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# The impact of azithromycin therapy on the airway microbiota in asthma

## INTRODUCTION

There is interest in the use of macrolide antibiotics in asthma. Macrolides have been shown to improve airway hyperresponsiveness (AHR) and measures of airway inflammation.[1] The degree of AHR may relate to the microbiota present in the airways,[2] with a recent study reporting that patients with asthma with a significant improvement in AHR following treatment with clarithromycin had a higher bacterial diversity prior to treatment.[3] To our knowledge, the impact on the asthmatic airway microbiota of an antibiotic has not been reported and we therefore set out to establish if macrolide therapy was associated with a change in airway microbiota in asthma.

## METHODS

Five adult patients with moderate/severe asthma (British Thoracic Society step 4–5) (see online supplementary table S1) and no evidence of respiratory infection or bronchiectasis underwent bronchoscopy before and after 6 weeks of daily 250 mg azithromycin therapy. Patients had consented to the study (REC 11/EM/0062). Saline washings of the right upper lobe were obtained following standard procedure, DNA was isolated from the samples (see online supplementary Methods) and the microbiota analysed by using pyrosequencing performed by Molecular Research DNA. Microbiota results were analysed after random resampling of the data[4] and calculation of two diversity indices; richness and Shannon's.

## RESULTS

A total of 5223 reads were analysed from five sample pairs (pretreatment and post-treatment). Eighty-nine distinct genera were detected. Bacteria from the genera *Staphylococcus* (10.49%), *Pseudomonas* (9.35%), *Streptococcus* (7.99%) and *Neisseria* (4.75%) were all found to be among the more abundant genera in the pretreatment samples (table 1).

The total abundance of each genus is given as a percentage of the total number of reads within each of the three groups. Parentheses represent the number of samples where the genus was present.

Many genera reduced in abundance after treatment including *Prevotella* (3.43%), *Staphylococcus* (4.59%) and *Haemophilus* (3.28%), with *Pseudomonas* not detected post-treatment. There was an increase in the relative number of *Anaerococcus* (39.18%) observed in two patients after treatment.

**Table 1** Relative abundance of each of the most dominant genera in samples pretreatment and post-treatment

| Genus | Total (n=10) | Pretreatment (n=5) | Post-treatment (n=5) |
|---|---|---|---|
| *Actinobacillus* | 2.97 (1) | 4.78 (1) | 0.00 (0) |
| *Anaerococcus* | 17.29 (5) | 3.89 (3) | 39.18 (2) |
| *Fusobacterium* | 1.82 (4) | 2.90 (3) | 0.05 (1) |
| *Haemophilus* | 7.91 (5) | 10.74 (3) | 3.28 (2) |
| *Neisseria* | 3.01 (2) | 4.75 (1) | 0.15 (1) |
| *Prevotella* | 4.12 (6) | 4.54 (4) | 3.43 (2) |
| *Pseudomonas* | 5.80 (2) | 9.35 (2) | 0.00 (0) |
| *Staphylococcus* | 8.25 (8) | 10.49 (5) | 4.59 (3) |
| *Streptococcus* | 8.08 (6) | 7.99 (3) | 8.22 (3) |
| *Veillonella* | 7.39 (6) | 4.10 (3) | 12.76 (3) |
| Other | 7.37 (3) | 7.99 (2) | 6.35 (1) |

Evaluation of richness revealed that the mean number of genera detected in the pretreatment samples was 19.37 genera (SD=5.68, n=5). This was higher than the mean number of genera post-treatment (mean=12.80 genera, SD=3.70, n=5). Equally, the mean Shannon's index in the pretreatment group was 1.62 (SD=0.20, n=5) compared with post-treatment (mean=1.22, SD=0.40, n=5). Non-parametric investigation found near significant differences between the patients pretreatment and post-treatment with richness and Shannon's index (both Kruskal-Wallis $\chi^2=3.15$, p=0.076; figure 1).

## CONCLUSION

This is the first study to examine longitudinal changes in airway microbiota following antibiotic treatment in asthma. Azithromycin therapy was associated with decreased bacterial richness in the airways and altered the airway microbiota leading to *Anaerococcus* becoming dominant within the bacterial community in some cases. Importantly, *Pseudomonas*, *Haemophilus* and *Staphylococcus* (three pathogenic genera associated with airway disease) were all reduced. This may explain the clinical improvement observed in asthma[5] and suggests a possible antibiotic as well as immunomodulatory effect of macrolides on AHR. Azithromycin has also been shown to decrease mucus secretion, airway neutrophil accumulation as well as specific

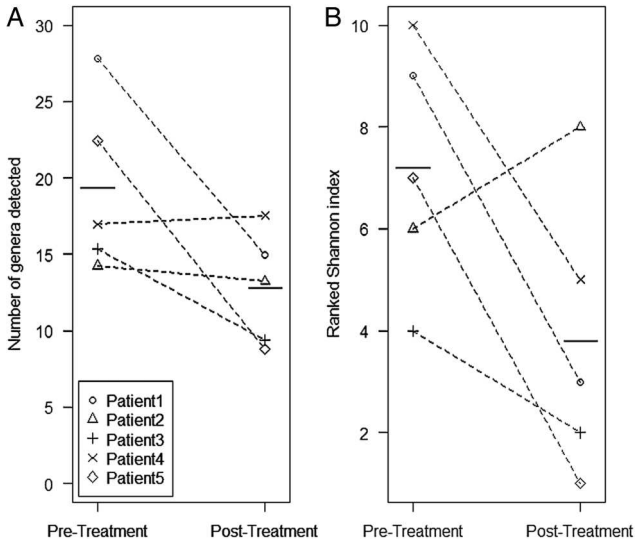


**Figure 1** Characteristics of the microbiota in patients prior to and after azithromycin treatment. Each data point represents a single sample with horizontal lines indicating the mean number of genera detected (A) and Shannon's index rank (B). Near significant differences were reported between groups for both measures ($\chi$2=3.15, p=0.076). Dotted lines are shown to indicate the change in measure for each patient.

antibiotic and antipseudomonal activity. This early work indicates that larger studies of the effect of treatments on the airway microbiota and clinical outcomes are now needed.

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