella* is recognized to be part of the normal oral flora, and at the same time, it is isolated from respiratory tract infections (Aas et al., 2005). The reduction of Bacteroidetes in our asthmatic patients compared with the non-asthmatic participants may be relevant to a disease. Also, it may be relevant that *Prevotella* spp. and particularly *Prevotella melaninogenica* immediately inhibited the growth of other bacteria such as *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterobacter cloacae*, and *Klebsiella species* (Murray and Rosenblatt, 1976). Furthermore, both *Prevotella* and *Veillonella* spp. could be characteristic components of the normal flora of the lung and other epithelia.

After adjusting for age and gender, among the independent variables that showed significant differences between the two study groups, no significant effect was found with regards to the output documented originally. Type of medicine, age and gender showed no significant effect on the dependent variables' mean of microbial species.

## 5. Conclusion

In conclusion, samples from the asthmatic patients have greater bacterial diversity than the samples from non-asthmatic subjects. The asthmatic patients showed an altered microbial composition in their LRT. The results of this study have demonstrated that all sputum samples contained five bacterial phyla: Proteobacteria, firmicutes, bacteroidetes, actinobacteria, and fusobacterium, with the first three phyla accounting for 90 % of the total sequences. Proteobacteria were present in higher proportions in the asthmatic patient, with a high abundance of the potential pathogen *Haemophilus influenza* in the asthmatics' airways. In contrast, Firmicutes and Bacteroidetes were found in higher relative abundances in samples from the non-asthmatic subjects. In addition, type of asthma medicine (oral or inhaler), age and gender showed no significant link with variation in the respiratory microbiota between asthmatic and non-asthmatic subjects. Therefore,

we propose to use the relative abundance of *Haemophilus influenza* as an indicator for asthma.

## 6. Authors' contributions

MAN: Planned the work, helped in analysing the sequencing data, reviewed the manuscript.

IB: Planned the work, reviewed the manuscript.

NR: collected the samples, performed DNA extraction, analysed the questionnaire, and wrote the manuscript.

Abj: helped in analysing the data and developing the figures, reviewed the manuscript.

RQ: helped in analysing the questionnaire, reviewed the manuscript.

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## 8. Ethics approval and consent to participate

Written in the Materials and Methods and will be provided in the supplementary information.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2022.103406>.

## References

- Aas, J.A., Paster, B.J., Stokes, L.N., Olsen, I., Dewhirst, F.E., 2005. Defining the normal bacterial flora of the oral cavity. *J. Clin. Microbiol.* 43 (11), 5721–5732.
- Accordini, S., Corsico, A.G., Braggion, M., Gerbase, M.W., Gislason, D., Gulsvik, A., Heinrich, J., Janson, C., Jarvis, D., Jögi, R., Pin, I., Schoefer, Y., Bugiani, M., Cazzoletti, L., Cerveri, I., Marcon, A., de Marco, R., 2012. The cost of persistent asthma in Europe: an international population-based study in adults. *Int. Arch. Allergy Immunol.* 160 (1), 93–101.
- Biggaard, H., Hermansen, M.N., Buchvald, F., Loland, L., Halkjaer, L.B., Bønnelykke, K., Brasholt, M., Heltberg, A., Vissing, N.H., Thorsen, S.V., Stage, M., Phipps, C.B., 2007. Childhood asthma after bacterial colonization of the airway in neonates. *N. Engl. J. Med.* 357 (15), 1487–1495.
- Blasi, F., Johnston, S.L., 2007. The role of antibiotics in asthma. *Int. J. Antimicrob. Agents* 29 (5), 485–493.
- Centers for Disease Control and Prevention (CDC), Specimen collection guidelines. Available [Online] from: <https://www.cdc.gov/urdo/downloads/speccollectionguidelines.pdf>.
- Chung, K.F., 2017. Airway microbial dysbiosis in asthmatic patients: a target for prevention and treatment? *J. Allergy Clin. Immunol.* 139 (4), 1071–1081.
- DeJong, T.M., 1975. A comparison of three diversity indices based on their components of richness and evenness. *Oikos* 26 (2), 222.
- Denner, D.R., Sangwan, N., Becker, J.B., Hogarth, D.K., Oldham, J., Castillo, J., Sperling, A.I., Solway, J., Naureckas, E.T., Gilbert, J.A., White, S.R., 2016. Corticosteroid therapy and airflow obstruction influence the bronchial microbiome, which is distinct from that of bronchoalveolar lavage in asthmatic airways. *J. Allergy Clin. Immunol.* 137 (5), 1398–1405.e3.

- Dickson, R.P., Huffnagle, G.B., 2015. The lung microbiome: new principles for respiratory bacteriology in health and disease. *PLoS Pathogens* 11 (7).
- Dickson, R.P., Erb-Downward, J.R., Freeman, C.M., McCloskey, L., Falkowski, N.R., Huffnagle, G.B., Curtis, J.L., Clemente, J.C., Molyneaux, P., Bogaert, D., 2017. Bacterial topography of the healthy human lower respiratory tract. *MBio* 8 (1).
- Didierlaurent, A., Goulding, J., Hussell, T., 2007. The impact of successive infections on the lung microenvironment. *Immunology* 122 (4), 457–465.
- Durack, J., Boushey, H.A., Lynch, S.V., 2016. Airway microbiota and the implications of dysbiosis in asthma. *Curr. Allergy Asthma Rep.* 16 (8), 52.
- Durack, J., Lynch, S.V., Nariya, S., Bhakta, N.R., Beigelman, A., Castro, M., Dyer, A.-M., Israel, E., Kraft, M., Martin, R.J., Mauger, D.T., Rosenberg, S.R., Sharp-King, T., White, S.R., Woodruff, P.G., Avila, P.C., Denlinger, L.C., Holguin, F., Lazarus, S.C., Lugogo, N., Moore, W.C., Peters, S.P., Que, L., Smith, L.J., Sorkness, C.A., Wechsler, M.E., Wenzel, S.E., Boushey, H.A., Huang, Y.J., 2017. Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment. *J. Allergy Clin. Immunol.* 140 (1), 63–75.
- Gedalia, A., Shetty, A.K., 2004. Chronic steroid and immunosuppressant therapy in children. *Pediatr. Rev.* 25, 425–434.
- Gollwitzer, E.S., Saglani, S., Trompette, A., Yadava, K., Sherburn, R., McCoy, K.D., Nicod, L.P., Lloyd, C.M., Marsland, B.J., 2014. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat. Med.* 20 (6), 642–647.
- Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., Davies, J., Ervine, A., Poulter, L., Pachter, L., Moffatt, M.F., Cookson, W.O.C., Neyrolles, O., 2010. Disordered microbial communities in asthmatic airways. *PLoS ONE* 5 (1), e8578.
- Huang, Y.J., Nelson, C.E., Brodie, E.L., DeSantis, T.Z., Baek, M.S., Liu, J., Woyke, T., Allgaier, M., Bristow, J., Wiener-Kronish, J.P., Sutherland, E.R., King, T.S., Icitovic, N., Martin, R.J., Calhoun, W.J., Castro, M., Denlinger, L.C., DiMango, E., Kraft, M., Peters, S.P., Wasserman, S.I., Wechsler, M.E., Boushey, H.A., Lynch, S.V., 2011. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J. Allergy Clin. Immunol.* 127 (2), 372–381.e3.
- Huang, Y.J., Nariya, S., Harris, J.M., Lynch, S.V., Choy, D.F., Arron, J.R., Boushey, H., 2015. The airway microbiome in patients with severe asthma: associations with disease features and severity. *J. Allergy Clin. Immunol.* 136 (4), 874–884.
- Kraft, M., 2000. The role of bacterial infections in asthma. *Clin. Chest Med.* 21 (2), 301–313.
- Marri, P.R., Stern, D.A., Wright, A.L., Billheimer, D., Martinez, F.D., 2013. Asthma-associated differences in microbial composition of induced sputum. *J. Allergy Clin. Immunol.* 131 (2), 346–352. <https://doi.org/10.1016/j.jaci.2012.11.013>.
- Martin, R.J., Kraft, M., Chu, H.W., Berns, E.A., Cassell, G.H., 2001. A link between chronic asthma and chronic infection. *J. Allergy Clin. Immunol.* 107 (4), 595–601.
- Murray, P.R., Rosenblatt, J.E., 1976. Bacterial interference by oropharyngeal and clinical isolates of anaerobic bacteria. *J. Infect. Dis.* 134 (3), 281–285.
- Schleifer, K.-H., 2009. Phylum XIII. Firmicutes Gibbons and Murray 1978, 5 (Firmacutes [sic] Gibbons and Murray 1978, 5). *Bergey's Manual® of Systematic Bacteriology*. Springer. 19–1317.
- Simpson, J.L., Daly, J., Baines, K.J., Yang, I.A., Upham, J.W., Reynolds, P.N., Hodge, S., James, A.L., Hugenholtz, P., Willner, D., Gibson, P.G., 2016. Airway dysbiosis: *Haemophilus influenzae* and *Tropheryma* in poorly controlled asthma. *Eur. Respir. J.* 47 (3), 792.
- Sutherland, E.R., Martin, R.J., 2007. Asthma and atypical bacterial infection. *Chest* 132 (6), 1962–1966.
- Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M., Michel, G., 2011. Environmental and gut bacteroidetes: the food connection. *Front. Microbiol.* 2, 93.
- Wong, E.H., Porter, J.D., Edwards, M.R., Johnston, S.L., 2014. The role of macrolides in asthma: current evidence and future directions. *Lancet Respiratory Med.* 2 (8), 657–670.
- Zhang, Q., Cox, M., Liang, Z., Brinkmann, F., Cardenas, P.A., Duff, R., Bhavsar, P., Cookson, W., Moffatt, M., Chung, K.F., Chalmers, J.D., 2016. Airway microbiota in severe asthma and relationship to asthma severity and phenotypes. *PLoS ONE* 11 (4), e0152724.