

# History Of Tonsillectomy Does Not Significantly Affect The Small Intestinal Microbiome

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## BACKGROUND

A prior history of tonsillectomy has been shown to be significantly associated with esophageal adenocarcinoma, an increase in upper respiratory tract diseases and a higher incidence of autoimmune diseases<sup>1-3</sup>. The short-term complications of tonsillectomy are well explored, whereas relatively little is known about the long-term complications. The absence of tonsillar tissue might cause pathogens to penetrate the alimentary tract.

The small bowel plays an important role in human immune function and metabolism, and to date, there is no study examining the effect of tonsillectomy on the small bowel microbiome. Here, we explore the influence of tonsillectomy on small bowel microbial profiles in those who have had their tonsils removed.

## AIM

In this study, we compare small bowel microbial profiles from those who underwent a tonsillectomy from the REIMAGINE study to those who did not undergo a tonsillectomy.

## METHODS

The REIMAGINE study is a novel large-scale study using validated methods for duodenal aspirate collection and microbiome sequencing in subjects undergoing upper endoscopy without colon prep. Subjects were divided into 2 groups: those who self-reported undergoing a tonsillectomy (T+), and those who did not undergo a tonsillectomy (NT). Groups were matched for gender, age  $\pm 5$  years, and BMI  $\pm 3$  kg/m<sup>2</sup>. Duodenal aspirates were collected, and microbial DNA was isolated using the MagAttract PowerSoilDNA Kit. V3V4 libraries were sequenced on MiSeq. Reference based Operational Taxonomic Unit clustering was performed using SILVA v132 database. Taxonomic analysis were performed with CLC Microbial Genomics Module v. 2.5 and MicrobiomeAnalyst<sup>2</sup>. Blood was also collected and levels of inflammatory biomarkers (IL1 $\beta$ , IL2, IL4, IL5, IL6, IL8, IL10, IL12p70, IL13, IFN $\gamma$ , MCP1, GMCSF and TNF $\alpha$ ), cholesterol, triglycerides, LDL, HDL, glucose, insulin, glucagon, GLP-1, vinculin and CdtB antibodies were checked.

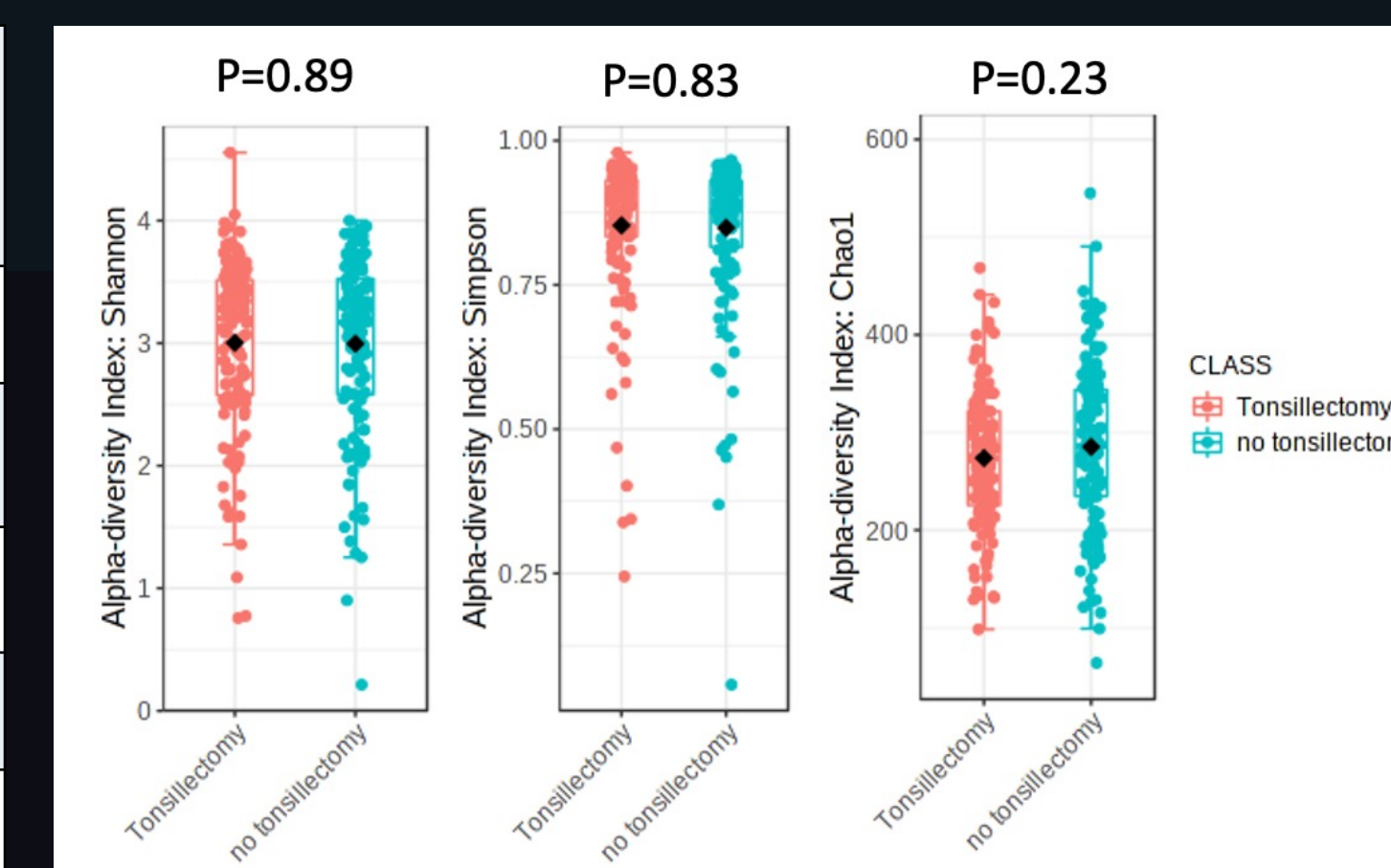
## RESULTS

This study included a total of 253 subjects, 126 in the Tonsillectomy (T+) group (mean age = 62 $\pm$ 15, mean BMI = 26.12 $\pm$ 5.55), and 127 in the No Tonsillectomy (NT) group (mean age = 63 $\pm$ 13, mean BMI = 25.62 $\pm$ 5.75) (Table 1). There was no significant difference in circulating levels of any analyte analyzed between groups. There was no statistical difference in duodenal microbial alpha-diversity or beta-diversity between the T+ group vs. NT group (Fig 1 & 2).

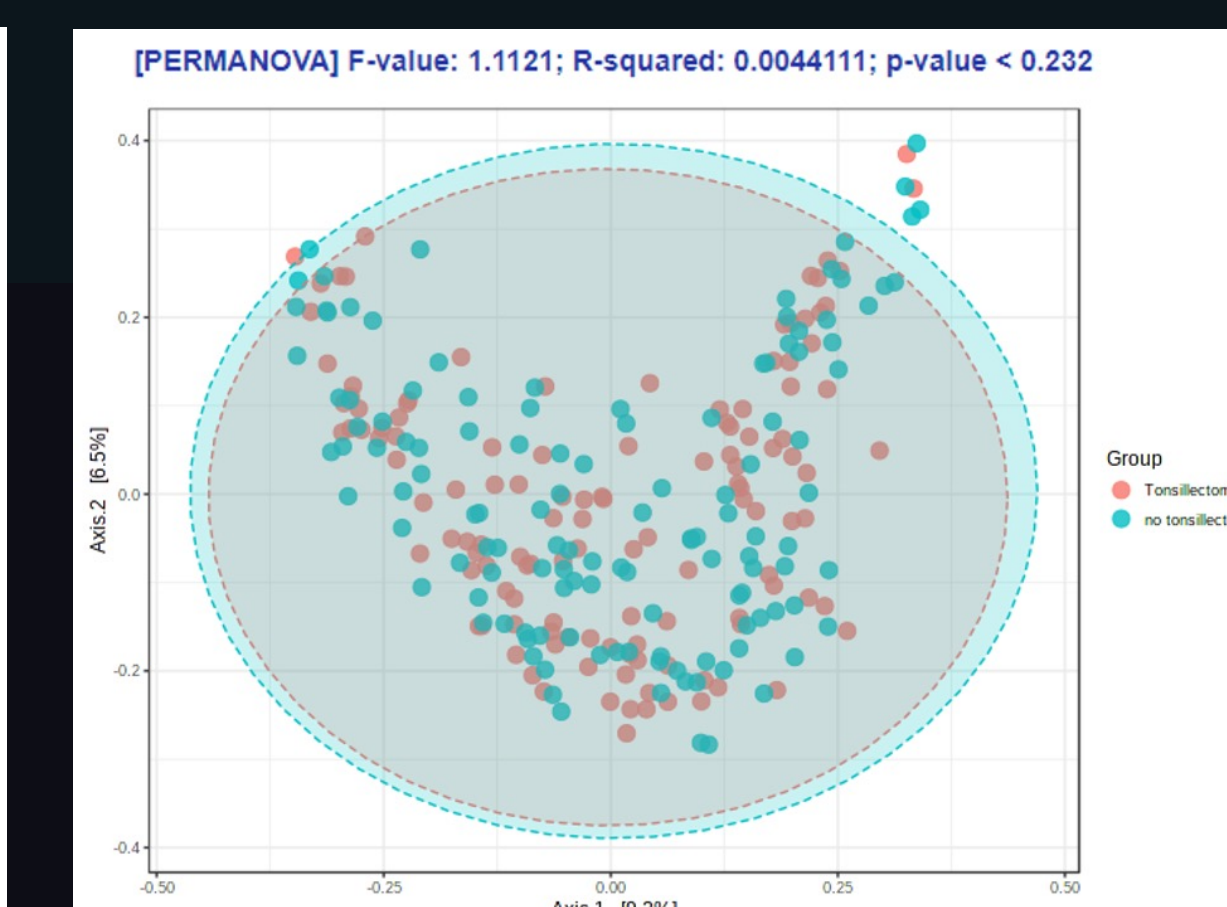
The most prevalent phylum in the core duodenal microbiome of the T+ group was Firmicutes, followed by Actinobacteria and Proteobacteria. While Firmicutes was also the most prevalent phylum in the NT group, Proteobacteria moved up in rank to the second most prevalent phylum, and Actinobacteria dropped to third most prevalent. There were no major higher order taxonomic differences between T+ and NT groups (Fig 3). Interestingly, at the genus level, *Streptococcus* (FC= 0.1, FDR P-value=1) and *Veillonella* (FC= 0.1, FDR P-value=1). These two genera are associated with the colonization of the upper respiratory tract, and were not significantly different between groups<sup>4</sup>. However, the relative abundance of the Gram-positive genus *Granulicatella* was significantly increased in the T+ group (FC= 2.02, FDR P-value=0.01).

Group	Total	Tonsillectomy (T+)	No Tonsillectomy (NT)
N	253	126	127
Female %	62%	63%	61%
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
BMI	26.11 $\pm$ 5.90	26.12 $\pm$ 5.55	25.62 $\pm$ 5.75
Age	62 $\pm$ 14	62 $\pm$ 15	63 $\pm$ 13

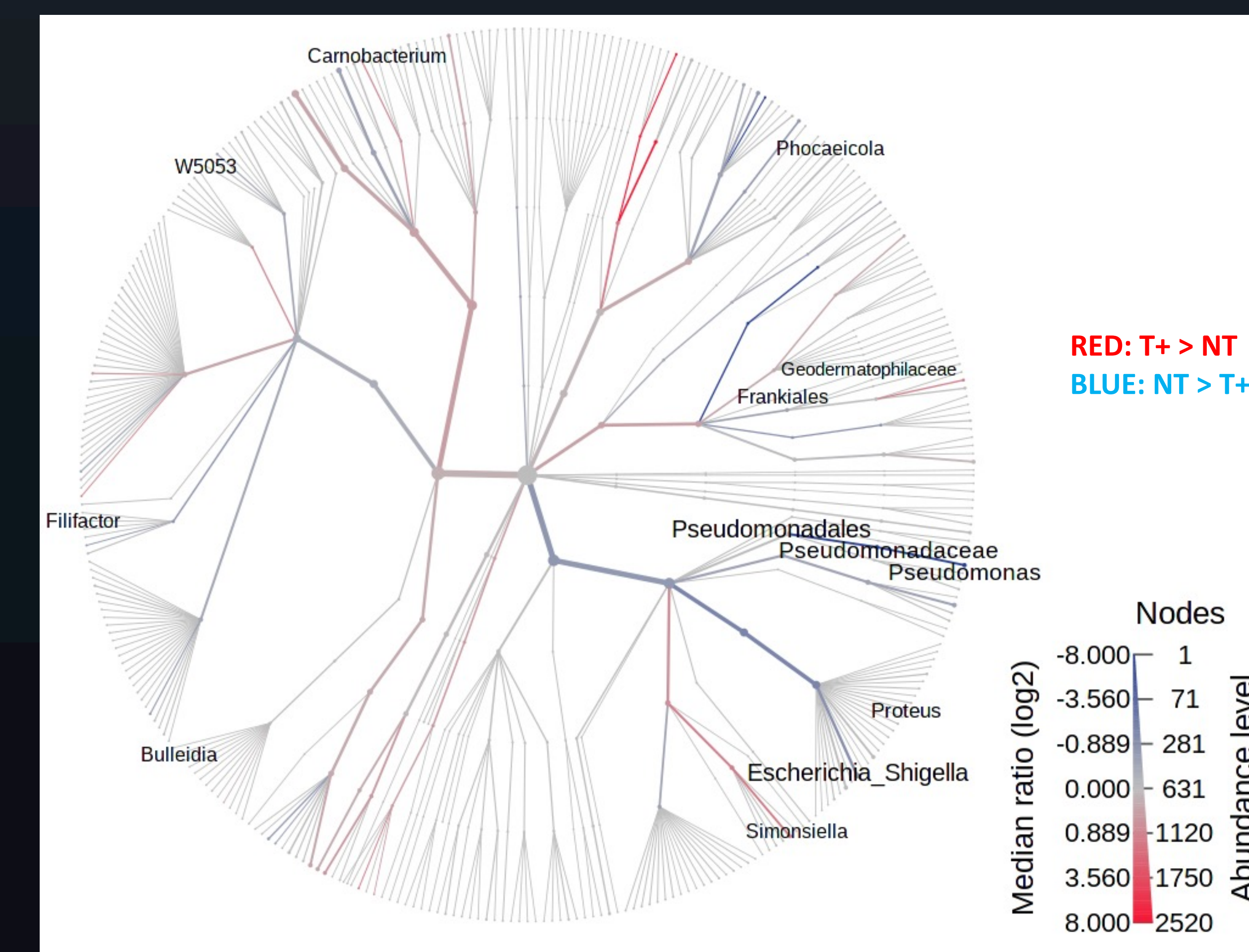
**Table 1:** Subject Demographics. None of the parameters were statistically different between groups.



**Figure 1:** Duodenal microbial alpha diversity in the T+ group, and the NT group, as determined using three different indices, Shannon's, Simpson's and Chao1.



**Figure 2:** Principal Component Analysis (PCA) plot of the duodenal microbiome beta-diversity of T+ group compared to NT group.



**Figure 3:** Heat tree of the duodenal microbiome in the T+ vs. NT groups. Nodes represent taxonomic rank. Taxa shown in red have higher RA in the T+ group, and taxa shown in blue have higher RA in the NT group. Only taxa that are statistically different between groups (P<0.05) are labeled.

## CONCLUSIONS

Our findings demonstrate that undergoing a tonsillectomy is not associated with significant changes in the small intestinal microbiome, despite tonsillar tissue being an important part of the immune system.

## REFERENCES

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