The effects of post-operative antibiotics in endoscopic sinus surgery

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Abstract

Previous research into post-operative antibiotic usage for chronic rhinosinusitis (CRS) patients generated contradictory results. Some studies found that post-operative antibiotics did not improve the short-term clinical outcomes (Jiang et al., 2008; Lehmann et al., 2020; Liang et al., 2011) while some did (Albu & Lucaciu, 2010). Those studies had also focused primarily on the clinical outcomes of the surgery, overlooking the potential impact of prophylactic antibiotics on the sinonasal microbiome. Using cultivation-independent methods, this study aimed to examine the impact of post-operative antibiotics on the sinonasal microbiome alongside short-term clinical outcomes of CRS patients undergoing endoscopic sinus surgery (ESS). Twelve patients undergoing ESS to treat their CRS were enrolled in this study and randomly distributed into two groups: an antibiotic treatment group and a placebo treatment group. The antibiotic treatment group received doxycycline for 28 days after surgery. Clinical information, including computed tomography scan, symptom questionnaires, and nasal endoscopy, were collected for each patient before and after ESS. Swab and tissue samples were collected and underwent DNA extraction, PCR amplification of the bacterial 16S rRNA gene, and amplicon sequencing analysis to longitudinally characterise each patient's sinonasal microbiome. There were no differences in symptom scores or endoscopic/radiological scores between treatment groups before or after the surgery, and there was no significant difference in the microbiome between the treatment groups after surgery. Our preliminary study showed that post-operative antibiotics did not improve short-term clinical outcomes for CRS patients undergoing ESS, and there was no significant difference in the microbiome between treatments, either. However, our preliminary findings reflect only short-term effects, and many of our analyses were statistically underpowered due to the modest sample size. A larger study is needed to explore specific patterns observed in our data with greater confidence.

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List of Abbreviations

ASV	Amplicon Sequence Variant
CRS	Chronic Rhinosinusitis
CRSsNP	Chronic Rhinosinusitis without Nasal Polyps
CRSwNP	Chronic Rhinosinusitis with Nasal Polyps
CT	Computed Tomography
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EPOS	European position statement on sinusitis
ESS	Endoscopic Sinus Surgery
ITs	Turbinoplasty
LMS	Lund-Mackay System
MLMES	Modified Lund Mackay Endoscopic Score
NMDS	Non-metric Multi-dimensional Scaling
PCR	Polymerase Chain Reaction
S. aureus	*Staphylococcus aureus*
Septo	Septoplasty
SNOT-22	Sinonasal outcome test 22

Chapter One: Introduction

1.1 Chronic rhinosinusitis

Chronic rhinosinusitis (CRS) is an inflammatory disease affecting an estimated 5% to 15% of populations in developed countries (Fokkens et al., 2012). This disease is characterised by inflammation of the paranasal sinuses and nasal cavity. Its symptoms include nasal congestion and discharge, facial pain/pressure, and a lack of smell. The presence of these symptoms for greater than 12 weeks is required to diagnose CRS; otherwise, it is classified as acute sinusitis (Barshak & Durand, 2017; Fokkens et al., 2012).

The current guidelines for the clinical diagnosis and standard research requirements for CRS are set by the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (EPOS) and defined as the following (Fokkens et al., 2012):

- Inflammation of the nose and paranasal sinuses with two or more symptoms which must include nasal discharge or nasal blockage/obstruction/congestion:
    - ± facial pain/pressure
    - ± reduction or loss of smell

  And either

- Endoscopic signs:
    - nasal polyps
    - mucopurulent discharge primarily from the middle meatus
    - oedema/mucosal obstruction

  and/or

- Computed tomography scan changes
    - mucosal changes within the ostiomeatal complex and/or sinuses.

CRS is often differentiated into two distinct subtypes based on the presence of nasal polyps (Yang et al., 2015). CRS with nasal polyps (CRSwNP) is diagnosed by the presence of polyps in the nasal and paranasal sinuses when examined endoscopically, whereas a lack of polyps is diagnosed as CRS without nasal polyps (CRSsNP) (Figure 1.1). CRSwNP accounts for approximately 20% of CRS cases (Hayes et al., 2015). Current studies suggest that these two subtypes may be distinct diseases based on the different inflammatory responses of the subtypes (Yang et al., 2015); however, the pathogenesis of these subtypes remains complex with some overlapping features between these subtypes (Lam et al., 2015; Yang et al., 2015). CRSwNP is associated with T helper$_2$ cells and eosinophilic inflammation (Foreman et al., 2011; Maxfield et al., 2017; Yang et al., 2015), whereas CRSsNP is driven by T helper$_1$ cells

and has been associated with less severe inflammation of the mucosa and lower eosinophils present compared to CRSwNP (Foreman et al., 2011; Hayes et al., 2015; Yang et al., 2015).

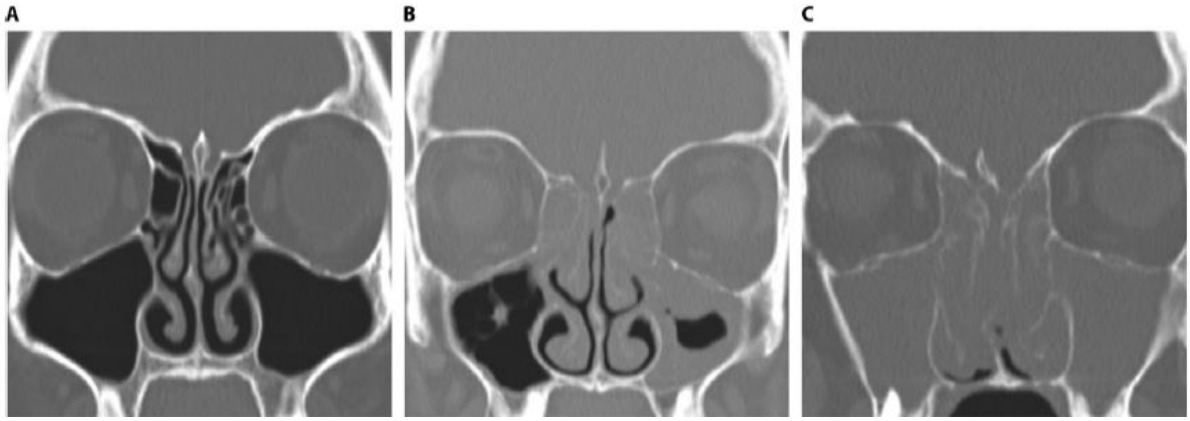

Figure 1.1: CT scan of sinonasal blockages in a healthy patient (A), CRS patient without nasal polyps (B), and CRS patient with nasal polyps (C) (Hoggard, Wagner Mackenzie, et al., 2017).

1.2 CRS assessment methods

1.2.1 22-item Sino-Nasal Outcome Test

A 22-item Sino-Nasal Outcome Test (SNOT-22) is a commonly used test to assess the severity of CRS and can be used to determine the likelihood of a patient willing to undergo surgical treatment (DeConde et al., 2014). This test is scored using 22 symptoms of the disease on a scale of 0 to 5 (5 for the most severe symptoms) with a maximum score of 110 (DeConde et al., 2014). Alongside assessing the likelihood that a patient will undergo surgery, this test has also been used in research and clinical settings to determine the effectiveness of CRS treatments. A reduction in a patient's SNOT-22 score indicates better clinical outcomes for the patient due to a reduction in the severity of their symptoms (DeConde et al., 2014).

1.2.2 Lund-Mackay System

The Lund-Mackay system (LMS) accesses the disease extent in patients with CRS (Lund & Mackay, 1993). LMS grades each sinus group (maxillary, anterior ethmoids, posterior ethmoids, sphenoid, frontal, ostiomeatal complex) using computed tomography (CT), on a scale of 0 to 2 (0: no abnormalities, 2: occluded) for the left and right sinus (Lund & Mackay, 1993). This test's total score range is 0 to 24 and can be used to assess the disease's severity, both pre- and post-operatively (Lund & Mackay, 1993). This assessment method has been

previously found to be correlated with the extent of the disease and offers an objective assessment of the disease severity (Hopkins et al., 2007). This method should be used alongside a SNOT-22 score to indicate disease severity and if surgical interventions are required (Hopkins et al., 2007).

1.2.3 Modified Lund-Mackay Postoperative Endoscopy Score

The Modified Lund-Mackay Postoperative Endoscopy Score (MLMES) is another commonly used method to assess the severity of CRS in post-operative sinus cavities. This method considers the sinuses' appearance when examined using an endoscope rather than using symptoms or CT scans, unlike the previous techniques (Snidvongs et al., 2013). MLMES scores all ten post-operative sinus cavities (left and right maxillary, ethmoid, sphenoid, frontal sinuses, and olfactory fossa) and gives an overall score ranging from 0-100 (the higher the number, the more severe the disease).

Both LMS and MLMES are used when assessing disease severity in research and for endoscopic sinus surgery (ESS) referrals; however, unlike self-reported SNOT-22 scores, LMS and MLMES scores are more objective and reproducible across multiple patients (Snidvongs et al., 2013). These methods can give an overall view of the severity of CRS in a patient based on both clinical and symptom severity.

1.3 Epidemiology

CRS is a common disease estimated to currently affect 14-30 million American adults (Anderson et al., 2016; DeConde & Soler, 2016; Hamilos, 2011). The National Health Interview Survey carried out in the U.S. reported a 12.1% prevalence of diagnosed sinusitis in adults by a health care provider in 2012 (Blackwell et al., 2014). This survey included 234,921 adults and found a higher prevalence of sinusitis in females and Caucasians (Blackwell et al., 2014). This survey, however, did not differentiate between chronic or acute sinusitis. Another U.S. survey using self-reported symptoms estimated a prevalence of 13.5%, which was confirmed by another survey suggesting 16% of adults in the U.S. had this condition (Blackwell et al., 2002; Collins, 1997). The prevalence of CRS also appeared to be affected by age, with increasing CRS rates in older populations; patients aged 20-29 had a prevalence of 2.7%, while patients aged 50-59 had a prevalence of 6.6% (Fokkens et al., 2012). A nationwide survey in Korea found a prevalence of 1% for CRS diagnosis (Min et al., 1996), while a U.S. study in

2000 based on medical records found a prevalence of 1.96% (Shashy et al., 2004). These results demonstrate the potentially exaggerated prevalence reported in self-reported studies and the geographic variability of the disease. Prevalence based on medical records also has a bias with reported numbers due to patients not seeking medical help for the disease, resulting in an underestimation of its prevalence. Accurate estimates regarding CRS's prevalence are also limited since the current diagnostic criteria for CRS were only recently developed (DeConde & Soler, 2016; Hamilos, 2011).

1.4 Pathogenesis of CRS

CRS is thought to be a heterogeneous disease resulting from multiple host and environmental factors (Foreman et al., 2011). There currently is no evident causative agent behind this disease; however, bacteria are thought to play an important role (Anderson et al., 2016; Jain et al., 2018). Historically, this disease was thought to have been due to infection, but recent advances in cultivation-independent techniques have suggested that the pathogenesis of CRS is comprised of several mechanisms, including anatomical abnormalities, genetic disorders, biofilms, pathogenic bacteria, and bacterial dysbiosis (Lam et al., 2015).

1.4.1 Bacterial

Bacterial dysbiosis has been suggested as a mechanism behind CRS (Cho et al., 2020; Copeland et al., 2018; Hoggard, Biswas, et al., 2017). According to this hypothesis, deviations from the typical microbial community (i.e., microbial homeostasis) can lead to the development or maintenance of CRS (Anderson et al., 2016; Copeland et al., 2018; Hoggard, Biswas, et al., 2017). A shift in community structure can be caused by a range of factors, including antibiotics that have been shown to affect microbial biodiversity (Feazel et al., 2012). It is thought that the abundances of keystone species change due to a range of different events such as a decrease in nasal epithelial integrity or antibiotics, allowing for the growth of rare pathogenic or opportunistic species (Copeland et al., 2018; Hoggard, Biswas, et al., 2017). The increased abundance of these pathogenic or opportunistic bacteria is presumed to contribute to the maintenance of the disease (Copeland et al., 2018; Hoggard, Biswas, et al., 2017).

Staphylococcus aureus has been suggested to have a role in CRS and is linked to poor surgical outcomes and prognosis (Anderson et al., 2016; Fokkens et al., 2012; Jain et al., 2017; Jervis-Bardy et al., 2009). *S. aureus* present in the submucosa has been found in high

abundance in CRS patients and is thought to impact the patient's immune system by releasing interleukin-6 and supporting other pathogenic evasions (Hoggard, Wagner Mackenzie, et al., 2017). The presence of *S. aureus* has also been suggested to reduce the presence of anti-inflammatory interleukin-10, thereby increasing inflammatory responses (Sivasubramaniam & Douglas, 2018). *S. aureus* super-antigens create exotoxins which are thought to affect T helper$_2$ swelling, mast cell activity, and promote eosinophil release, leading to an increase in inflammation and tissue damage in the sinuses (Fokkens et al., 2012). These super-antigens are thought to be disease modifiers rather than the primary aetiology. *S. aureus* has also been associated with increased risk of CRS and increased chance of relapse after endoscopic sinus surgery (ESS) (Hoggard, Biswas, et al., 2017). It is thought that *S. aureus* enters the patient's cells in the form of small colonies, allowing these bacteria to escape the host's immune response and act as a reservoir for super-antigen release into the sinuses (Foreman et al., 2011; Kim et al., 2015). There is, however, contradictory evidence regarding the role of *S. aureus* in CRS. Some studies have found an increased abundance of *S. aureus* in CRS patients, while other studies found similar abundances between healthy and CRS patients (Anderson et al., 2016; Feazel et al., 2012; Hoggard, Wagner Mackenzie, et al., 2017). These conflicting results make it difficult to draw any conclusions about the role of *S. aureus* in CRS and whether this bacterium is a modifier, causative agent, or just a local species present in the sinuses.

Biofilms, both fungal and bacterial, have also been speculated to be a factor in CRS and have been suggested to play a role in maintaining inflammation in the sinuses (Fokkens et al., 2012; Hayes et al., 2015; Wood & Douglas, 2010). A study by Dlugaszewska et al. (2016) found that 77% of CRS patients had evidence of biofilm presence while the EPOS reported biofilm presence rates of 30-100% (Fokkens et al., 2012). The large variability of detected biofilms may be due to the different methodologies used for detection. Biofilms are hypothesised to worsen CRS by releasing free-floating bacteria from a reservoir formed in these biofilms, which protect bacteria from antibiotics and other host defences (Fokkens et al., 2012; Kim et al., 2015). *S. aureus* has also been identified as a common biofilm-forming bacterium in CRS patients with a colonisation rate of 27% in CRSsNP patients and 60% of CRSwNP patients (Hayes et al., 2015; Wood & Douglas, 2010). The presence of biofilm-forming *S. aureus* may explain why patients may still have inflammation even after antibiotic treatment, as medications would be unable to breach the biofilm and therefore not affect *S. aureus* (Wood & Douglas, 2010). It is still uncertain if biofilms are a factor in the development of CRS, but biofilms in other diseases have been shown to reduce the effect of antibiotics and

the patient's immune response, leading to a worse prognosis (Fokkens et al., 2012; Hoggard, Wagner Mackenzie, et al., 2017).

1.4.2 Non-bacterial

Non-bacterial factors that have been suggested as causative agents or to impact severity of CRS include aspirin sensitivity, allergy, asthma, immune deficiencies, genetic and anatomical abnormalities (Anderson et al., 2016; Copeland et al., 2018). The patient's immune system is thought to contribute to the inflammatory characteristics of CRS (Stevens et al., 2015). The anatomy of the sinuses can play a role in CRS incidences since it can influence the sinuses' clearance and increase the risk of developing inflammation. Narrow sinus drainage pathways have been suggested to predispose patients to CRS, while other anatomical abnormalities that have also been linked to CRS include septal deviation, atypical ethmoid cells, concha bullosa, etc. (Wood & Douglas, 2010). Several disorders and conditions predispose the patient to CRS, including cystic fibrosis, Kartagener's syndrome, and primary ciliary dyskinesia (Fokkens et al., 2012; Lam et al., 2015). These factors may be associated with CRS due to the effect these conditions have on ciliary and mucus transport (Fokkens et al., 2012). However, most CRS cases do not have these underlying conditions, and the cause of the disease is currently unknown in these cases (Wood & Douglas, 2010).

Allergic rhinitis has been suggested to predispose individuals to CRS. However, some studies have suggested that allergic rhinitis is a superimposed problem that can contribute to CRS inflammation rather than predisposing (Lam et al., 2015). Allergic rhinitis can aid in obstructing the sinuses due to the inflammation of the nasal mucosa, which can hinder ventilation and retain mucus (Fokkens et al., 2012; Lam et al., 2015; Wood & Douglas, 2010). The role of allergies in CRS remains uncertain; however, the prevalence of CRS is noted to be higher in those with allergies than without (Fokkens et al., 2012).

1.5 CRS management and treatment

1.5.1 Medical management of CRS

Patients who suffer from CRS initially manage their symptoms through maximal medical therapy, which typically consists of corticosteroids, antibiotics, and saline nasal irrigation. These treatments have varying effectiveness due to the heterogeneity of this disease. The treatment used may also vary depending on the institution and patient consent. The

treatment is generally the same for both subtypes of CRS (CRSwNP and CRSsNP), except that antibiotic treatment is not recommended for patients with CRSwNP (Hamilos, 2011).

Glucocorticoids are used for the treatment of CRS due to their anti-inflammatory effects. This medication has been shown to significantly improve patient symptoms when compared to patients who were treated with a placebo (Fokkens et al., 2012; Guilemany et al., 2010; Hamilos, 2011). Glucocorticoids reduce eosinophil activity and viability, which leads to a reduction in the chemotactic cytokines secreted by the nasal mucosa and polyp epithelial, reducing eosinophil function (Fokkens et al., 2012; Guilemany et al., 2010). The delivery of this treatment is dependent on a range of different factors, including surgical state, delivery technique and device, and fluid dynamics (Fokkens et al., 2012). ESS has been shown to improve topical glucocorticoids' delivery to the sinuses, allowing for more effective treatment (Fokkens et al., 2012).

Nasal irrigation is an effective complementary treatment for CRS and has been recommended in recent CRS consensus documents (Hamilos, 2011). Irrigation reduces postnasal drainage, rinses away allergens and irritants, removes secretions, improves mucociliary clearance, and can be performed with various over-the-counter devices (Hamilos, 2011). Nasal irrigation has been shown to reduce symptoms and improves the endoscopic appearance of the nasal cavity and sinuses (Guilemany et al., 2010).

Antibiotics use for CRS treatment remains controversial due to a lack of evidence supporting the use of this medication for CRS (Guilemany et al., 2010). Some studies have found that short-term oral antibiotics improve symptoms, while other studies found no significant differences between control and treated patients (Guilemany et al., 2010). There is a concern about the development of antibiotic resistance and the other potential side effects of this medication, such as gastrointestinal upset, skin rash, allergic reaction, and elevation of liver enzymes (Fokkens et al., 2012). Long-term antibiotics, such as macrolides, have also been a suggested treatment due to the anti-inflammatory and antibacterial effects. These antibiotics, however, have also been proposed to affect the host immune response, which could lead to antibiotic resistance and, as such, is only recommended for patients who do not respond to other medical interventions (Fokkens et al., 2012; Guilemany et al., 2010; Hamilos, 2011). There is no FDA-approved antibiotic for the treatment of CRS currently due to the uncertainty about the role of antibiotics in the treatment of CRS (Barshak & Durand, 2017).

1.5.2 Surgical management of CRS

Patients who do not respond to medical treatments may be offered the opportunity to undergo surgical intervention to help reduce their symptoms (Murthy & Banerjee, 2013). A common surgery to undergo as a treatment for difficult to treat CRS is ESS (Sethi & Chakravarti, 2016; Wood & Douglas, 2010). During an ESS, tissue, nasal polyps, and bone are removed from the sinus ostia to allow for more ventilation and greater mucociliary clearance (Fokkens et al., 2012). Other obstructions and inflamed tissue are also removed during this process (Fokkens et al., 2012; Hoggard, Wagner Mackenzie, et al., 2017; Jervis-Bardy et al., 2009). The purpose of this surgery is to open up the sinuses to improve the drainage and airflow through the nose, decrease the severity of the disease, and improve the delivery of medication into the sinuses. ESS, in general, has a low complication rate of 0.69-2%; however, complications include infections, toxic shock syndrome, ecchymosis, and epistaxis (Shafik & Youssef, 2013).

ESS has been shown to reduce the severity of symptoms for individuals who suffer from CRS, with one study showing an average improvement rate of 91% (Terris & Davidson, 1994). Of the 91% improvement rate, 63% reported very good results, while another 28% reported good results (Terris & Davidson, 1994). Another study saw a 97.5% improvement, with 85% of patients reporting significant symptom improvement (Senior et al., 1998). This study also found that after an average of 7.8 years (6.3 to 11 years), there was a decline in antibiotic usage by 82% after the ESS (Senior et al., 1998). A study by Smith et al. (2013) found an improvement in quality of life for patients who underwent ESS compared to patients who continued with medical therapy. The success of the surgery for patients with CRSwNP is dependent on what caused the formation of these polyps – whether formed by idiopathic CRS pathways or underlying conditions such as cystic fibrosis (Fokkens et al., 2012). One study found 12% of patients who undergo this surgery are likely to have revision surgery, while another reported revision surgery as high as 20% (Fokkens et al., 2012; Terris & Davidson, 1994). Revision surgeries have increased risk of complications and an increased risk of revision surgery, with success rates ranging from 50-70% in revision surgeries (Fokkens et al., 2012).

Certain conditions such as asthma, cystic fibrosis, and allergies have been linked to worse surgical outcomes and higher chances of undergoing revision surgery (Fokkens et al., 2012; Hopkins et al., 2007; Murthy & Banerjee, 2013; Senior et al., 1998). Patients who also have a high olfactory disruption have also been shown to have worse surgical outcomes (Murthy & Banerjee, 2013). The presence of *S. aureus* has also been linked to unfavourable

post-operative outcomes (Fokkens et al., 2012). A decrease in bacterial diversity has also been linked to unfavourable post-operative outcomes, however, the cause behind this phenomenon remains uncertain (Hoggard, Wagner Mackenzie, et al., 2017).

For some patients, additional procedures are recommended while undergoing ESS. These adjunct surgeries work together with the ESS to give better outcomes for the patient than what would have been achieved with just ESS. These surgeries are often used to reconstruct the sinuses. Common examples of adjunct surgeries for CRS include septoplasty (septo) and turbinoplasty (ITs). Septoplasty is a surgical procedure that can be done alongside ESS to correct nasal septal deformities (Hwang et al., 1999; Orabona et al., 2018). Nasal septal deformities have previously been implicated with diseases such as sinusitis and can cause a basal obstruction in the patient (Orabona et al., 2018). For this procedure, the septal is straightened by removing the inner lining and removing bent cartilage (Hwang et al., 1999). Turbinoplasty is a procedure used to reduce the inferior turbinate bone, which can become swollen in CRS patients (Friedman et al., 1999). The techniques used for this process can differ depending on the clinician, but the overall procedure involves removing lining tissue of the turbinate to allow access to the bone, which is then reduced in size (Friedman et al., 1999). These surgeries are used to reduce nasal obstruction further to allow for better clinical outcomes for the patient (Friedman et al., 1999; Orabona et al., 2018).

1.6 Microbiome of CRS

The human microbiome has been shown to significantly impact an individual's health and diseases (Cho & Blaser, 2012). Previous research into the gut microbiota has demonstrated that the microbiome plays a role in the immune system, protection against pathogens, susceptibility to inflammation, diseases such as obesity, and more (Cho & Blaser, 2012; Ramakrishnan et al., 2015). Research investigating CRS has suggested that this disease might also be influenced or maintained by the microbial community present in the paranasal sinuses, with several studies finding links between the sinus microbiome and CRS (Ramakrishnan et al., 2015).

Until surprisingly recently, the sinuses were thought to be sterile in healthy patients, whereas the presence of bacteria indicated a diseased state (Sivasubramaniam & Douglas, 2018). This theory was disproved as new molecular techniques arose, identifying diverse microbial communities present in both diseased and healthy sinuses. Studies have found that the microbial communities for CRS and healthy patients are similar and composed primarily

of *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* (Copeland et al., 2018; Hoggard, Biswas, et al., 2017; Jain et al., 2017; Sivasubramaniam & Douglas, 2018). Patients with CRS were found to have a higher relative abundance of *Proteobacteria* (Copeland et al., 2018). *Corynebacterium* has been found at a higher reactive abundance for the healthy patients, while *Escherichia* was found in a higher relative abundance for CRS patients (Copeland et al., 2018; Hoggard, Biswas, et al., 2017). Healthy patients tend to have a lower abundance of pathogenic organisms present than patients with CRS (Sivasubramaniam & Douglas, 2018). A study by Feazel et al. (2012) found a significant difference at a community level between patients with CRS and healthy patients; however, this difference may have been due to treatment of CRS, i.e., antibiotics, rather than the disease. There is a high amount of inter-individual variation for healthy and diseased states, but this variation is expected given the different host and environmental factors influencing individuals' microbiome (Ursell et al., 2012).

Feazel et al. (2012) found that *S. aureus* had a similar prevalence between healthy patients and CRS patients, but found a higher relative abundance of *S. aureus* in patients with CRS than healthy patients. A review by Wang et al. (2020) suggested that the role *S. aureus* has in the microbiome of CRS patients is not as significant as other studies have suggested previously, due to the relative abundance of *S. aureus* in some studies being found in similar or at higher abundances in healthy patients. The overall role *S. aureus* has in the microbiome of CRS patients currently remains unclear. Other specific bacteria such as *Pseudomonas* and some species of *Corynebacterium* have been associated with worse symptoms or quality-of-life in patients with CRS (Wang et al., 2020). Aurora et al. (2013) also found *Corynebacterium accolens* and *Curtobacterium* at higher relative abundances in CRS patients than the healthy control patients. This study also found that *Alicycliphilus* and *Cloacibacterium* had a lower relative abundance in CRS patients (Aurora et al., 2013).

Several studies have found evidence of microbial dysbiosis occurring in patients with CRS, which has been suggested to play a role in this disease's mechanism (Cho et al., 2020; Copeland et al., 2018; Ramakrishnan et al., 2015). Copeland et al. (2018) suggest that external influences on the sinonasal microbiome result in a shift from a stable to unstable microbiome (dysbiosis), leading to a prevalence of *Staphylococcus*, *Propionibacterium*, and *Corynebacterium* in CRS patients along with changes in the alpha diversity. A study by Ramakrishnan et al. (2015) found that while a large proportion of bacteria species identified in their study were present in both healthy and diseased patients, some of the bacteria present also correlated with a population variable and the patient's diseased state (Ramakrishnan et al.,

2015). This study suggested that *Bacteroides* and *Fusobacteria* may act as opportunistic pathogens in some CRS patients, while phyla such as *Actinobacteria* and genus *Corynebacterium* may be predictive of the surgical outcomes in CRS patients (Ramakrishnan et al., 2015). High interindividual variation present within healthy and CRS patients has made it difficult to completely understand what a stable or unstable microbial community may look like, which makes it challenging to determine if dysbiosis plays a role, and what microbial species are involved with dysbiosis (Cho et al., 2020; Ramakrishnan et al., 2015). In general, studies have noted an increased abundance of opportunistic pathogens in CRS patients, suggesting that dysbiosis may play a vital role in the maintenance or development of CRS (Cho et al., 2020).

The microbiome of CRS has been investigated using molecular techniques in multiple studies; however, the role of post-operative antibiotics after ESS has not been thoroughly investigated using molecular techniques. Previous studies have investigated the role of post-operative antibiotic for CRS patients undergoing ESS using symptom questionnaires and endoscopic images of the sinuses, but there is a lack of understanding of changes in the microbial communities during this process (Albu & Lucaciu, 2010; Hauser et al., 2016; Jiang et al., 2008; Lehmann et al., 2020; Liang et al., 2011; Wu et al., 2019). Investigating the microbiome of patients with CRS who received post-operative antibiotics compared to those who received placebo may elucidate the role antibiotics play in the recovery process for ESS, and the role the sinonasal microbiome plays in recovery from ESS. Alongside this information, investigations into post-operative antibiotics after ESS may also give insight into the general role of the sinonasal microbiome in CRS and identify the role microbial dysbiosis plays in this disease. Overall, more research is needed using molecular techniques to understand the role microbial communities play in CRS, including post-operative antibiotic usage after ESS.

1.7 Current research into antibiotic use associated with ESS

It is currently standard practice for patients to receive 7 to 14 days of post-operative antibiotics after ESS to reduce the risk of post-operative infection, which could affect healing time and improve surgical outcomes. However, there is little research to support the prophylactic use of post-operative antibiotics, and questions of the efficiency of post-operative antibiotics have arisen in recent research (Jiang et al., 2008; Lehmann et al., 2020; Lux et al., 2020; Saleh et al., 2012). Current research on antibiotics has identified an increasing trend of antibiotic resistance present in bacteria due to many factors, including the use and over-

prescription of antibiotics in the medical field (Lehmann et al., 2020; MacGowan & Macnaughton, 2017). A study by Harbarth et al. (2000) investigated the prolonged use of prophylactic antibiotics after cardiovascular surgery and found that post-surgical antibiotic usage after 48 hours increased the risk of acquired antibiotic resistance and did not reduce rates of surgical site infection. The World Health Organisation has described increased incidences of antibiotic resistance as a major public health concern, potentially resulting in a post-antibiotic era where minor injuries and infections can kill (World Health Organization, 2014). An estimated 25,000 people in the European Union already die each year due to antibiotic-resistant infections (Baym et al., 2016). Another risk of antibiotics is the potential adverse side effects, including low white blood cells and blood platelet counts, anaphylaxis, gastrointestinal pain, nausea, and infections (Cunha, 2001).

A study by Jiang et al. (2008) investigated prophylactic antibiotic use (amoxicillin/clavulanate) after ESS using LMS, swab cultures (anaerobic and aerobic cultures), antibiotic sensitivity testing, and symptom severity. The study found that symptoms scores for both the control group and those treated with antibiotics significantly decreased over time and that there was no significant difference between the two groups regarding endoscopic scores and culture results (Jiang et al., 2008). The bacterial growth in culture samples collected from antibiotic-treated patients increased after surgery, and the bacteria identified from the cultures varied significantly before and after the ESS (Jiang et al., 2008). This study concluded that their results demonstrated that antibiotics did not cause an improved outcome for patients compared to those treated without antibiotics. However, it should be noted that this study did not investigate the long-term impact of antibiotics usage and that investigators were not blinded for this study, which may have led to a bias in the results.

Liang et al. (2011) investigated the differences in ESS outcomes for groups treated using amoxicillin, Chinese herbal medicine, and a placebo. This study compared pre- and post-operative bacterial culture rates (anaerobic and aerobic) alongside CT scores, endoscopic scores, and a rhinosinusitis outcome measure. Bacterial culture rates increased significantly for patients treated with amoxicillin, whereas patients treated with Chinese herbal medicine or the placebo only had a slight increase in bacterial culture rates. *S. aureus* was the most common bacterium present in all three groups after the ESS (Liang et al., 2011). There was no significant difference between the three groups for endoscopic scores, CT scores, and rhinosinusitis outcomes (Liang et al., 2011). This study concluded that there was no significant benefit to using antibiotics or Chinese herbal medicine for post-operative ESS care in CRS patients.

Unlike the other studies, Albu and Lucaciu (2010) found that post-operative antibiotics may have improved ESS outcome regarding patient symptoms and moderately accelerated the healing. This study investigated bacterial culture rates, symptom scores, and endoscopic scores under double-blind, randomised conditions (Albu & Lucaciu, 2010). Both groups show significant improvements regarding self-reported symptoms scores, but individuals treated with antibiotics had a larger improvement for symptoms scores of nasal obstruction and discharge (Albu & Lucaciu, 2010). The endoscopic scores from days 5 to 12 were significantly different between the two groups, with patients treated with antibiotics recovering quicker (Albu & Lucaciu, 2010). This study did not discuss the bacteria cultures in detail and primarily focused on the patients' endoscopic and physical symptoms (Albu & Lucaciu, 2010; Rudmik et al., 2011). The authors attempted to justify using post-operative antibiotics due to bacterial adhesion to the healing wound that cannot be avoided, and the constant risk of infection present to all patients (Albu & Lucaciu, 2010).

A study by Wu et al. (2019) compared the symptoms, endoscopic score, and swab cultures between patients using erythromycin and patients using intranasal steroids after ESS. When comparing steroid treatment to erythromycin treatment following ESS, the erythromycin treatment improved the endoscopic score in patients and symptoms such as smell (Wu et al., 2019). The bacterial cultures for those treated with erythromycin and steroids had similar results. This study found that both treatments were effective and improved surgical outcomes; however, no conclusion about which treatment was better for post-operative care in CRS could be made as both treatments were effective (Wu et al., 2019).

A study by Lehmann et al. (2020) also investigated post-operative antibiotics after ESS; unlike the previous studies, this was a noninferiority clinical trial. A noninferiority clinical trial determines if a new treatment is noninferior (not worse) to the standard treatment that is currently being used. In the study by Lehmann et al. (2020), the new treatment was a placebo ($n = 37$) whereas the standard treatment was post-operative antibiotics ($n = 40$). Each patient underwent a Lund-Kennedy endoscopic score and SNOT-22 test to determine the surgery's effect on the patient's quality of life and clinical outcomes (Lehmann et al., 2020). Cultures were also collected from the sinuses once the ESS was completed; however, these were not discussed in detail (Lehmann et al., 2020). The study was performed under double-blind and randomised conditions. This study found that the placebo treatment group was non-inferior (i.e., not worse) to the antibiotic treatment group when only considering the patients' sinonasal-specific quality of life (Lehmann et al., 2020). This study also found no significant difference in post-operative infection rates or endoscopic scores, but it noted a significantly higher rate of

diarrhoea in the antibiotic treatment group (Lehmann et al., 2020). Lehmann et al. (2020) did note that it was statistically underpowered due to the modest sample size and could not make any conclusive statements; however, this study did suggest that post-operative antibiotics may be unnecessary.

The difference in these studies' outcomes may be due to variation in treatment such as steroids, nasal packaging, how extensive the surgery may have been, and time points used in their studies. A comparison of the Albu and Lucaciu (2010) and Jiang et al. (2008) suggests that post-operative antibiotics are an option for CRS patients undergoing ESS (Rudmik et al., 2011). Still, professional judgement is needed from the clinician for each patient to determine the most appropriate treatment at the time, since the option of post-operative antibiotics should not be applied to all patients (Rudmik et al., 2011). Other studies and reviews such as Patel et al. (2018) and Saleh et al. (2012) suggest that the use of antibiotics should be withheld unless signs of infection are present.

A limitation of the earlier studies is that they focused primarily on symptom and endoscopic results and did not adequately address the sinonasal microbiome (Albu & Lucaciu, 2010; Jiang et al., 2008; Lehmann et al., 2020; Liang et al., 2011). When bacteria were investigated, culture-based methods were used; however, culture-based methods are not as effective as DNA-based molecular genetic methods (Albu & Lucaciu, 2010; Jiang et al., 2008; Lehmann et al., 2020; Liang et al., 2011). Previous research has indicated that 60 to 99% of microorganisms are uncultivable (Anderson et al., 2016). One study compared the results of clinical cultures to a molecular-based technique and found that the molecular-based approach detected a greater number of microorganisms (Feazel et al., 2012). Samples using molecular-based techniques could detect organisms both found and missed when using culture-based techniques (Feazel et al., 2012). While culture-based methods may give some indication of what bacteria are present in the nasal cavity of patients, molecular-based methods have been shown to be more sensitive than culture-based methods, allowing for a complete understanding of the effects of antibiotics and the role the nasal microbiome may play in the recovery after ESS (Anderson et al., 2016; Feazel et al., 2012; Zapka et al., 2017).

A study by Hauser et al. (2016) investigated the short-term effects of post-operative antibiotics on the sinonasal microbiome. This study used molecular-based techniques, 16S rRNA gene PCR amplicon sequencing and quantitative PCR, to examine the changes in the microbiome of 13 patients at three time-points: pre-operative, 2 weeks post-operative, and 6 weeks post-operative (Hauser et al., 2016). All patients in this study received a two-week course of post-operative antibiotics (Hauser et al., 2016). Hauser et al. (2016) found that the

overall bacteria burden (determined using quantitative PCR) was higher at the two-week timepoint compared to pre-operative and six weeks post-operative timepoints (Hauser et al., 2016). This study also found that the bacterial composition was similar for the pre-operative and six-week post-operative samples, indicating that the microbiome was repopulated or returned to baseline after surgery (Hauser et al., 2016). Unfortunately, Hauser et al. (2016) did not include a placebo control, and its findings therefore cannot inform or rationalise the use or disuse of post-operative antibiotics.

In summary, more research is needed to rationalise post-operative antibiotic use or disuse after ESS. Although several studies have shown that antibiotics have no impact on symptoms or endoscopic outcomes of the surgery, other studies have demonstrated better surgical outcomes for patients treated with antibiotics (Albu & Lucaciu, 2010; Jiang et al., 2008; Lehmann et al., 2020; Liang et al., 2011). Alongside these contradictions, there has been no placebo-controlled clinical trial for post-operative antibiotic in CRS patients using molecular techniques. This lack of a comprehensive understanding of the bacteria present in a patient's sinonasal microbiome makes it challenging to understand the role of bacterial dysbiosis that could occur due to antibiotics and its role in patient recovery. A greater understanding of the sinonasal microbiome could also enable associations between recovery and certain bacteria and give insight into the role of bacteria in this disease and its recovery. Overall, a cultivation-independent, placebo-controlled, double-blinded study investigating the microbial ecology in patients undergoing ESS is needed to further understand both the usefulness of antibiotics in this surgery and the potential role in the recovery of the patient.

1.8 Sample collection location

Mucus samples collected using swabs of the sinus mucosa are frequently utilised when investigating CRS and make up the majority of studies in this field (Kim et al., 2015). Other sampling methods, such as tissue samples collected during surgery, have also been used in the literature but much less frequently (Kim et al., 2015; Roediger et al., 2010). Sampling sites and techniques can vary significantly between studies, which may contribute to the lack of consensus on the role of bacteria in CRS (Kim et al., 2015; Roediger et al., 2010). The middle meatus is the most frequently used site when collecting swab samples from patients since this site is easily accessible, has high agreement (82% similarity) to microbial cultures collected from maxillary sinus aspirates, and because this location has drainage from three major sinuses (maxillary, anterior ethmoid, and frontal) (Cho et al., 2020; Dubin et al., 2005). A study by

Kim et al. (2015) investigated the bacterial composition of swab samples compared to tissue samples from the middle meatus using the 16S rRNA gene. This study found that the bacterial communities identified in the swab samples were also identified in the tissue samples, but not all bacteria present in the tissue samples were present in the swab samples (Kim et al., 2015). Statistical analysis comparing the swabs to tissue samples found a significant difference between the bacterial communities; however, further research is needed to validate these results (Kim et al., 2015). Kim et al. (2015) suggest that this difference may be caused by the protected environment found in tissue along with the impact of the patients' distinct immune responses to each environment. These factors may have led to distinct bacterial communities (Kim et al., 2015). In contrast to Kim et al. (2015), a study by Bassiouni et al. (2015) found that there was no significant difference between microbial composition in swab and tissue samples collected from the sinuses. Bassiouni et al. (2015) found that tissue samples had a higher richness; however, this relationship was not significant. Overall, Bassiouni et al. (2015) found that swab and tissue samples had similar microbiomes. These results show that further studies are needed to determine whether swab samples are representative of the 'true' sinonasal microbiome as suggested by Bassiouni et al. (2015), or if swab samples may not identify all the bacteria present in this diseased environment as suggested by Kim et al. (2015).

1.9 Research aims

Due to the lack of research investigating the sinonasal microbiome in CRS patients undergoing ESS, the effects of post-operative antibiotics on the sinonasal microbiome are largely unknown. This research aims to longitudinally characterise the sinonasal microbial ecology of CRS patients undergoing ESS using molecular techniques and to investigate the differences in the sinonasal microbiome between post-operative antibiotic and placebo treatments. Previous research indicates that there may be differences between tissue and swab samples due to the formation of biofilms and bacterial communities in the underlying tissue, so a baseline comparison between swab and tissue samples was conducted.

Chapter Two: Methods

2.1 Clinical samples and data collection

2.1.1 Patient recruitment

Twelve patients undergoing ESS were recruited for this study from November 2019 to November 2020. Patient recruitment and sample collection were suspended from April to June 2020 due to the COVID-19 pandemic. The participants were under the coordinating investigator's care before recruitment and identified from a surgical waiting list. Patients were contacted by the coordinating investigator about potential involvement in this study. If the patient was confirmed to be eligible for and interested in participating in the study, a patient information sheet and consent form were emailed to the patient. The patient received a follow-up phone call from a research nurse to answer further questions and inquire if they wished to participate in the study. For patients who agreed to participate in this study, signed informed consent was collected on the surgery day. Patients were excluded from this study if they had one or more of the following exclusion criteria:

- prior sinus surgery
- underlying condition predisposing to CRS (e.g., cystic fibrosis, vasculitis)
- unilateral sinusitis or a LMS (a pre-operative scan indicating the severity of CRS) score lower than 10 (i.e., radiological evidence of mild sinusitis)
- antibiotic usage within 12 weeks prior to ESS
- known allergy to study drugs
- confirmed or possible pregnancy

Patients were randomised in a double-blinded fashion and allocated to a treatment (placebo/antibiotic) group using Microsoft Excel's RANDBETWEEN function by the hospital pharmacist. For 28 days after the ESS, six patients received doxycycline 100 mg by mouth twice daily, while the remaining six received a placebo by mouth twice daily. The following demographic information and clinical details about the patients were collected using hospital records prior to the ESS: age, sex, LMS score, disease subgroup (CRSwNP, presence of co-morbid asthma), and any adjunctive surgical procedures (i.e., septoplasty, inferior turbinoplasties).

This study received approval from the New Zealand Health and Disability Ethics Committee and the University of Waikato (reference number: 19/NTA/64/AM01, universal

trial number: U1111-1229-8735). Written and informed consent was obtained from all the participants prior to inclusion in this study. This study was registered online with the Australian and New Zealand Clinical Trial Registry (Reference number: ACTRN12619000505101p).

2.1.2 Sample collection

Swab samples of the middle meatus were collected from the nose's left and right side using sterile rayon-tipped swabs (Copan Diagnostics Inc., Murrieta, CA) at three time points: during the ESS, two weeks after ESS, and three months after ESS. Some samples for the final time point were collected earlier or later than expected (ranging from 6 weeks to 6 months) due to the COVID-19 pandemic. Tissue samples from the nasal mucosa were also collected during ESS. No prophylactic antibiotics or other topical medications were used prior to sample collection during ESS. Once all intra-operative samples had been collected, all patients received a single dose of intravenous cefazolin. The swab and tissue samples collected were immediately placed in separate 2-mL screw-cap tubes containing 450 µL of DNA/RNA Shield (Zymo Research, Irvine, CA, U.S). Samples were then transported to the Thermophile Research Unit at the University of Waikato, where the samples were stored at 4°C until processed within 4 days of collection. The remaining swab samples were collected in follow-up appointments at an outpatient clinic. The nasal cavity was treated with a co-phenylcaine nasal spray which acted as an anaesthetic and decongestant. Swab samples from the middle meatus from the left and right side of the nose were then collected and placed in 450 µl of DNA/RNA Shield then transported to the Thermophile Research Unit at the University of Waikato. Prior to ESS and during later follow-up appointments, patients also completed a 22-item Sinonasal outcome tool (SNOT-22 score). The endoscopic appearance of the sinus mucosa was graded at the 3-month follow-up appointment using MLMES.

2.1.3 Surgical treatment and post-operative care

All patients underwent the same routine clinical and surgical care during and after the surgery except for the randomised doxycycline or placebo medication. Each patient was also prescribed prednisone (20 mg) for 10 days post-operatively with frequent use of saline nasal spray and saline lavage in the initial post-operative period. At post-operative follow-up appointments, patients were examined with a rigid nasal endoscope, and debridement of clot and crusting was performed. After the two-week follow-up appointment, patients were

encouraged to continue with twice-daily saline lavage and topical steroids. All participants were provided with contact details of the research team to report any side effects that they believe may have been attributed to the study medication, which was documented. Patients were asked to return any unused study medications so that compliance could be monitored.

2.2 Molecular genetic analyses

2.2.1 DNA extraction

Total DNA was extracted from tissue and swab samples using the ZymoBIOMICS DNA Microprep Kit (catalogue no. D4301, Ngaio Diagnostics, Nelson) according to the manufacturer's protocol for samples preserved in DNA/RNA Shield. Extraction blanks were also extracted alongside the tissue and swab samples. These extraction blanks were created during each extraction using only the reagents from the ZymoBIOMICS DNA Microprep Kit. The extracted DNA was quantified using a Qubit Fluorometer following the dsDNA HS protocol (Thermo Fisher Scientific, Auckland) and stored at -20°C until further analysed.

2.2.2 DNA amplification of 16S rRNA gene

A two-step polymerase chain reaction (PCR) was used to amplify the V4 hypervariable region of the 16S rRNA gene from the extracted DNA. The Earth Microbiome Project primers with adaptor sequence added (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [515FB] and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [806RB]) as described in the Quick-16S NGS Library Prep Kit (catalogue no. D6400, Ngaio Diagnostics, Nelson) were used for the first PCR. For the first PCR step, the reactions (20 µL) consisted of 0.4 µL of bovine serum albumin (BSA) (0.8 mg/mL), 2 µL of dNTPs (2mM), 2 µL of 10X PCR buffer, 2 µL $MgCl_2$ (50 mM), 0.5 µL of each primer (10 mM), 0.08 µL of Platinum *Taq* DNA Polymerase (Thermo Fisher Scientific, Auckland), 2 µL of template DNA (5 ng/uL), and 10.52 µL molecular-grade DNA-free ultra-pure water (Thermo Fisher Scientific, Auckland). Triplicate reactions were done and pooled for each sample using the following thermocycler conditions: an initial 3-minute denaturation step at 94°C followed by 35 cycles of 45 seconds at 94°C, 60 seconds at 50°C, and 90 seconds at 72°C, with a final extension step at 72°C for 10 minutes. All reactions were run on an Applied Biosystems ProFlex PCR System (Thermo Fisher Scientific, Auckland). Positive (1 ng of *E. Coli* genomic DNA) and negative (ultra-pure water) controls were run with every PCR.

An agarose gel electrophoresis was used to confirm successful PCR amplification. For this step, 1 µL of SYBR Safe (Thermo Fisher Scientific, Auckland) was combined with 5 µL of the pooled PCR product and loaded into the wells of a 1% agarose gel. 10 µL of a 1 Kb Plus DNA Ladder (Thermo Fisher Scientific, Auckland) was also loaded. The gel was run for 30 minutes at 70 V then visualised using an Alpha Innotech Imaging System (Alpha Innotech, California, USA).

To remove mitochondrial DNA that was also amplified during the first PCR, the PCR products underwent gel extraction using an E.Z.N.A. Gel Extraction Kit (catalogue no. D2500-01, Custom Science, Auckland) according to a manufacturer's protocol with some modification. These modifications to the manufacturer's protocol include the following changes: extra 20% of binding buffer was combined with the gel band, the gel band was incubated for a longer time (additional 8 minutes), samples were centrifuged at a lower RCF (4,000 RCF) when the sample was initially transferred onto the column (later centrifuge steps were as specified in the protocol), the elution buffer was heated (60ºC) before being added to the column, and the elution buffer was incubated on the column for longer (additional 3 minutes).

The extracted 16S rRNA gene PCR amplicons underwent barcode addition using the Quick-16S NGS Library Prep Kit according to its non-quantitative protocol for barcode addition. An agarose gel electrophoresis was used to confirm that barcode addition was successful.

2.2.3 Next-generation sequencing

Following the manufacturer's protocol, the barcoded PCR amplicons were processed and normalised using SequelPrep Normalization Plate Kit (Thermo Fisher Scientific, Auckland). Once library normalisation was completed, 3 µL of each sample was pooled into a sterile 1.5 µL Eppendorf tube to form an amplicon library. 150 µL of the library was combined with 10 µL of 50X ethylenediaminetetraacetic acid (EDTA), and the EDTA-treated amplicon library was sent at room temperature to GENEWIZ in Suzhou, China. At GENEWIZ, the amplicon library was sequenced using an Illumina MiSeq DNA sequencer in a 2 x 250 bp paired-end configuration using the MiSeq Reagent Kit v2.

2.3 Data analysis

2.3.1 Bioinformatic analysis

The raw FASTQ files from the Illumina MiSeq were imported into R (v4.0.2). ASV inference and initial filtering were performed using the 'DADA2' package (v.1.16.0) (Callahan et al., 2016). The forward and reverse reads' quality profiles were visually examined, and it was determined that truncLen trimming was unnecessary. Reads were then filtered to allow no "N" nucleotides using the following filtering parameters: maxN=0, (DADA2 requires no Ns) truncQ=2, rm.phix=TRUE, maxEE=2 (maximum number of "expected errors" allowed in a read). The reads were then truncated and trimmed using the following parameters: filterAndTrim(fnFs, filtFs, fnRs, filtRs), truncLen=c(240,160), maxN=0, maxEE=c(2,2), truncQ=2. Parametric error rates for the reads were visualised and evaluated, which showed that the estimated error rate had a good fit for the observed error rate. Forward and reverse reads were merged and denoised, and the amplicon sequence variants were constructed. Chimeras were removed with the removeBimeraDenovo function using the method "consensus" (appendix, Table 6-1). Taxonomy was assigned with the native implementation of the naive Bayesian classifier and a DADA2-formatted reference database for the SILVA v138 database (Quast et al., 2013).

Analysis of the sequence data was conducted in RStudio (v4.0.2), while clinical data analysis was conducted in Microsoft Excel. The taxonomic data and ASV data created using DADA2 were merged in R into a phlyoseq object along with a metadata file created using Excel (composed of patient information). This phyloseq object was statistically analysed using a range of R packages including: phlyoseq (v1.32.0) (McMurdie & Holmes, 2013), ggplot2 (v3.3.3) (Wickham, 2016), vegan (v2.5-6) (Oksanen et al., 2007), knitr (v1.31) (Xie, 2015), tidyverse (v1.3.0) (Wickham et al., 2019), decontam (v1.8.0) (Davis et al., 2018), pairwiseAdonis (v0.0.1) (Martinez Arbizu, 2020) and breakaway (v4.7.3) (Willis & Bunge, 2015). Contaminated taxa were identified from extraction blanks and the PCR negative control using the decontam package. These contaminated taxa were then removed from the dataset. Samples were removed from the dataset if they had fewer than 25,000 reads, while the remaining samples were rarefied to 25,000 reads. Before rarefaction, the samples' microbial diversity was investigated using the breakaway package to estimate species richness for each sample. Species richness was analysed in Excel using Two-Sample Assuming Unequal Variances or Paired Two Sample for Means in the Excel Add-in 'Analysis ToolPak'. Non-metric multi-dimensional scaling (NMDS) plots based on Bray-Curtis dissimilarities were used

to examine the dissimilarity between samples. Bacterial community composition was investigated using relative abundance of the most abundant genus (abundance >1%). Adonis2 and pairwise Adonis were used to determine statistical significance between variable or treatment groups.

Chapter Three: Results

3.1 General descriptions of data

3.1.1 Sequencing data

The 94 samples included in this study were composed of (for each patient) two swab and two tissue samples collected on the day of the ESS (T1), two swab samples collected 13 days after the surgery (except for patient 2, which was collected after 15 days) (T2), and two swab samples collected after an average of 105 days (41-188 days) (T3). The T3 samples were collected once the patient had recovered from the surgery (a minimum of six weeks after surgery). No T2 samples were collected for patient 6 due to the COVID-19 lockdown. The T3 samples had initially been intended to be collected after three months when the patients would undergo a routine check-up after the surgery; however, due to the COVID-19 pandemic, some patient samples could not be collected at the three-month point. This led to large variations in the time between ESS and the T3 sampling. Alongside the 94 samples, 22 extraction blanks and one PCR negative control were also processed and analysed. These samples underwent 16S rRNA gene PCR amplicon sequencing. The sequencing data then underwent de-noising and quality filtering using the DADA2 pipeline. This process yielded 11,340 amplicon sequence variants (ASVs) across the 94 samples, 22 extraction blanks, and one PCR negative control.

3.1.2 Clinical and demographical data

Clinical data such as demographical data, SNOT-22 scores (collected before the ESS, two weeks post-operatively, and three months post-operatively), radiological scores (pre-operative disease severity assessment through CT scans [LMS]), and endoscopic scores (assessing healing after surgery [MLMES]) were collected for all 12 patients (Table 3-1). SNOT-22 scores are a self-reported questionnaire used to assess the patient's symptoms and understand their recovery after ESS. LMS and MLMES are both clinical tests used to evaluate the severity of CRS in patients; however, unlike a SNOT-22 score, these tests are based on clinical results rather than self-reporting. LMS is measured through CT scans, whereas MLMES is measured using endoscopic images. The average age of the twelve patients was 44±12.1 (26-59), seven patients had CRSwNP, and five patients had asthma (Table 3-1). Eight

of these patients also underwent adjunct procedures (septoplasty [septo] and turbinoplasty [ITs])[1]. Three patients in this study were female, while the remaining nine patients were male.

92% of the patients showed an improvement in their symptoms at their final check-up. Patient 4, however, showed worse outcomes with an increased SNOT-22 score (Figure 3.1 and Table 3-1). Patient 1 did not show a significant improvement and still had a high SNOT-22 score after three months (Figure 3.1). Patients 1 and 4 both received antibiotics. The average pre-operative SNOT-22 score for patients who received the antibiotic treatment was 43, compared with 54 for patients who received the placebo control (Figure 3.2). The average SNOT-22 score at the three-month check-up was 24 for patients receiving antibiotics and 15 for patients receiving the placebo (Figure 3.2). Overall, both treatment and control groups showed improved results based on the patient's self-reported symptoms (Figure 3.1). There was no significant difference in the pre-operative (unpaired t-test, p-value = 0.29) and three-month (p-value = 0.27) SNOT-22 scores between the treatment groups, and both groups showed significant improvements in their SNOT-22 following ESS (paired t-test, p-value = 0.0002).

[1] Adjunct procedures are additional surgeries undertaken while undergoing ESS to help alleviate symptoms of CRS in patients who have nasal abnormalities (septoplasty straightens the septum; turbinoplasty reduces size of inferior turbinates).

Patient	Age	Sex	Adjacent Procedure	Nasal Polyps	Asthma	Treatment	SNOT pre-op	SNOT 2w	SNOT 3m	LMS	MLMES 3m
1	57	male	Septo	CRSsNP	Nil	Antibiotic	64	12	44	10	0
2	26	male	Septo	CRSwNP	Asthma	Placebo	81	14	20	21	45
3	27	female	ITs	CRSsNP	Asthma	Antibiotic	50	57	5	10	2
4	51	male	Nil	CRSwNP	Nil	Antibiotic	43	40	49	20	50
5	59	male	Nil	CRSsNP	Nil	Antibiotic	19	7	13	16	46
6	59	male	Nil	CRSwNP	Nil	Placebo	37	4	5	15	10
7	56	male	Nil	CRSwNP	Asthma	Placebo	65	12	21	14	4
8	45	male	Septo	CRSwNP	Nil	Placebo	62	43	14	14	5
9	30	female	Septo	CRSsNP	Nil	Placebo	46	31	20	12	6
10	34	male	Septo	CRSwNP	Asthma	Antibiotic	39	37	15	11	8
11	37	male	Septo / ITs	CRSsNP	Nil	Placebo	33	47	9	14	0
12	43	female	ITs	CRSwNP	Asthma	Antibiotic	44	32	20	14	2

Table 3-1:Clinical and demographic data collected from patients across 3 months during this study. Patients in yellow did not significantly improve their symptoms after recovering from surgery according to their pre-op and 3-month SNOT-22 scores. Low SNOT-22 scores (total score range = 0-110), LMS scores (total score range = 0-24), and MLMES scores (total score range = 0-100) indicate better surgical outcomes.

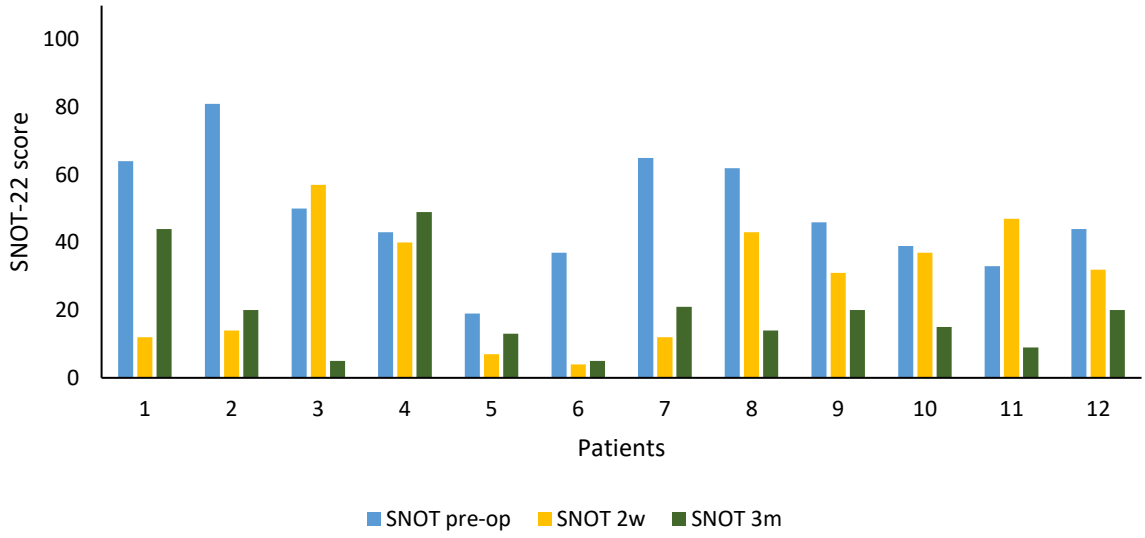

Figure 3.1: SNOT-22 scores for each patient over the course of 3 months. A high SNOT-22 score indicates worse CRS symptoms while a decrease in SNOT-22 over time indicates that the ESS has been successful.

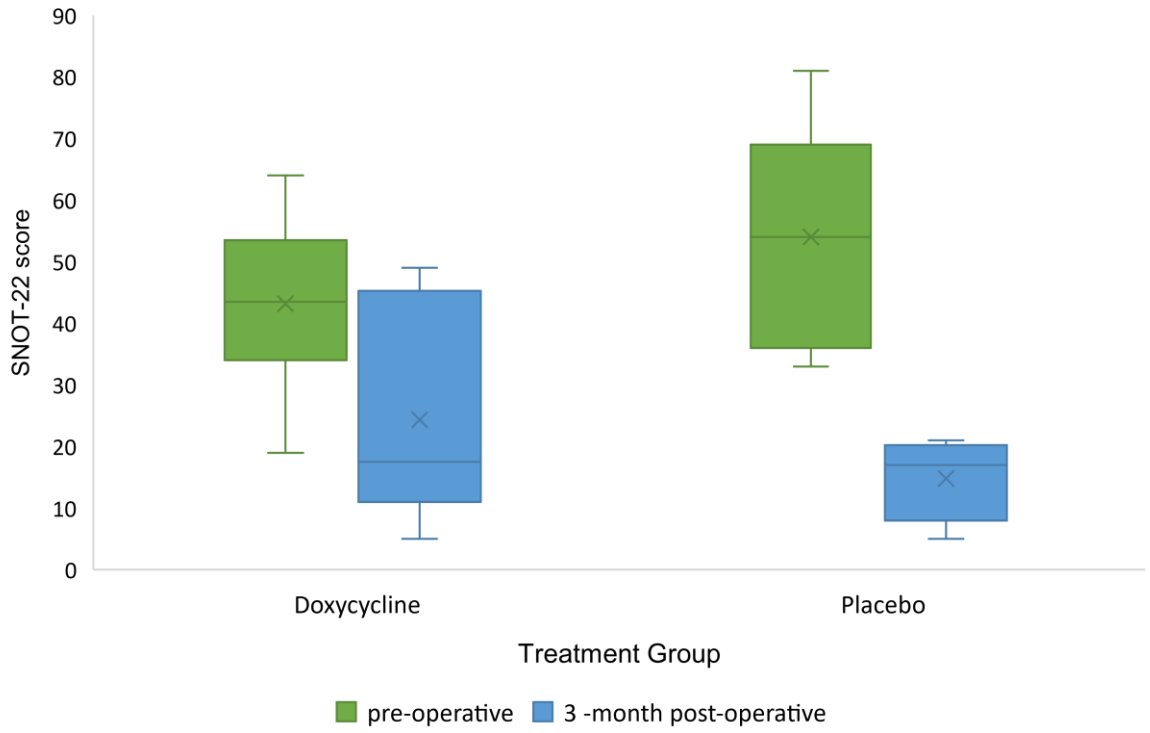

Figure 3.2: Pre-operative and 3-month SNOT-22 score of each patient sorted according to treatment group.

The average LMS score for patients treated with antibiotics was 13.5 and 15 for patients receiving the placebo (Figure 3.3). Three months after surgery, the average MLMES score for

patients receiving antibiotics was 18, compared with 12 for patients receiving the placebo medication (Figure 3.3). There was no statistical difference between the two treatment groups for their LMS scores (unpaired t-test, *p*-value =0.49). The placebo group appears to have better endoscopic results at the three-month check-up; however, this is not statistically robust (unpaired t-test, *p*-value = 0.60).

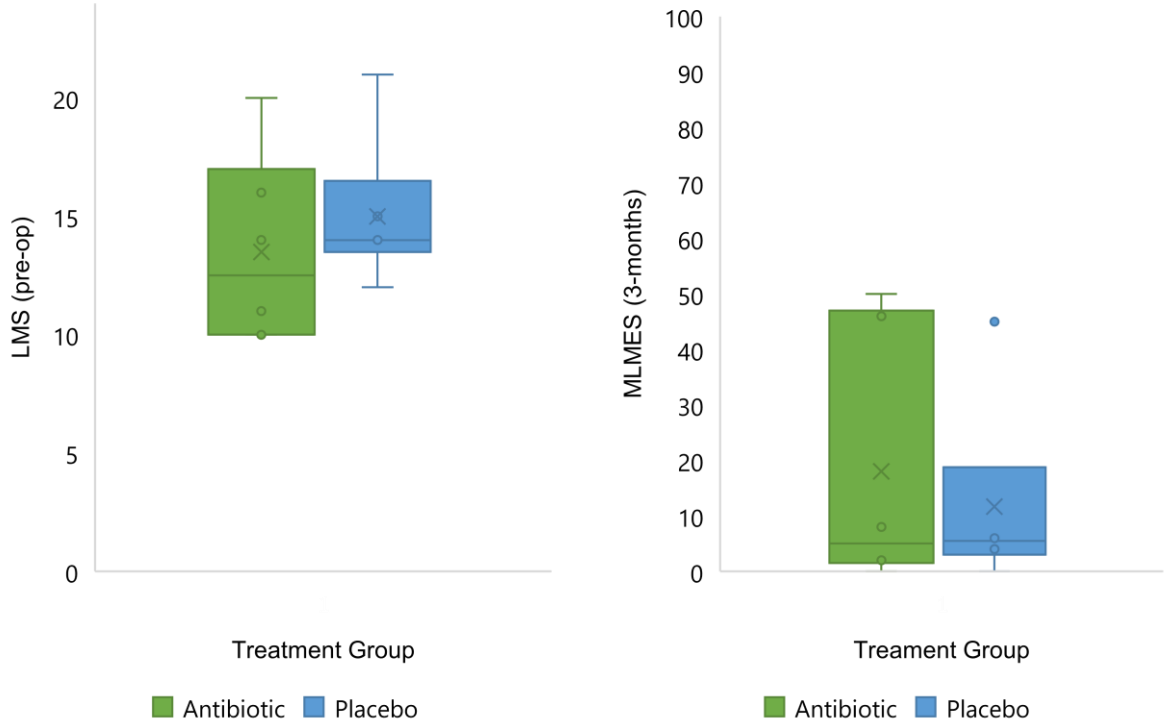

Figure 3.3: Pre-operative LMS scores and 3-month MLMES for each patient, sorted according to treatment group.

Four patients reported side effects of the medication, with three of the four patients receiving antibiotics and reporting common side effects of doxycycline (Table 3-2) (Medsafe, 2019). Patient 11, who received a placebo treatment, reported a sore throat on day seven and was then given Augmentin (amoxicillin and clavulanic acid) by their general practitioner. Patient 3 presented with vomiting and stopped taking the antibiotics after 11 days due to the severity of their symptom.

Patient	Treatment	Side effects
1	Antibiotic	nil
2	Placebo	nil
3	Antibiotic	vomiting - patient stopped study medication on day 11
4	Antibiotic	dizziness, tiredness, diarrhoea
5	Antibiotic	nil
6	Placebo	nil
7	Placebo	nil
8	Placebo	nil
9	Placebo	nil
10	Antibiotic	nil
11	Placebo	developed sore throat (day 7 post-op) - given Augmentin as treatment.
12	Antibiotic	facial rash - day 1 post-op

Table 3-2: Side effects reported by patients throughout this study.

3.2 Quality control and decontamination of the sequencing data

Contamination was observed in the DNA extraction blanks when they underwent 16S rRNA gene PCR amplification alongside the extracted DNA from patients before sequencing. These blanks were created during each batch of DNA extraction, containing no samples but instead composed only of the reagents present in the ZymoBIOMICS DNA Microprep Kit. Contamination was also observed in the PCR negative control once sequenced. The PCR negative control was created using ultra-pure water instead of sample DNA. The blanks and PCR negative control underwent the same procedures as the DNA collected from the patients to allow the contaminating taxa present to be removed later during the data analysis process.

There did not appear to be much overlap between ASVs present in the extraction blanks and PCR control when compared to the patient samples (Figure 3.4), indicating that the contaminants were relatively minor components of the data for patient samples. The most abundant ASVs present in the extraction blanks were affiliated with *Pseudomonas*, *Staphylococcus*, and *Thermus* [data not shown].

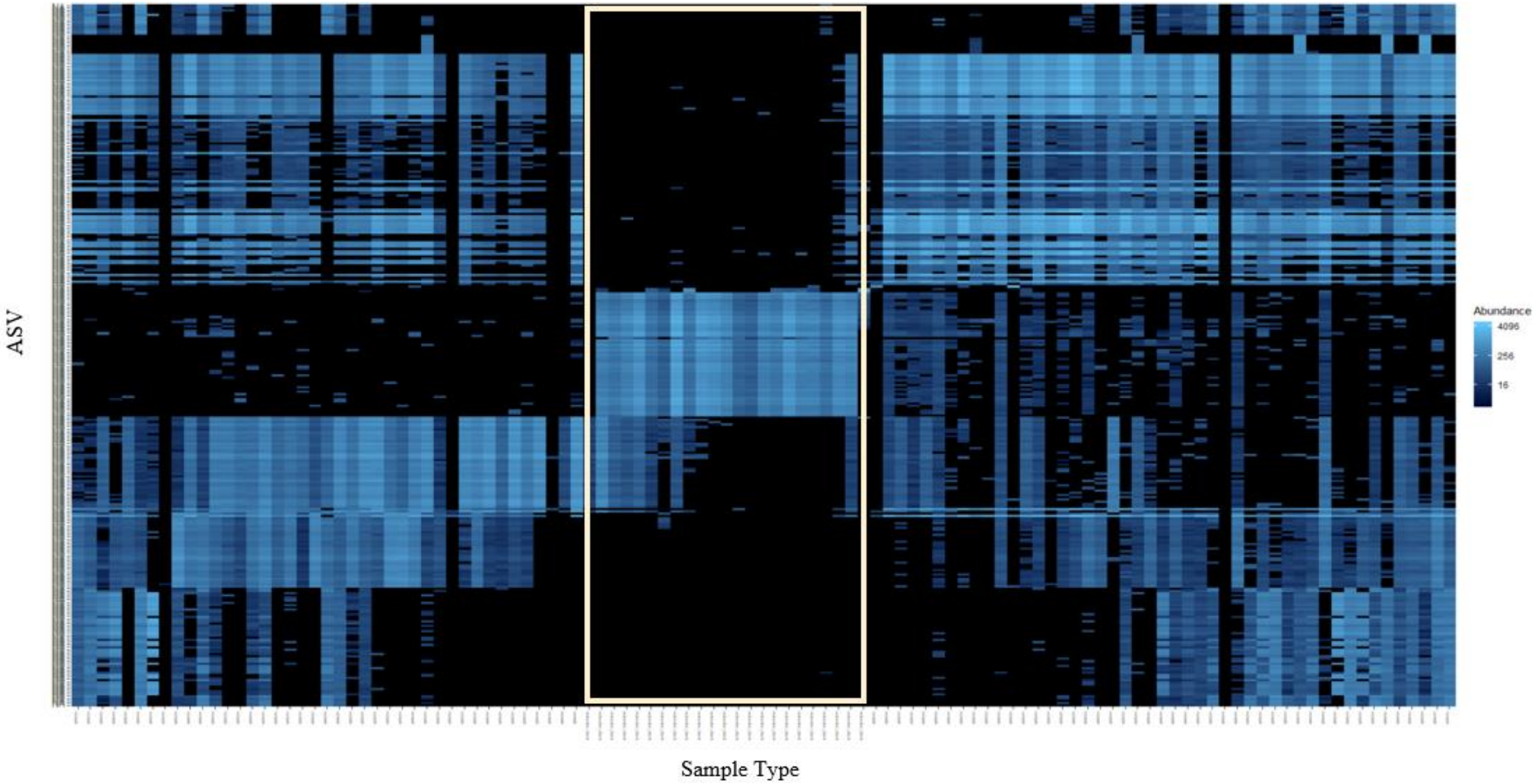

Figure 3.4: Heatmap of ASV present in extraction blanks and patient samples. Samples were sorted horizontally by sample type. ASV present in the white box are abundant in extraction blanks whereas ASV abundant outside of this box are patient samples.

To remove contaminant taxa from the patient samples, the ‘decontam’ package was used according to the prevalence-based method (Davis et al., 2018). This method uses the extraction blanks to calculate the prevalence of known contaminants in clinical samples while also considering that clinical samples will have competing template DNA (from the sinus microbiome), causing some contaminants to be absent in the clinical samples (Davis et al., 2018). Using chi-square statistics (presence-absence table) or Fisher’s exact tests, the probability that a taxon is a contaminant or non-contaminant is calculated (Davis et al., 2018). ‘decontam’ identified 889 taxa as contaminants at a 0.5 threshold (higher sensitivity compared with the default threshold of 0.1) (Davis et al., 2018). This threshold was selected since the extraction blanks had a high number of reads. The taxa identified as contaminants (all relatively rare taxa in patient samples) were then removed.

Samples with fewer than 25,000 reads appear to be under-sampled based on diversity indices (data not shown) so were considered anomalous (Figure 3.5) and removed using the pruning function in the ‘phyloseq’ package. Following this step, 16 samples were removed from the dataset; six of these samples had no reads, while the remaining ten samples had low read counts (Table 3-3). Correspondingly, 36 ASVs were removed, leaving a total of 6,595 ASVs across 78 samples, which were rarefied to 25,000 reads each, resulting in 5,888 ASVs present in the dataset. Further filtering of this dataset was performed to remove other contaminants such as mitochondria and chloroplasts, ASV that did not appear more than twice across all samples, and ASVs that were not assigned to Bacteria at the domain level (i.e., non-16S rRNA gene sequences). This filtering resulted in a total of 2,609 ASV present in the dataset.

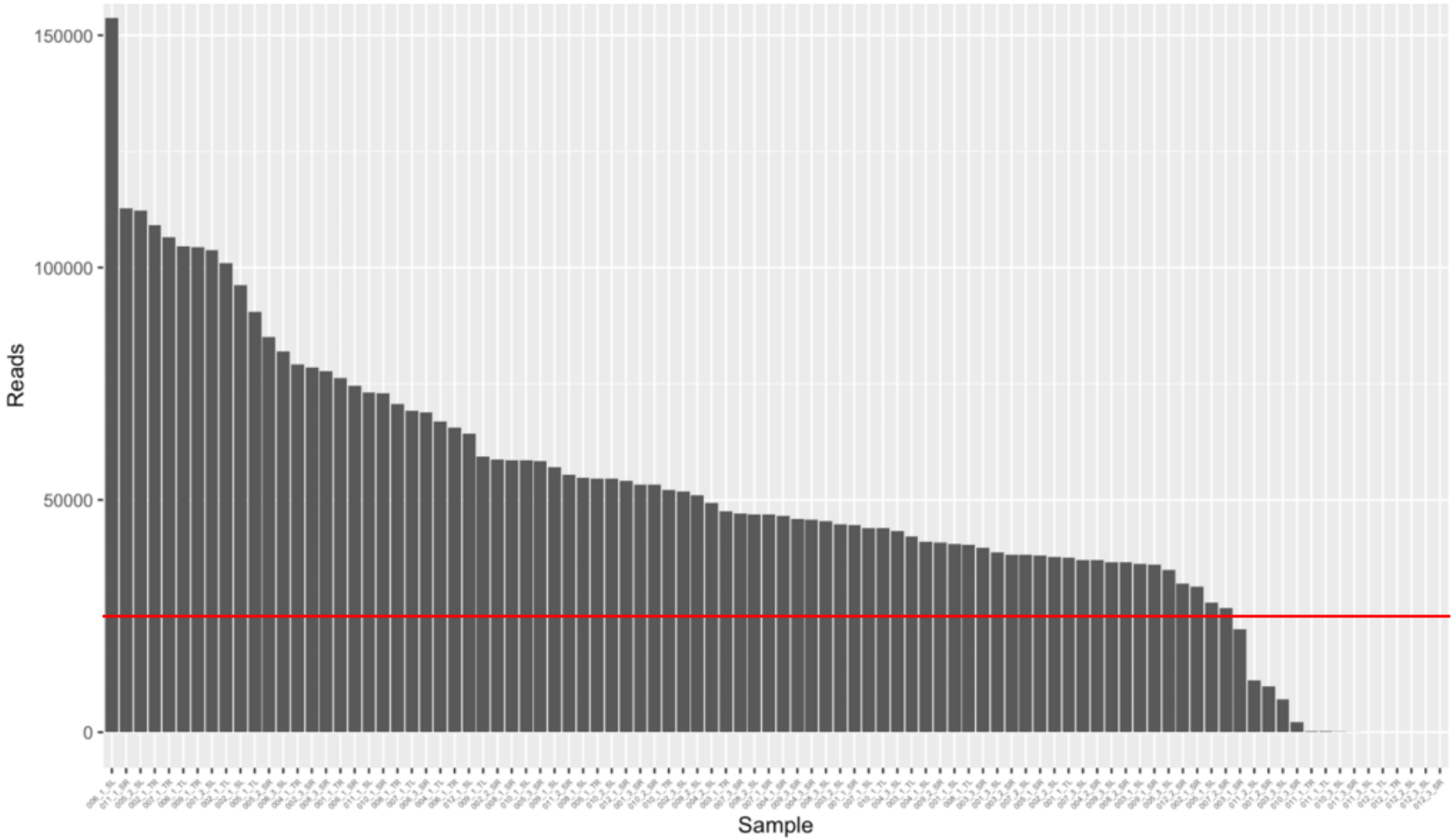

Figure 3.5: Number of reads present in each sample prior to any filtering or sample removal. Samples under the red line had less than 25,000 reads present.

Sample	Read count
001_2_SR	9829
003_1_SR	21769
003_3_SL	6879
010_3_SR	2103
010_3_SL	87
011_1_TR	212
011_1_TL	147
011_2_SR	24077
011_2_SL	894
011_3_SR	7
011_3_SL	0
012_1_TR	0
012_1_TL	0
012_2_SL	0
012_3_SR	0
012_3_SL	0

Table 3-3: Samples removed during the trimming process due to low read count (<25,000 reads).

3.3 Comparing sample position

3.3.1 Community richness

The alpha diversity of swab and tissue samples collected from the left and right sinuses were compared using observed richness, breakaway estimates, and Shannon indices (appendix, Table 6-2). Patients 11 and 12 were excluded from this analysis since only two samples from patient 11, and three from patient 12 remained in the quality-filtered final data set (Table 3-3).

According to all three indices, the average alpha diversity was similar for left and right sinuses (paired t-test, *p*-value =0.461) (Figure 3.6).

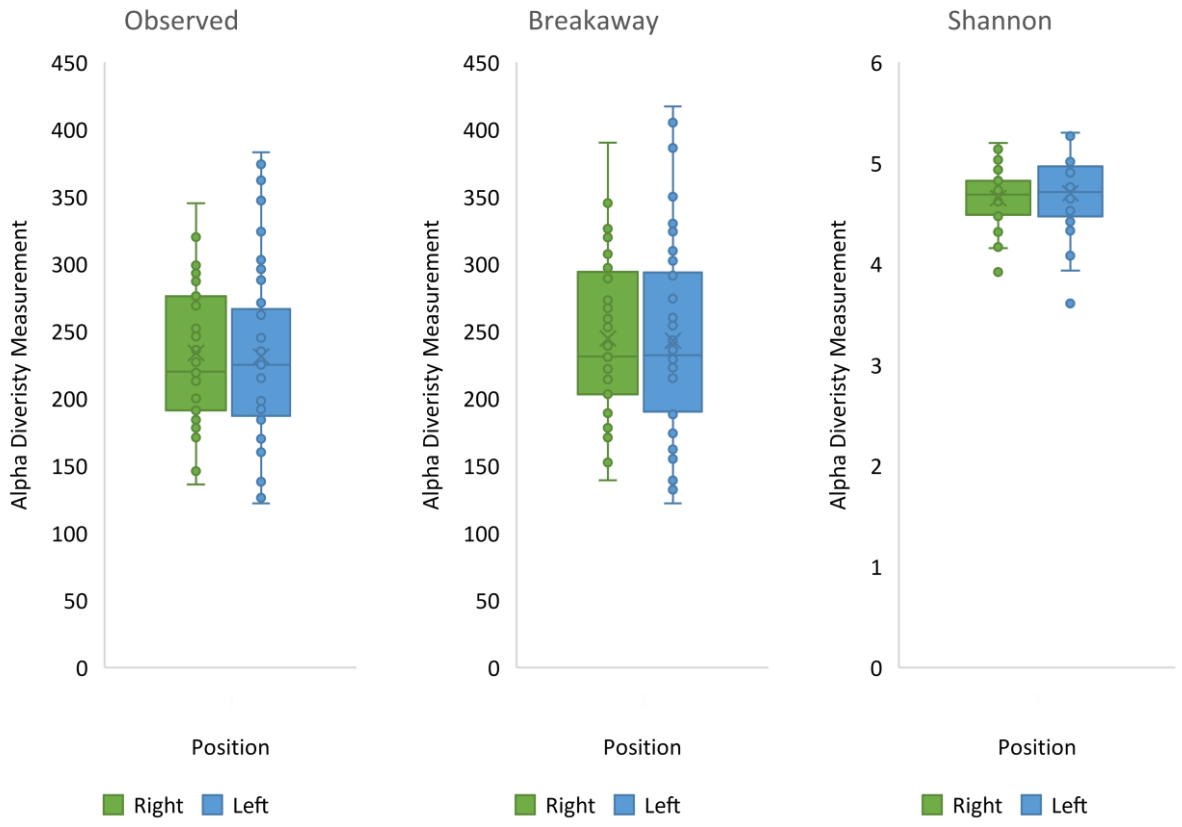

Figure 3.6: Alpha diversity of samples sorted according to sample position using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were made using unrarefied data.

3.3.2 Community structure

A non-metric multi-dimensional scaling (NMDS) plot was used to visualise the similarities between samples collected from the left and right sinus (Figure 3.7). There was no apparent clustering observed, with left and right samples intermixing throughout the graph.

Adonis2 analysis showed no significant difference between the left and right samples (R^2 = 0.0164, *p*-value =0.298).

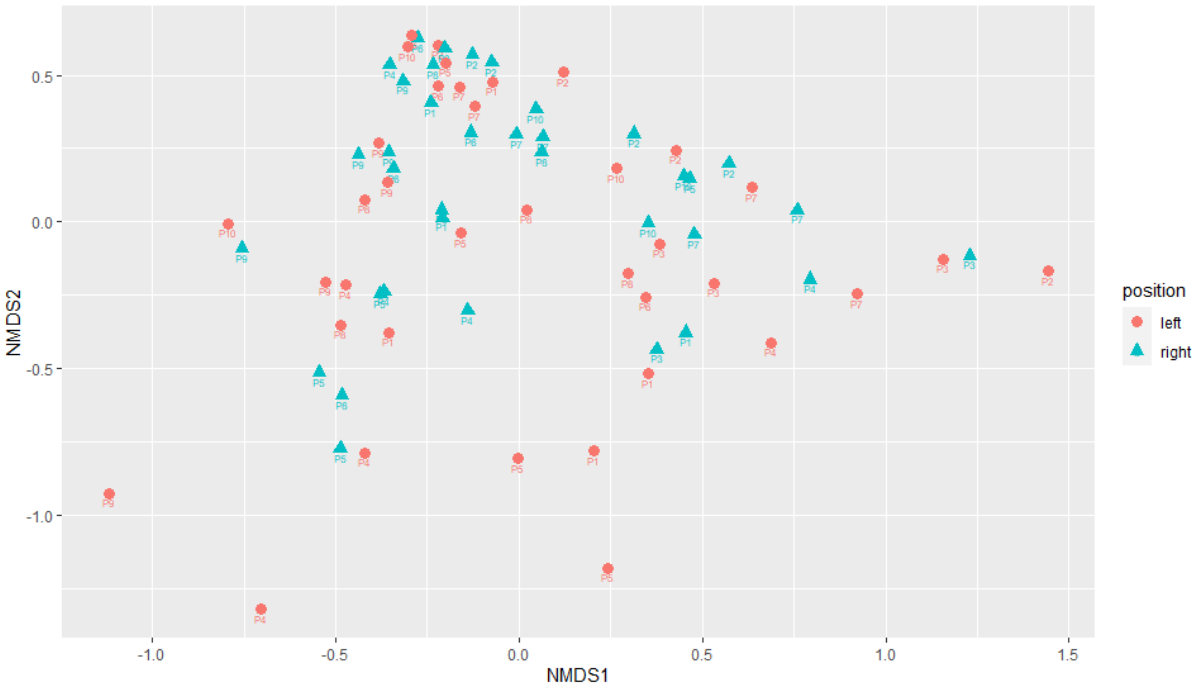

Figure 3.7: NMDS plot of samples from patient 1-10, labelled according to sample position. Ordination based on Bray-Curtis dissimilarities across T1 samples. 2D stress = 0.25, max residual = 0.00013.

3.4 Comparing tissue samples with swab samples

3.4.1 Community richness

The alpha diversity for tissue samples and swab samples was compared using observed richness, breakaway estimates, and Shannon indices. Patients 11 and 12 were excluded from this analysis since tissue samples from these patients were removed from the final data set due to low read counts (Table 3-3). For this analysis, T2 and T3 swab samples were excluded as there was no tissue counterpart for these samples.

The observed richness and breakaway estimates showed that tissue samples, on average, had significantly higher diversity than the swab samples (paired t-test for breakaway estimates, *p*-value = 0.016). In contrast, the Shannon index showed that swab samples had a higher diversity on average (Figure 3.8).

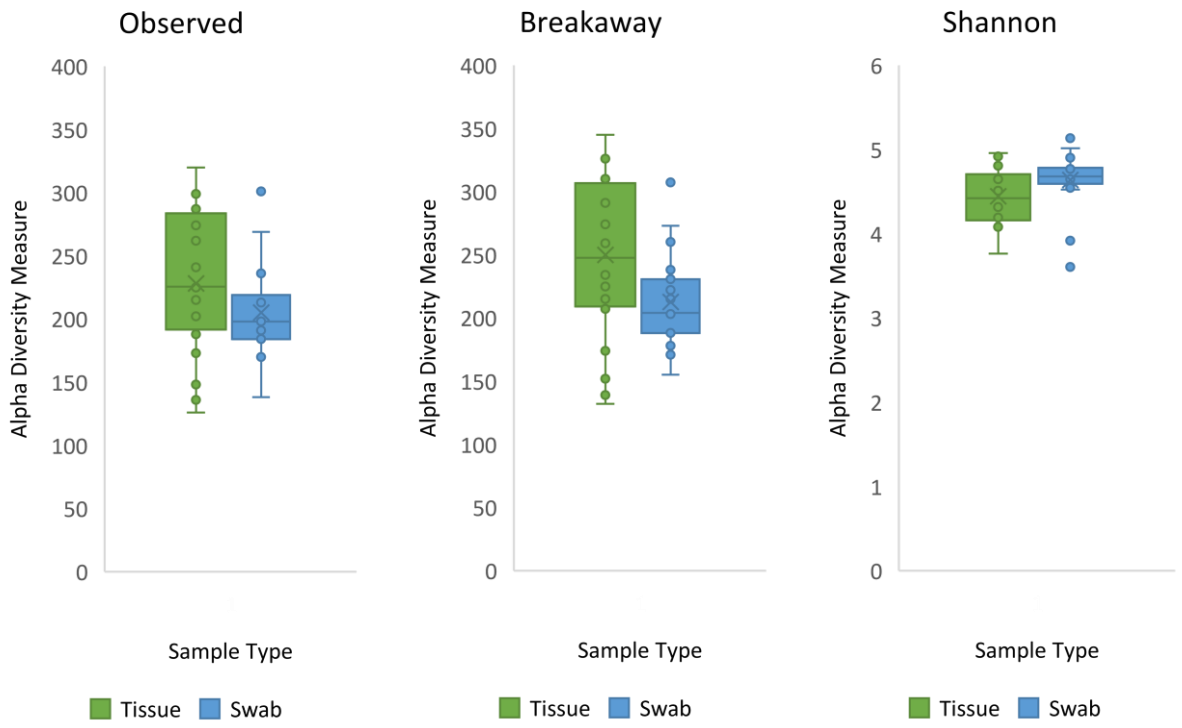

Figure 3.8: Alpha diversity of samples sorted according to sample type using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were made using unrarefied data.

3.4.2 Community structure

NMDS plot was used to visualise the similarity between the tissue and swab samples (Figure 3.9). The NMDS plot showed that tissue samples, in general, tended to cluster more closely together than the swab samples. When examining tissue and swab samples for each individual, there was no clear trend between swab and tissue samples (data not shown). Adonis2 analysis showed significant differences between tissue and swab samples (*p*-value = 0.009).

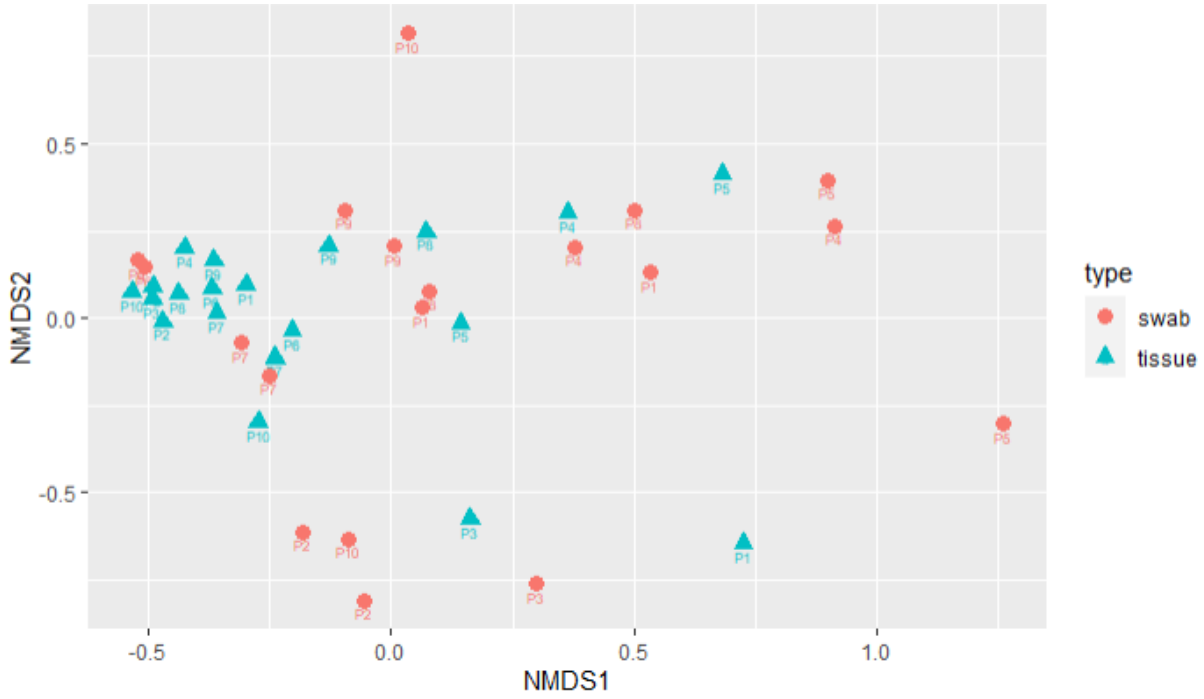

Figure 3.9: NMDS plot of samples from patient 1-10, labelled according to sample type. Ordination based on Bray-Curtis dissimilarities across T1 samples. 2D stress = 0.14, max residual = 0.025.

3.4.3 Community composition

Both tissue and swab samples were composed primarily of *Corynebacterium*, *Dolosigranulum*, and *Staphylococcus* (Figure 3.10). *Moraxella* had high relative abundance in patient 10 for both swab and tissue samples. Patients 1, 3, 5, and 9 all had similar community compositions for tissue and swab samples. In contrast, the community compositions for tissue and swab samples from patient 2 were dissimilar. There was a high relative abundance of *Escherichia-Shigella* in the tissue sample 002_1_TL, while the swab samples for patient 2 were composed primarily of *Staphylococcus* with some *Corynebacterium*. The tissue samples from patient 4 also differed from swabs since sample 004_1_TR had *Lawsonella* present while the swab samples did not. The remaining samples for patient 4 had similar microbial composition. Patients 6 and 7 had a greater diversity of genera present in their tissue samples compared to their swab samples. Tissue samples from patient 8 had *Streptococcus* present, whereas swab samples did not. Tissue samples from patient 8 also had a higher relative abundance of *Staphylococcus* than their swab counterparts. Samples from Patient 10 differed depending on sample type; tissue samples had a higher relative abundance of *Moraxella* and lower relative abundance of *Staphylococcus* than the swab samples for this patient. Overall, both swab and tissue samples were primarily composed of *Staphylococcus*, *Corynebacterium*, and

Dolosigranulum, with some exceptions (patients 6, 7, and 10). The relative abundance of these genera differed for the sample type in patients 2, 4, 6, 7, 8, and 10. However, Patients 1, 3, 5, and 9 had similar relative abundance between sample types (Figure 3.10).

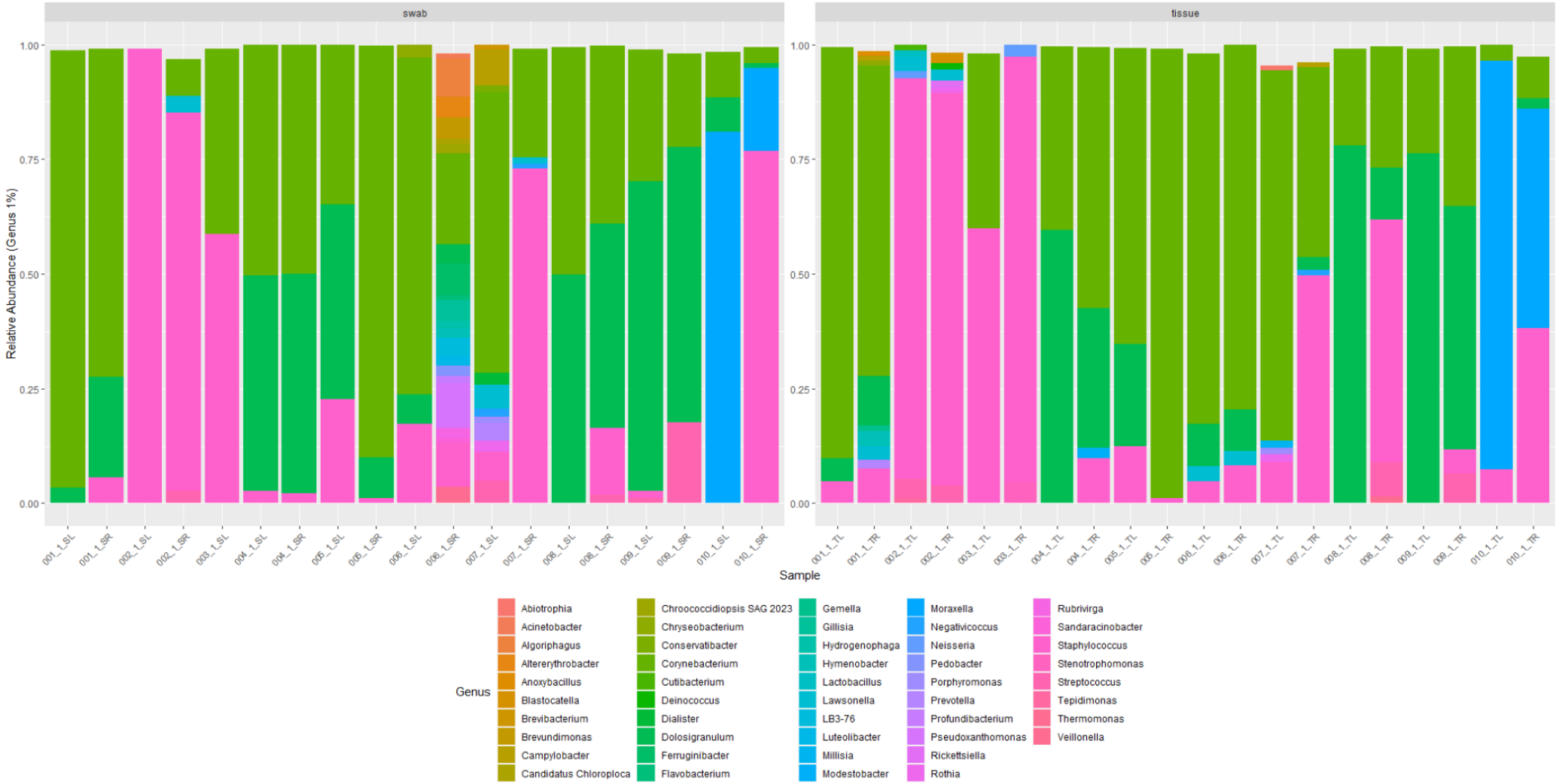

Figure 3.10: Relative abundance (>1%) of T1 samples for patients 1-10 separated into type of sample (tissue vs swab).

3.5 Exploring pre-treatment microbiome patterns against patient clinical data

To further understand what factors could influence the patients' sinonasal microbiome, clinical data was investigated to see if any patterns correlated with the sinonasal microbiome. Swab samples were examined to see if categorical clinical factors could influence the sinonasal microbiome. Tissue samples were analysed separately in patients with asthma or nasal polyps. This is because tissue samples may be influenced differently to swab samples due to the effects of the immune system and biofilms that may have been present in these tissue samples.

3.5.1 Pre-treatment microbiome patterns associated with asthma

The alpha diversity for patients with and without asthma was compared for both swab and tissue samples. Alpha diversity indices were compared using observed richness, breakaway estimates, and Shannon. The alpha diversity for swab samples showed that patients with asthma had, on average, a significantly higher diversity than patients without asthma (unpaired t-test for breakaway estimates, p-value = 0.026) (Figure 3.11).

The alpha diversity for tissue samples according to observed richness was similar (Figure 3.12). The Shannon index indicated that patients without asthma had a higher diversity on average. The relationship for patients with/without asthma was not statistically significant in tissue samples (unpaired t-test for breakaway estimates, p-value = 0.50).

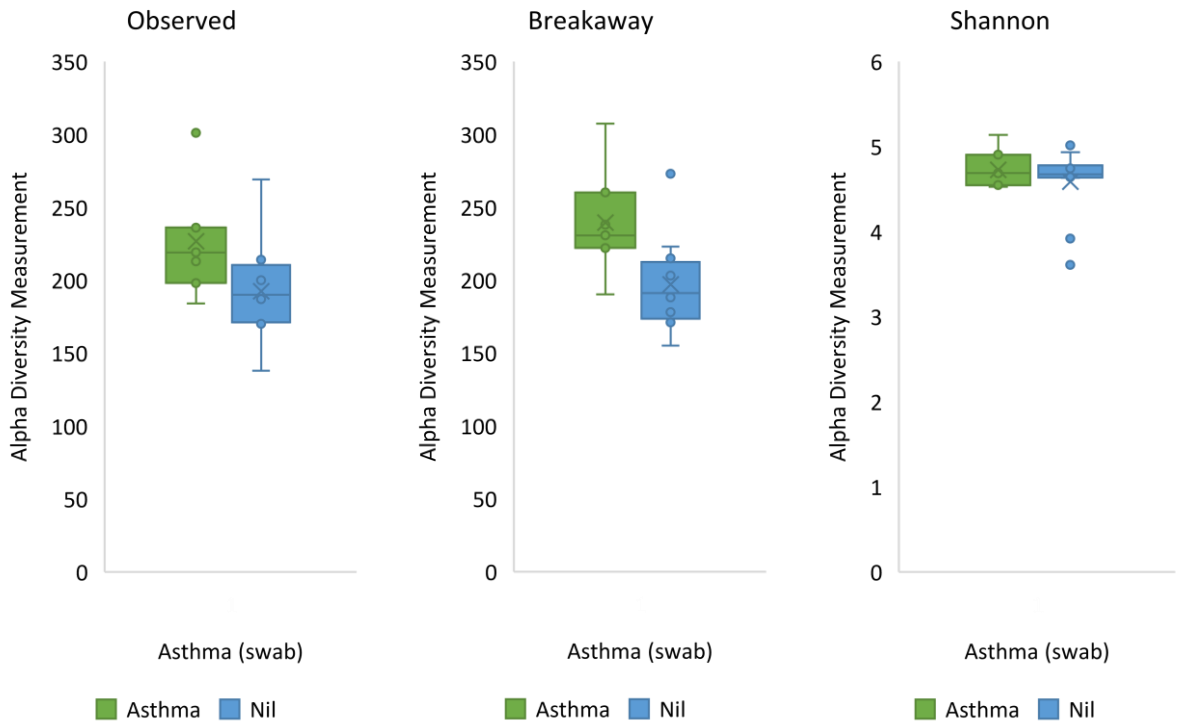

Figure 3.11: Alpha diversity of swab samples for patients with/without asthma using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

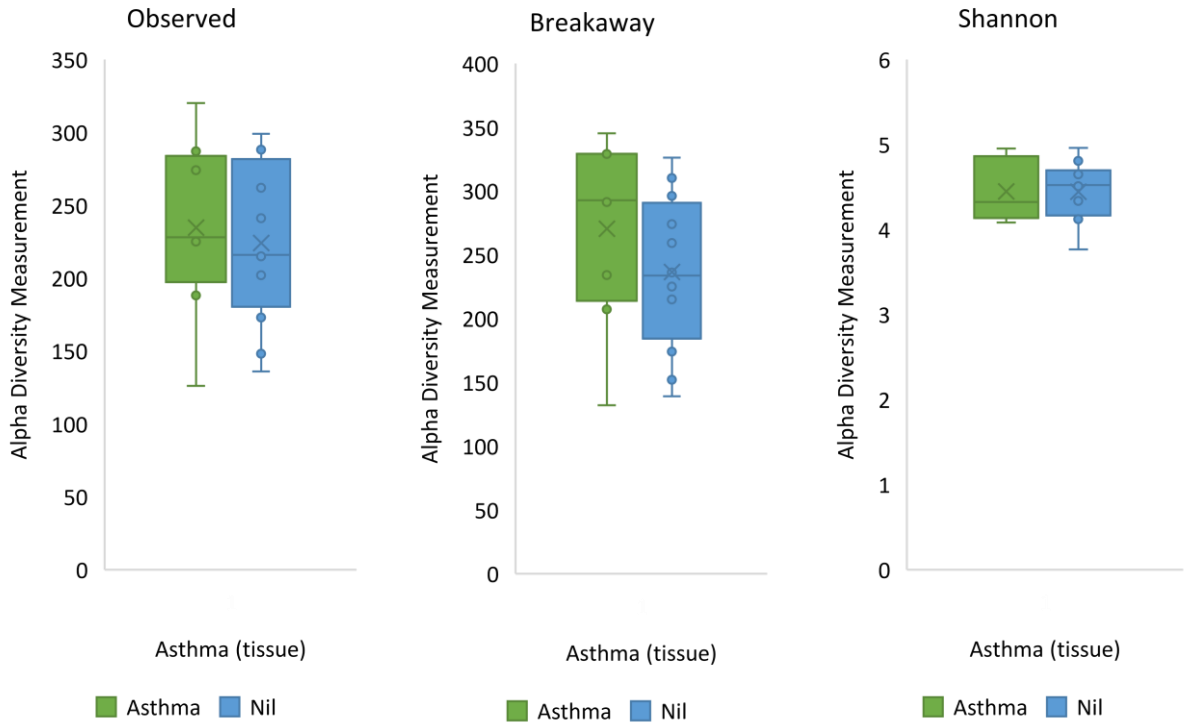

Figure 3.12: Alpha diversity of tissue samples for patients with/without asthma using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

The NMDS plots of patients with/without asthma for both tissue and swab samples showed patients with asthma clustering together while patients without asthma (nil) were spread throughout the graphs (Figure 3.13). This indicated that patients with asthma all had microbiomes that were relatively similar to each other. Some patients without asthma had similar microbiomes to asthma patients, but the majority were dissimilar to asthma patients. Adonis 2 analysis of asthma in swab samples also showed a significant difference between patients with/without asthma ($R^2 = 0.24$, *p*-value = 0.005). The same analysis for tissue samples showed less significant differences between patients with/without asthma ($R^2 = 0.11$, *p*-value = 0.056).

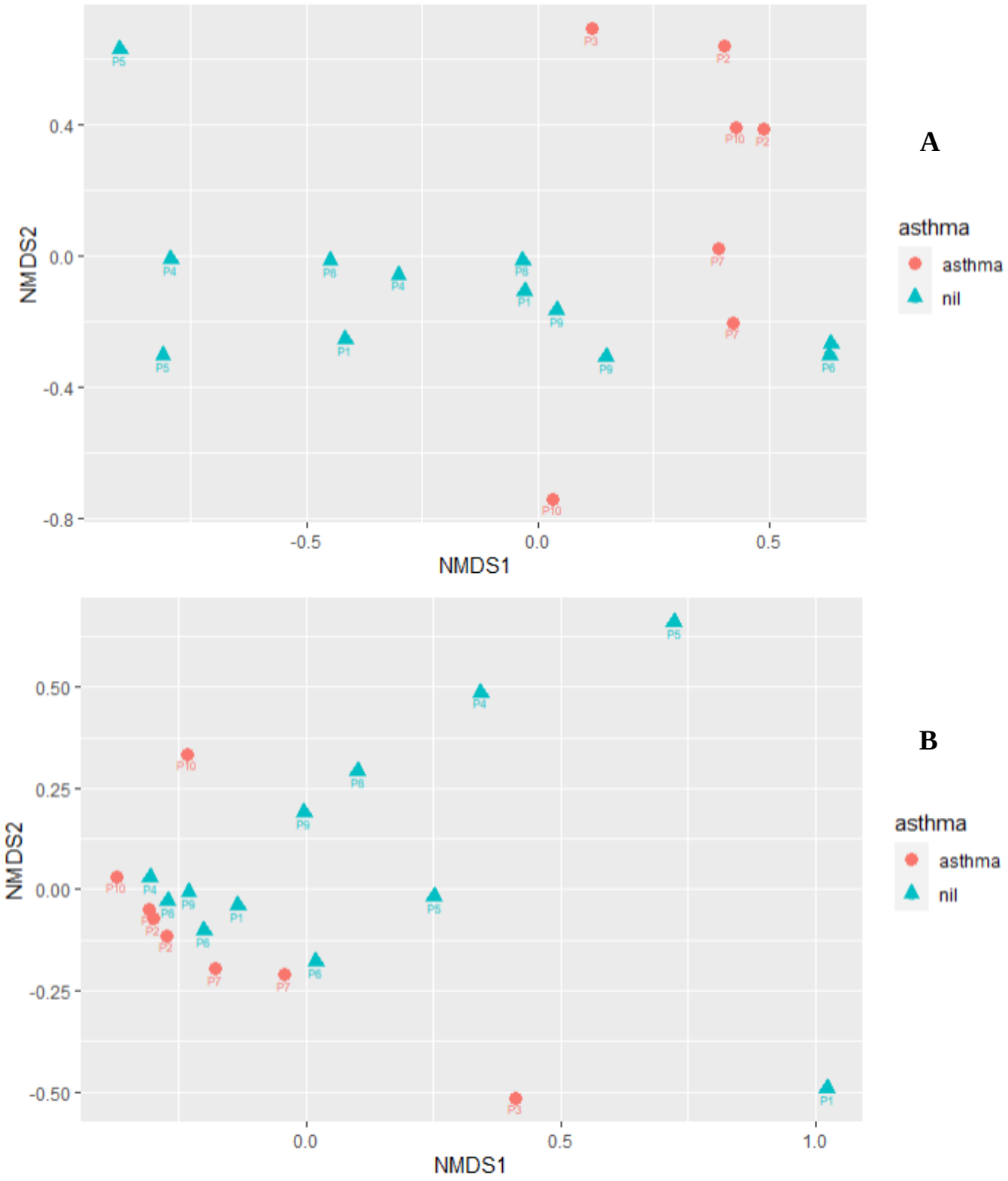

Figure 3.13: NMDS plot of swab samples (A) and tissue samples (B) sorted according to asthma. Ordination based on Bray-Curtis dissimilarities. Plot A: 2D stress = 020, max residual = 0.0000092. Plot B: 2D stress = 0.092, max residual = 0.00081.

The community composition for swab samples in patients differs depending on whether the patients had asthma or not (Figure 3.14). *Staphylococcus* dominated asthma patients except for two samples (007_1_SL and 010_1_SL); they were dominated by *Moraxella* and *Corynebacterium.* The sinonasal microbiomes of patients who did not have asthma were dominated by *Dolosigranulum*, with four exceptions. The exceptions were dominated by *Corynebacterium* or did not appear to have a dominant genus. *Staphylococcus* was present at a low relative abundance in every sample with three exceptions: two nil samples (001_1_SL and 008_1_SL) and one asthma sample (010_1_SL).

Tissue samples showed a similar trend to swab samples, with distinct microbiomes that depended on whether the patient had asthma or not (Figure 3.15). The microbial composition for tissue samples in patients with asthma was similar to the composition in swab samples. A majority of the samples were dominated by *Staphylococcus*. Tissue samples in patients without asthma, however, differed from the swab samples. *Corynebacterium* dominated the majority of these samples*;* however, some samples were dominated by *Dolosigranulum* and *Staphylococcus.*

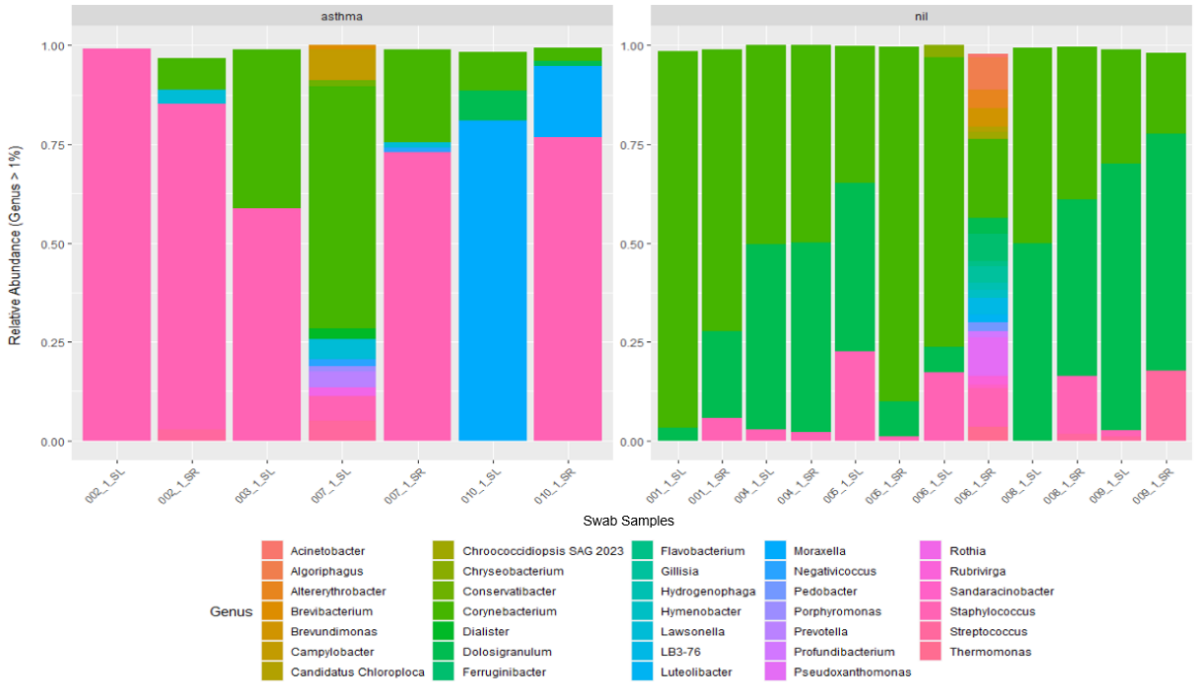

Figure 3.14: Relative abundance (>1%) of swab samples for patients with/without asthma.

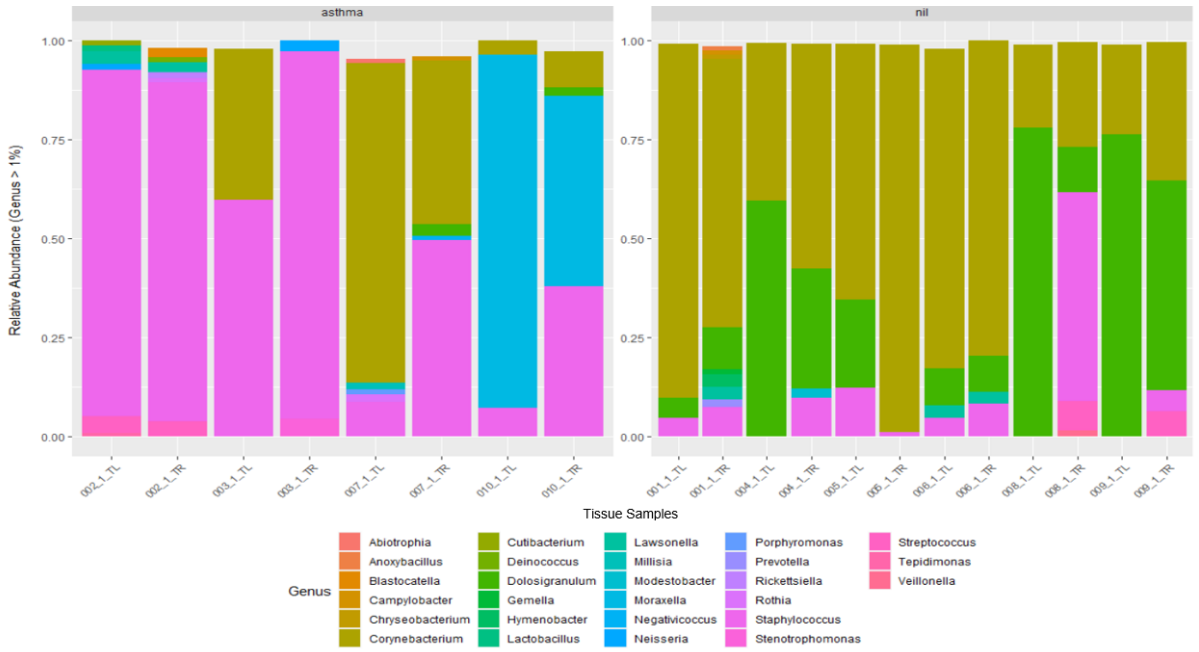

Figure 3.15: Relative abundance (>1%) of tissue samples for patients with/without asthma.

3.5.2 Pre-treatment microbiome patterns associated with nasal polyps

The alpha diversities for swab samples comparing patients with or without nasal polyps did not appear to show a significant difference between the means of patients with or without nasal polyps (unpaired t-test for breakaway estimates, *p*-value = 0.63) (Figure 3.16). The alpha diversities for the tissue samples of patients with or without nasal polyps showed that, on average, patients with or without nasal polyps had a similar richness (Figure 3.17) (unpaired t-test for breakaway estimates, *p*-value = 0.93).

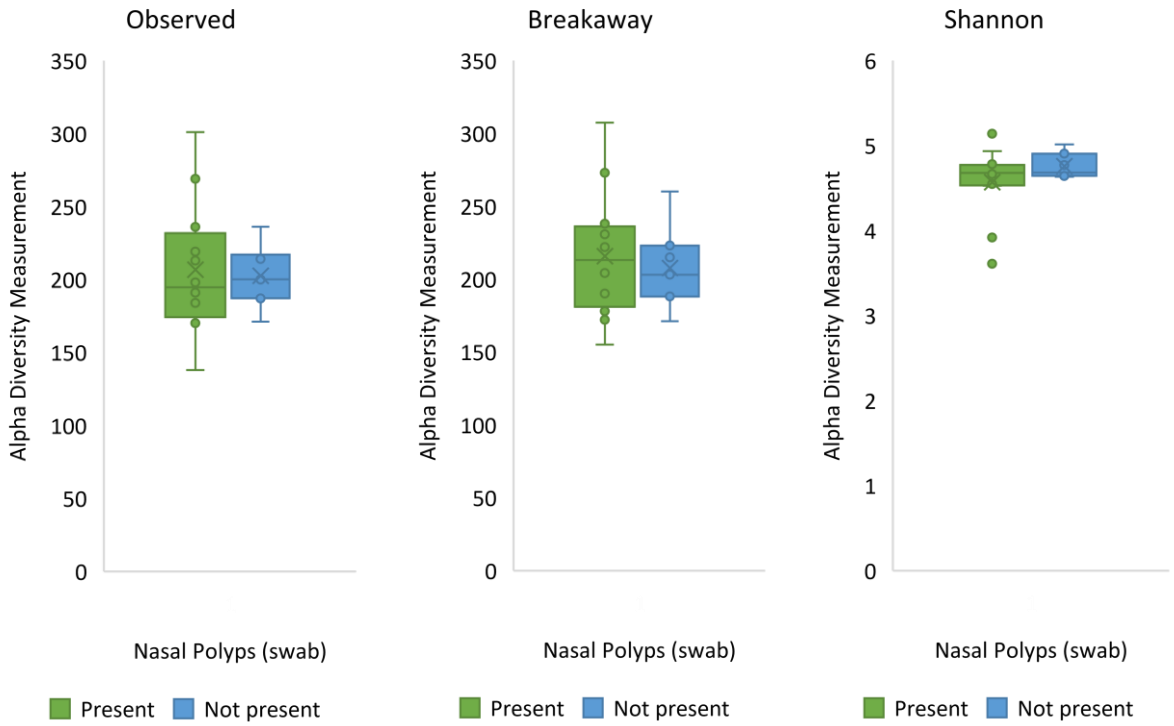

Figure 3.16: Alpha diversity of swab samples for patients with/without nasal polyps using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

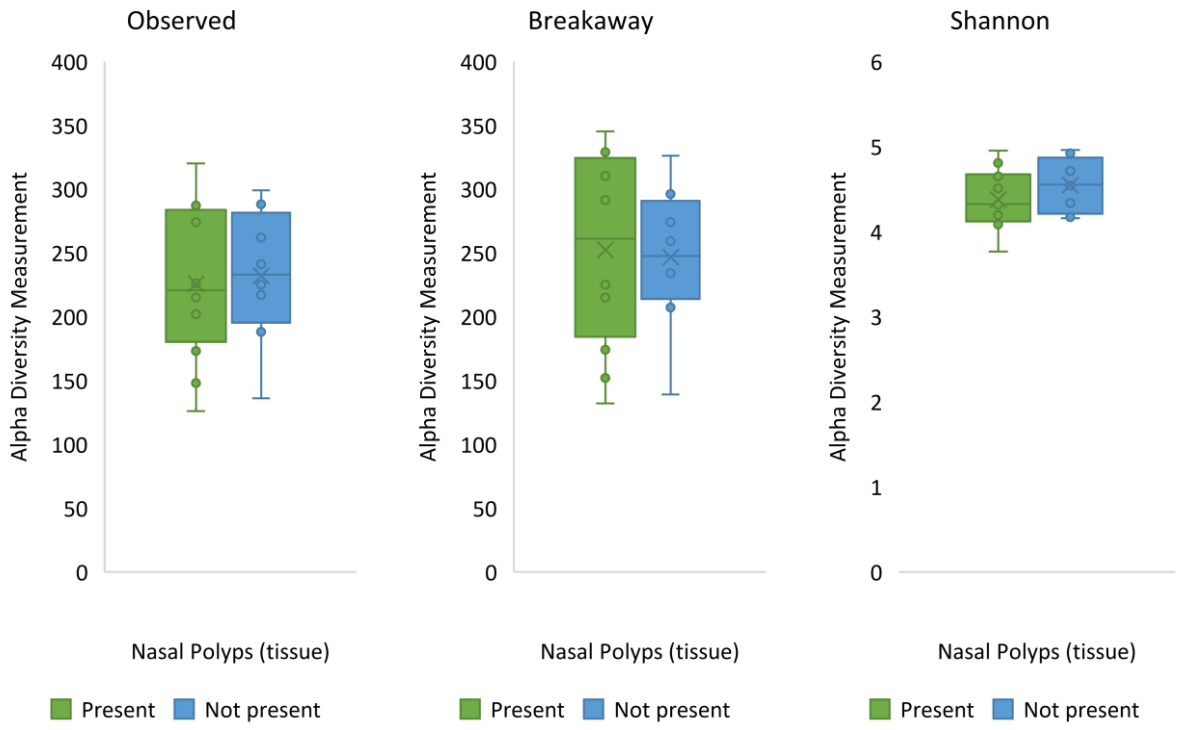

Figure 3.17: Alpha diversity of tissue samples for patients with/without nasal polyps using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

Ordination plotting (NMDS) of patients with or without nasal polyps did not show any distinct clustering in swab samples or tissue samples (Figure 3.18). This was further confirmed with Adonis2 analysis, which showed no significant difference between patients with/without nasal polyps in the swab samples ($R^2 = 0.074$, *p*-value = 0.219) and tissue samples ($R^2 = 0.086$, *p*-value = 0.129).

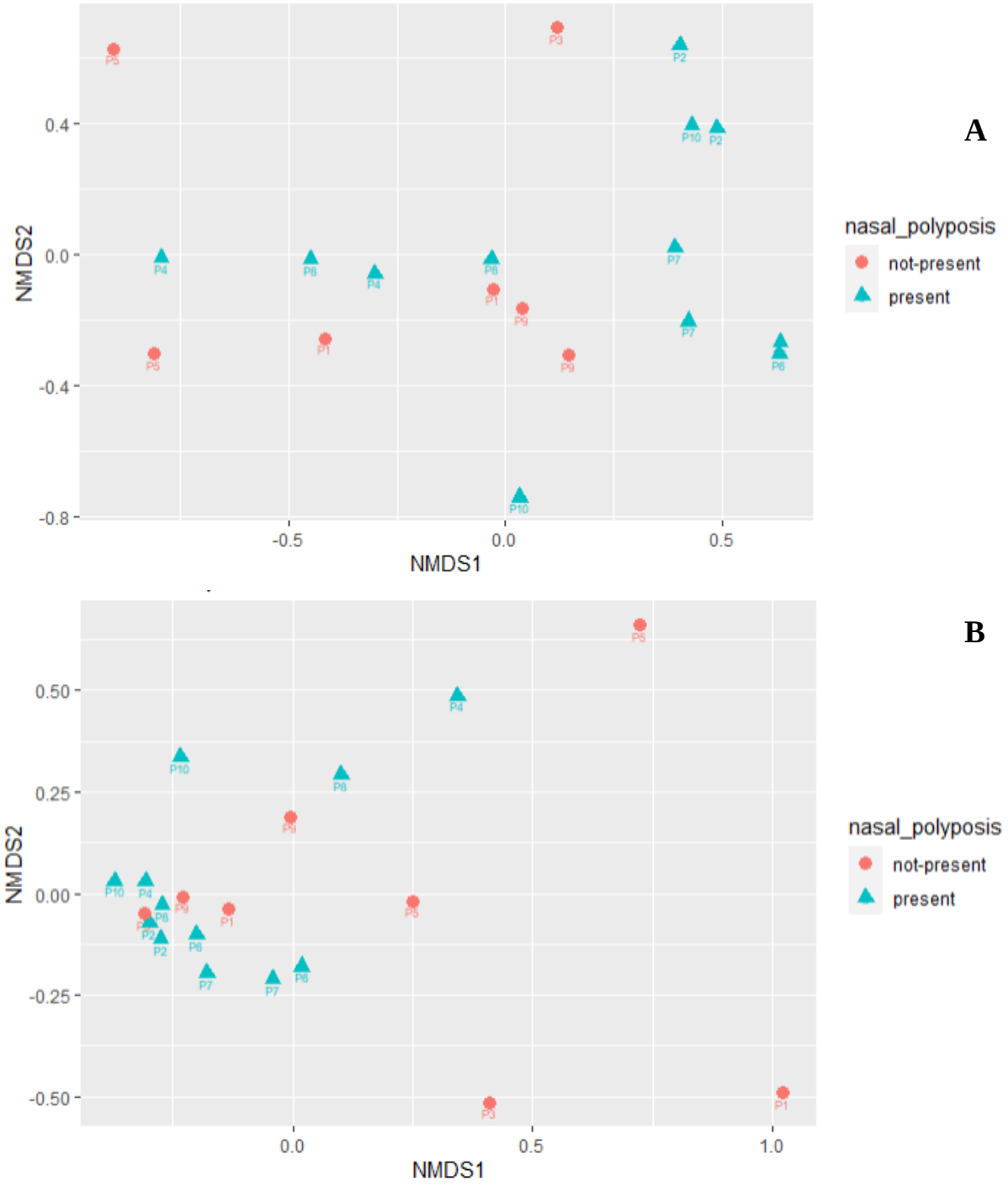

Figure 3.18: NMDS plot of swab samples (A) and tissue samples (B) sorted according to presence of nasal polyps. Ordination based on Bray-Curtis dissimilarities. Plot A: 2D stress = 0.13, max residual = 0. 000018. Plot B: 2D stress = 0.091, max residual = 0.00042.

3.5.3 Pre-treatment microbiome patterns of patients undergoing adjunct surgeries

Adjunctive surgeries were also investigated to see if these procedures would impact the microbiome. Patients undergoing these procedures may have a different sinonasal microbiome due to anatomical abnormalities treated with adjunct surgery. Only swab samples were used for this analysis, as biofilms were not thought to play a role in the need for these surgeries.

On average, the alpha diversity for patients who underwent septoplasty was higher than patients who did not undergo adjunctive surgery (Figure 3.19). However, the relationship between septoplasty and nil was not statistically significant (Unpaired t-test for breakaway estimates, *p*-value =0.099). No alpha diversity statistical analysis could be calculated for ITs patients due to the lack of samples. Using NMDS, no apparent clustering was observed (Figure 3.20). Pairwise Adonis testing showed no significant difference between the different procedures (Table 3-4).

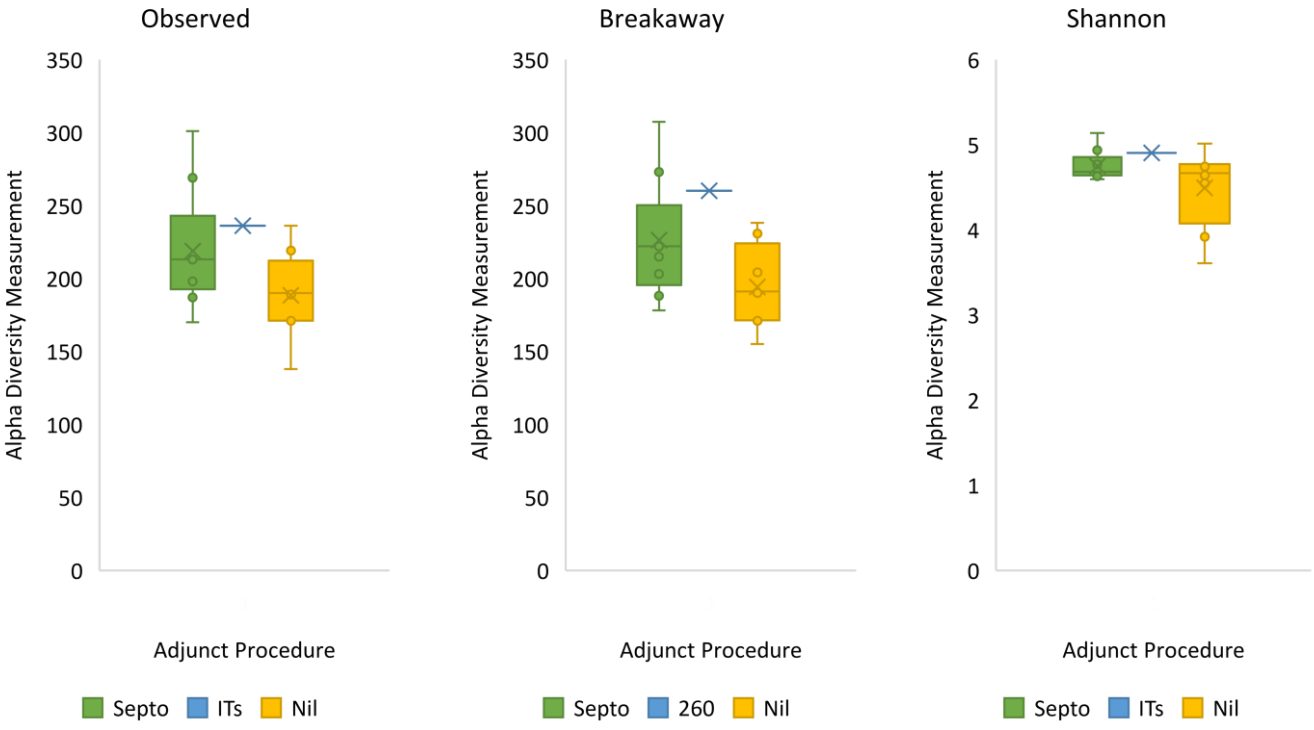

Figure 3.19: Alpha diversity of T1 swab samples sorted according to adjunct procedures using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

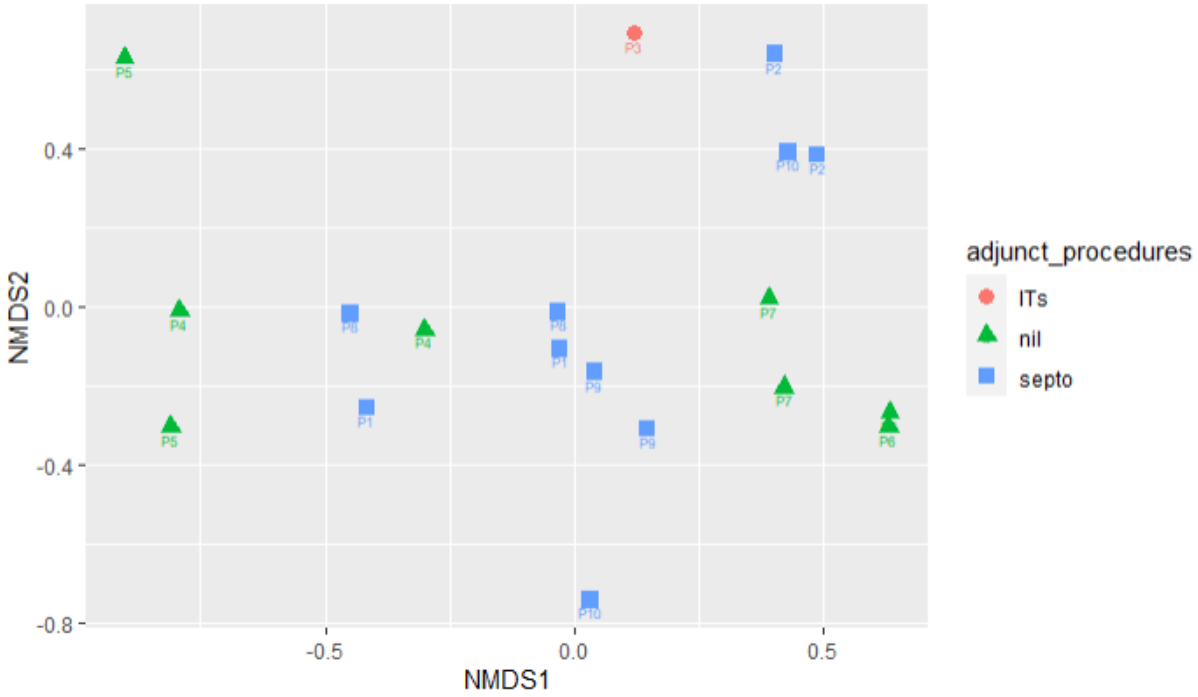

Figure 3.20: NMDS plot of T1 swab samples sorted according to adjunct procedures undertaken along with ESS. Ordination based on Bray-Curtis dissimilarities (2D stress = 0.13, max residual = 0.000014).

Procedure	R^2	*p*-value
Septo vs ITs	0.145	0.546
Septo vs nil	0.051	1.00
ITs vs nil	0.134	1.00

Table 3-4: Pairwise Adonis analysis comparing adjacent procedures undertaken by patients using T1 swab samples.

3.6 Exploring patterns between treatment groups

3.6.1 Community analysis of the treatment groups

To determine if antibiotics affected the outcome of ESS or influenced the sinonasal microbiome, the bacterial community structure and composition were compared between patients who received antibiotics and patients who received placebo. For this analysis, the T1 samples were excluded as the medication had not been administered. Patient 11 was excluded as they had received antibiotics midway through the study (Table 3-2) and due to the lack of T2 or T3 samples. Patient 12 was also excluded from this analysis due to a lack of T2 or T3 samples.

The different treatment groups were compared using alpha diversity indices: observed richness, breakaway estimates, and Shannon (Figure 3.21). On average, the placebo treatment had a higher diversity according to all three indices after ESS; however, this relationship was not significant (unpaired t-test from breakaway estimates, *p*-value = 0.25). Using an NMDS plot, there did not appear to be any distinct cluster between the treatment groups (Figure 3.22). Both the placebo and treatment groups were intermixed throughout this plot. Adonis2 analysis also showed no significant difference between these two groups (R^2 = 0.026, *p*-value = 0.504).

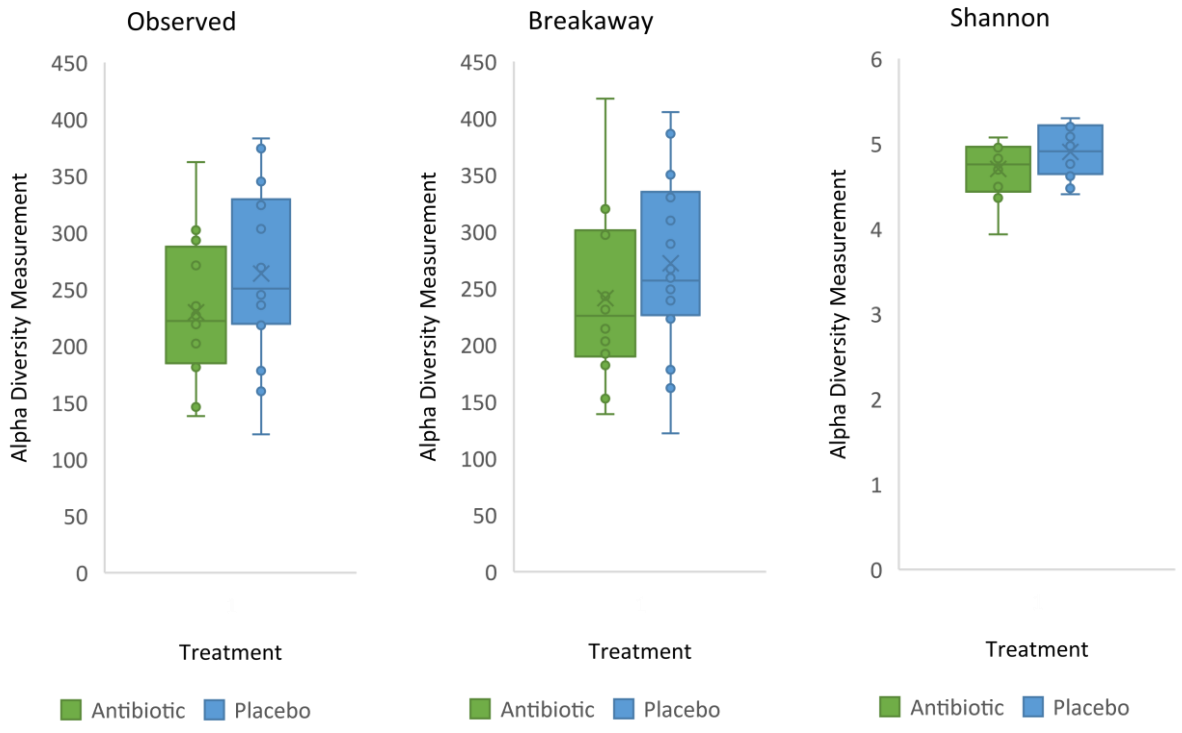

Figure 3.21: Alpha diversity of T2 and T3 swab samples sorted according to treatment undertaken using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

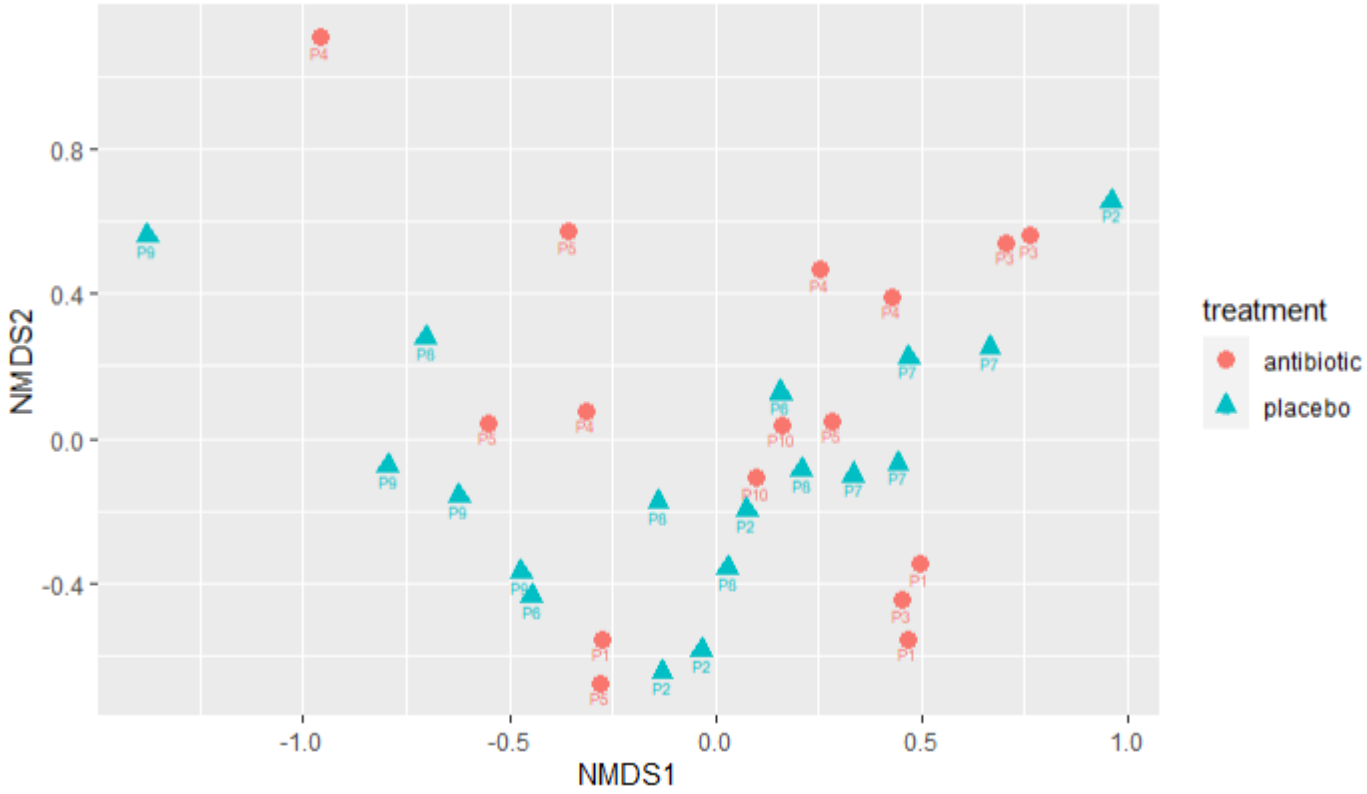

Figure 3.22: NMDS plot of T2 and T3 swab samples sorted according to treatment undertaken after ESS. Ordination based on Bray-Curtis dissimilarities (2D stress = 0.16, max residual = 0.0038).

3.6.2 Comparing timepoints for each treatment group

The different treatment groups were further analysed by comparing placebo and antibiotics samples at separate timepoints. (T1, T2, and T3). The alpha diversity for the T1 samples indicated that the placebo samples had a higher diversity on average according to the observed and breakaway estimate indices (Figure 3.23). This relationship was not shown to be statistically significant (unpaired t-test for breakaway estimates, *p*-value =0.17).

The alpha diversities for the T2 timepoint showed that patients who received the placebo, on average, had higher diversity (Figure 3.24). This relationship was especially noticeable in the Shannon index; however, this relationship was not statistically significant (unpaired t-test for breakaway estimates, *p*-value =0.82).

According to the observed and breakaway estimate indices, patients who received antibiotics had lower diversity than patients who received the placebo at the T3 timepoint

(Figure 3.25). There was no statistical difference between the two treatments at the T3 (unpaired t-test for breakaway estimates, *p*-value =0.16).

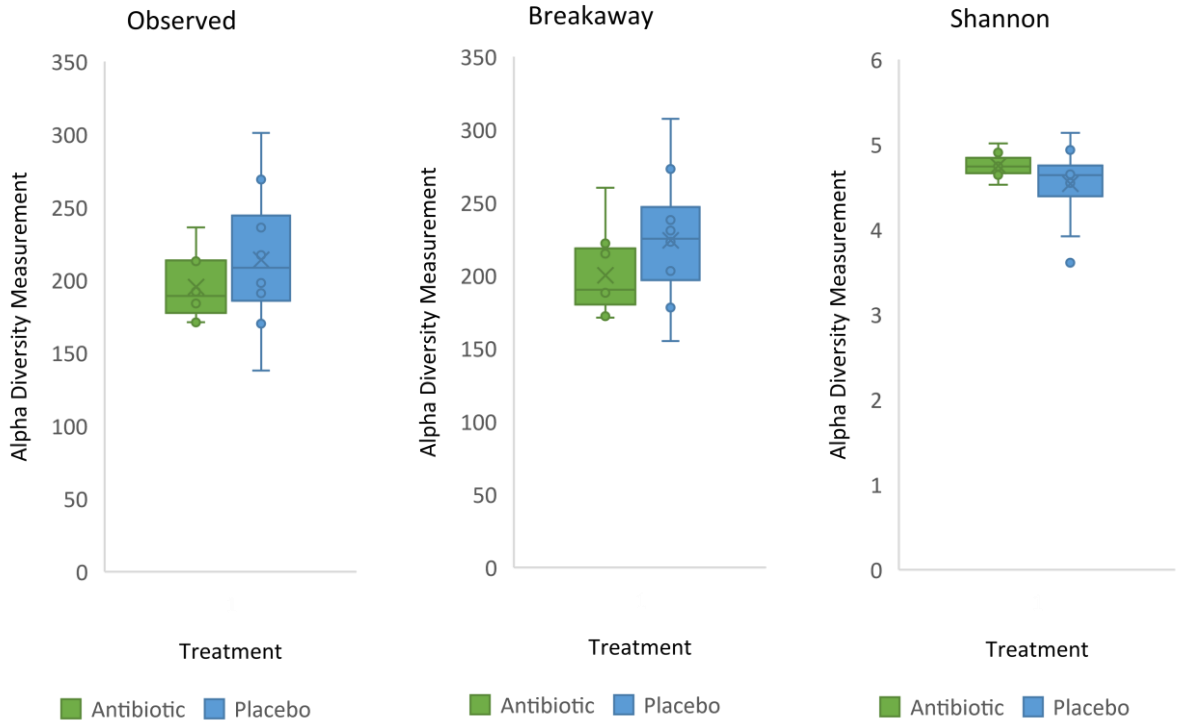

Figure 3.23: Alpha diversity of T1 samples sorted according to the treatment group using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

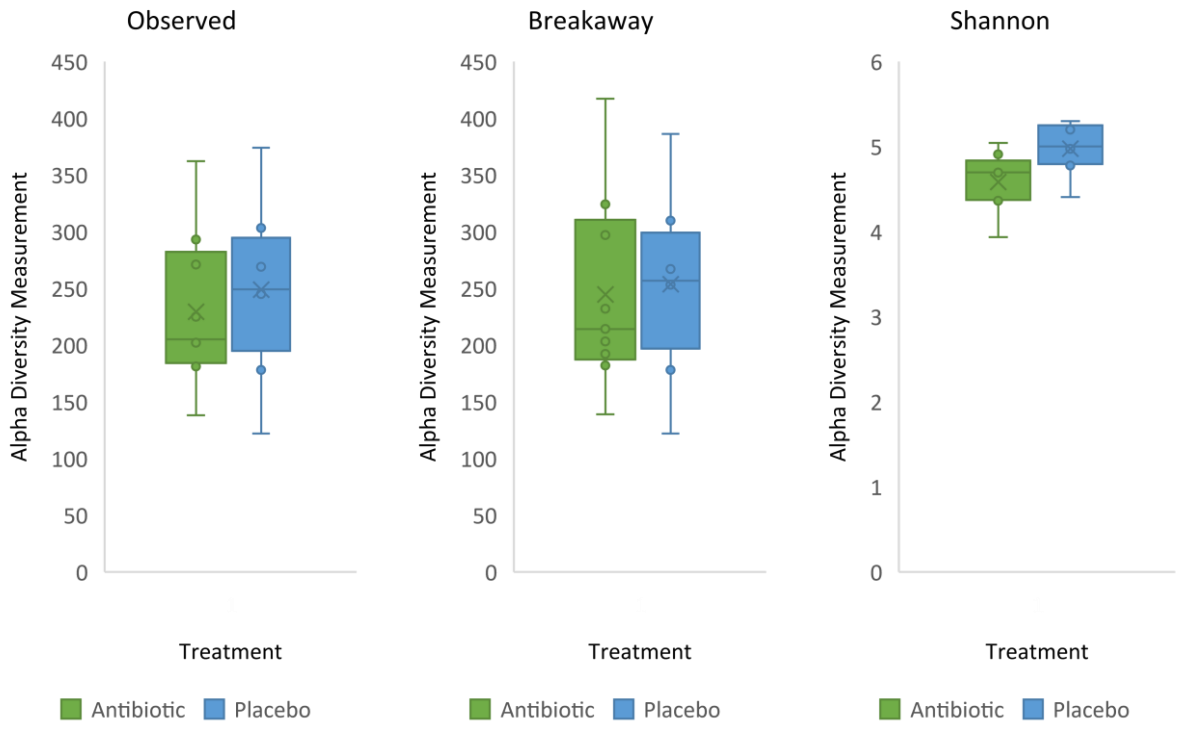

Figure 3.24: Alpha diversity of T2 samples sorted according to the treatment group using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

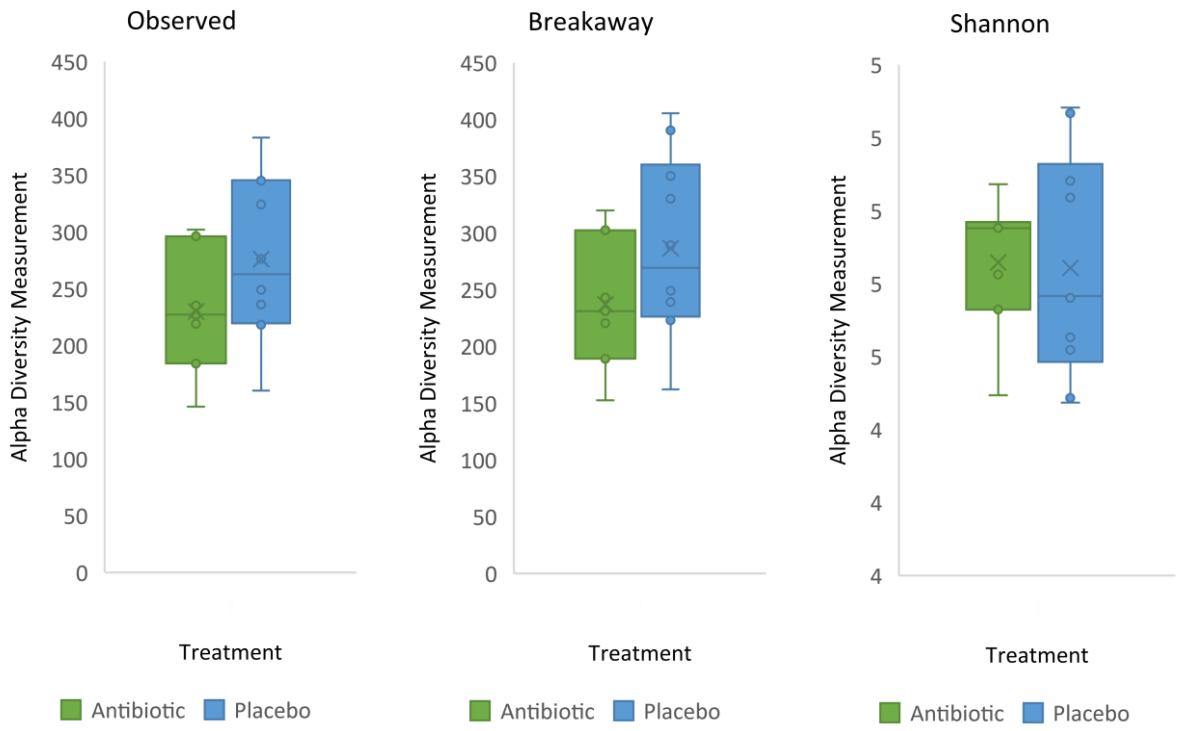

Figure 3.25: Alpha diversity of T3 samples sorted according to the treatment group using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

An NMDS plot for the T1 samples did not show any distinct clustering between the different treatment groups; however, a general trend appeared of the antibiotic samples grouping to the left axis and the placebo samples to the right (Figure 3.26). Adonis2 analysis for the T1 timepoint showed a statistically significant difference between the treatment groups (R^2 = 0.19, *p*-value =0.008). The NMDS plot for the T2 samples showed no distinct clustering between the treatment groups (Figure 3.27). Unlike the T1 samples, Adonis2 analysis of the T2 timepoint did not show a significant difference between the treatment groups (R^2=0.042, *p*-value =0.604). The T3 samples also did not show any distinct clustering in the NMDS plot (Figure 3.28), which was confirmed using Adonis2 analysis (R^2=0.096, *p*-value =0.188).

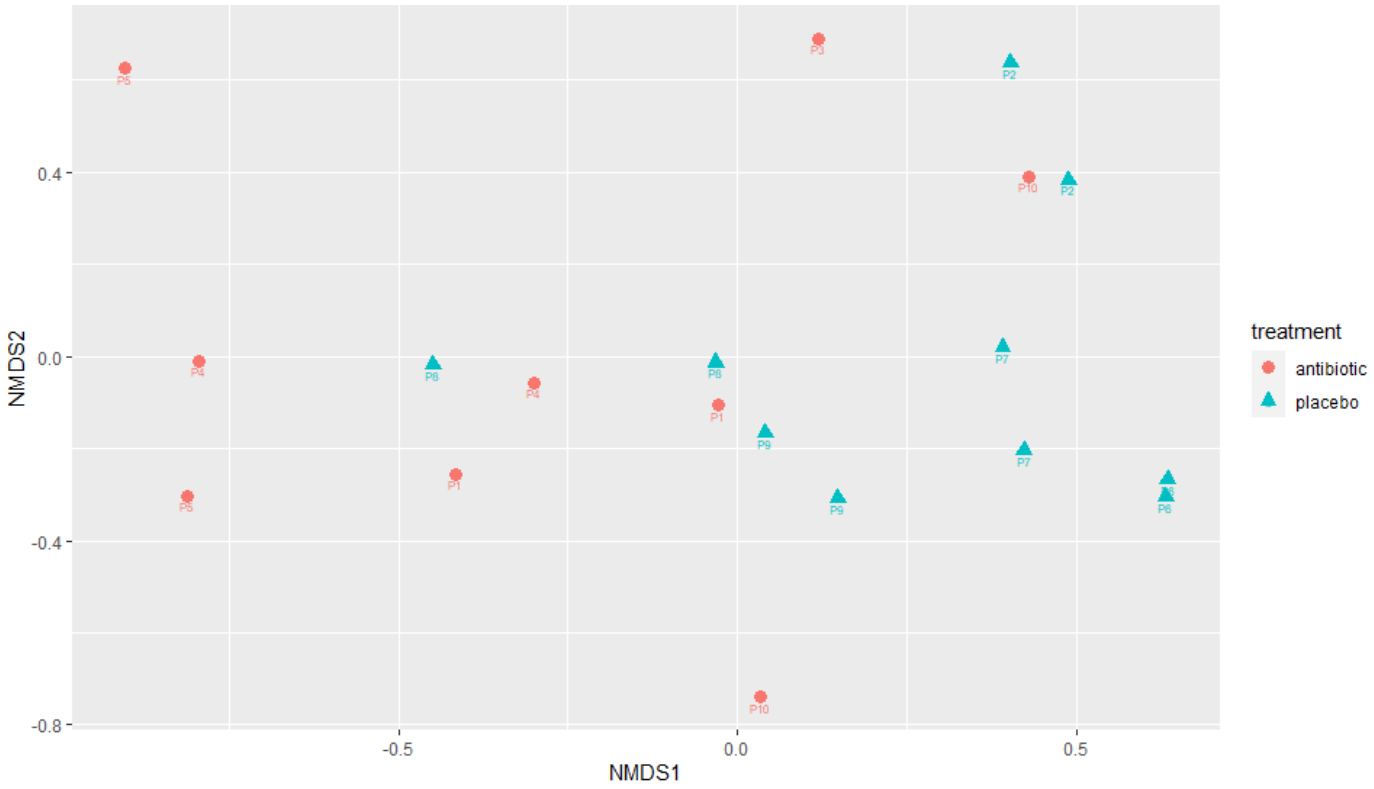

Figure 3.26: NMDS plot of T1 samples sorted according to treatment group. Ordination based on Bray-Curtis dissimilarities (2D stress = 0.18, max residual = 0.000019).

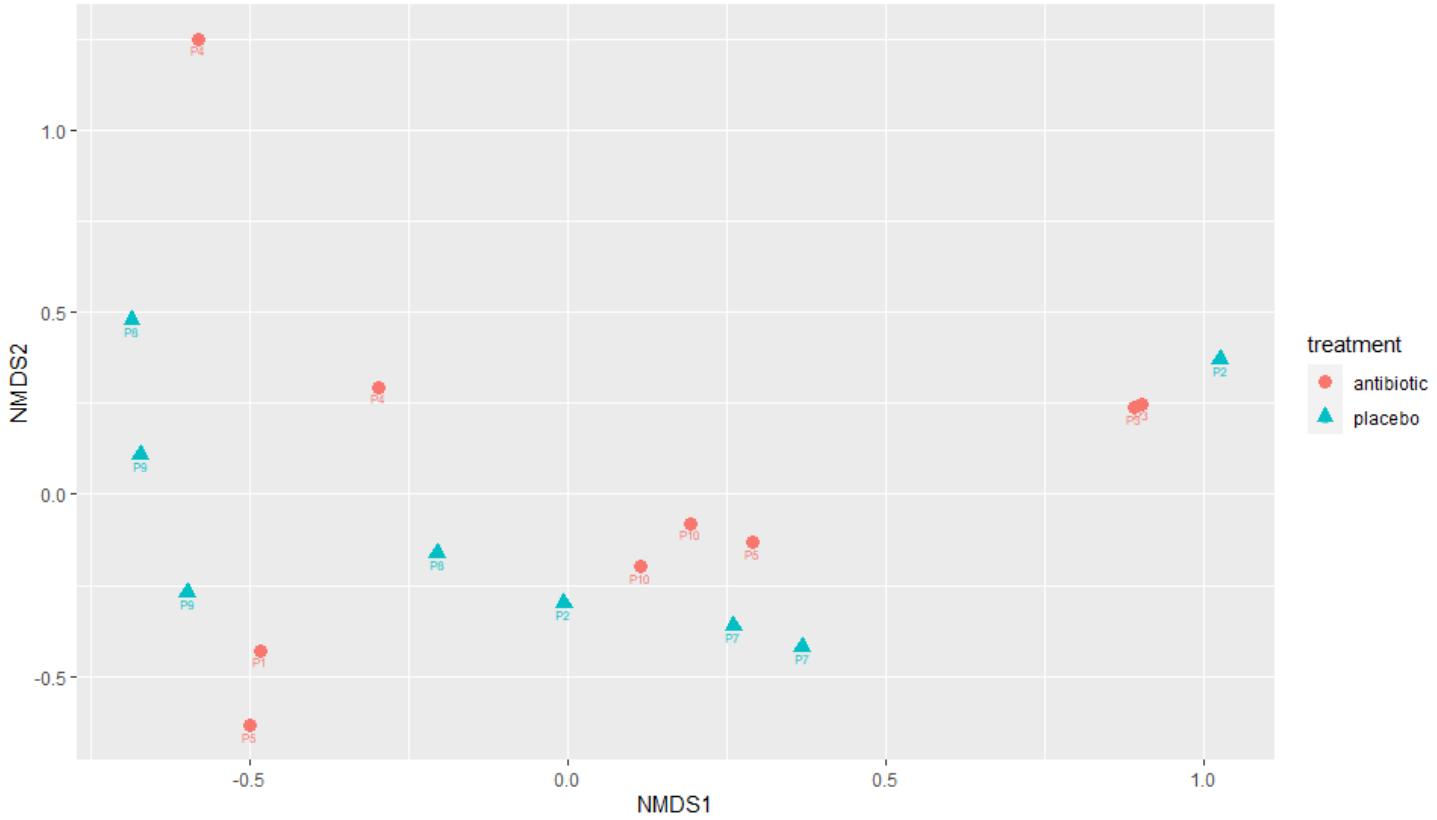

Figure 3.27: NMDS plot of T2 samples sorted according to treatment group. Ordination based on Bray-Curtis dissimilarities (2D stress = 0.13, max residual = 0.00095).

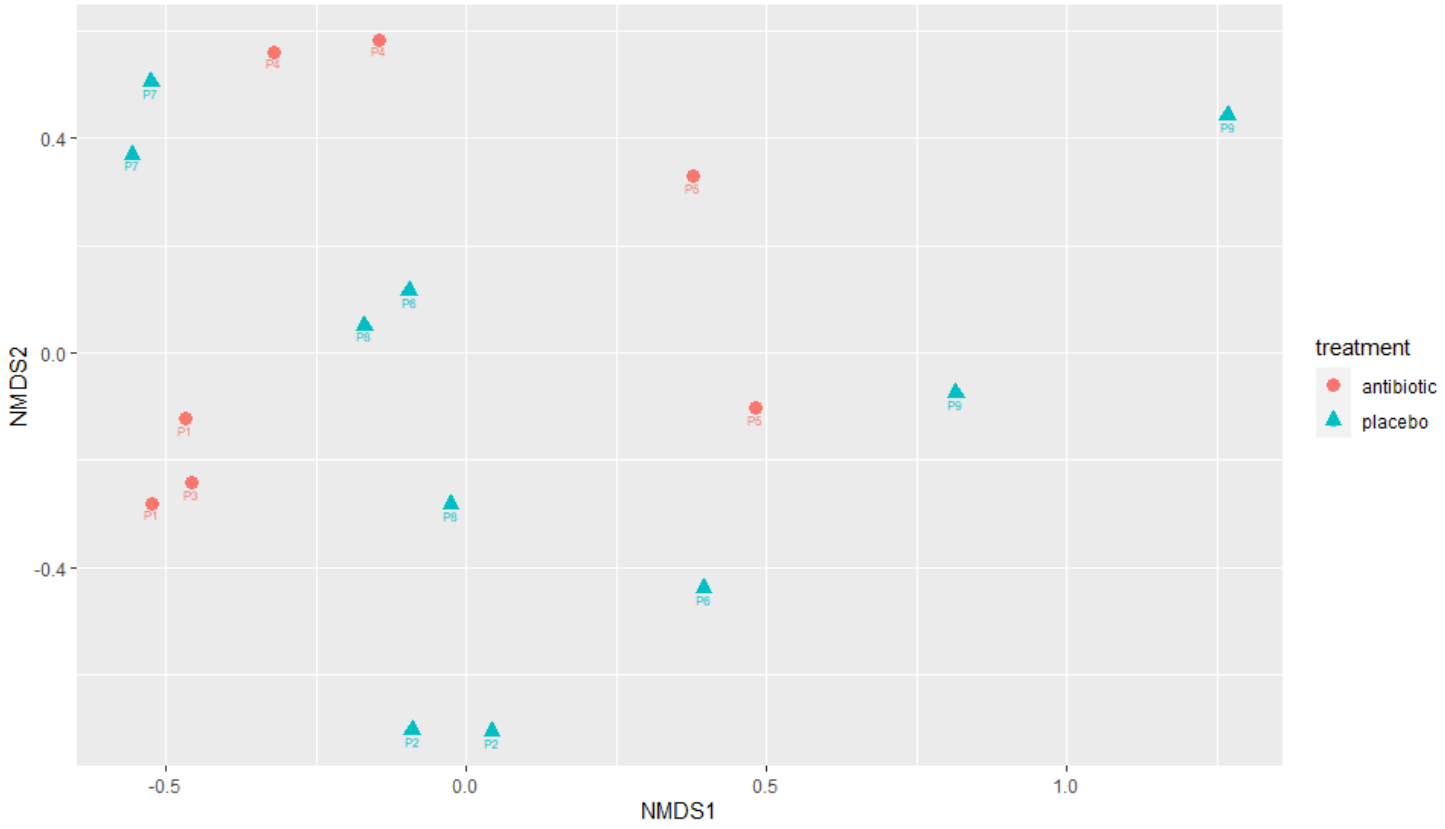

Figure 3.28: NMDS plot of T3 samples sorted according to treatment group. Ordination based on Bray-Curtis dissimilarities (2D stress = 0.071, max residual = 0.000014).

All three timepoints were primarily dominated by *Staphylococcus*, *Corynebacterium*, and *Dolosigranulum* (Figure 3.29, Figure 3.30, and Figure 3.31). The antibiotic T1 samples were dominated by *Corynebacterium* and *Dolosigranulum* (Figure 3.29). The placebo T1 samples were dominated by a variety of genus, including *Staphylococcus* and *Dolosigranulum.* *Corynebacterium* was present in both T1 treatments; however, had a higher relative abundance in antibiotic samples. *Staphylococcus* was found at higher relative abundances and higher prevalence in the T1 placebo samples. The T2 samples for both treatments were dominated by *Staphylococcus* (Figure 3.30). *Corynebacterium* dominated the six T2 samples that were not dominated by *Staphylococcus*. A variety of genus dominated the T3 samples for both treatments. *Staphylococcus* dominated the majority of the T3 placebo samples (Figure 3.31). *Corynebacterium* was present in all of the T3 samples.

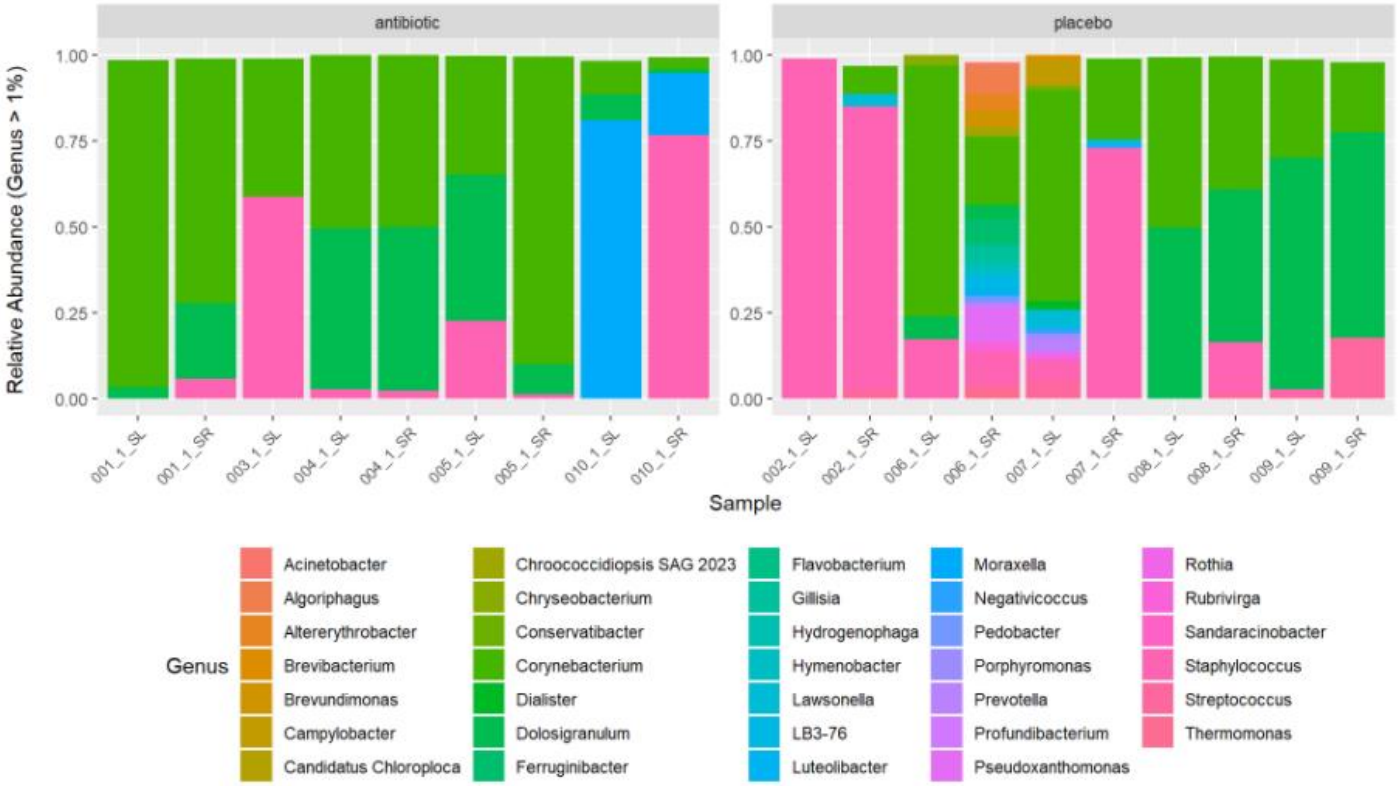

Figure 3.29: Relative abundance (>1%) of T1 samples, sorted according to treatment.

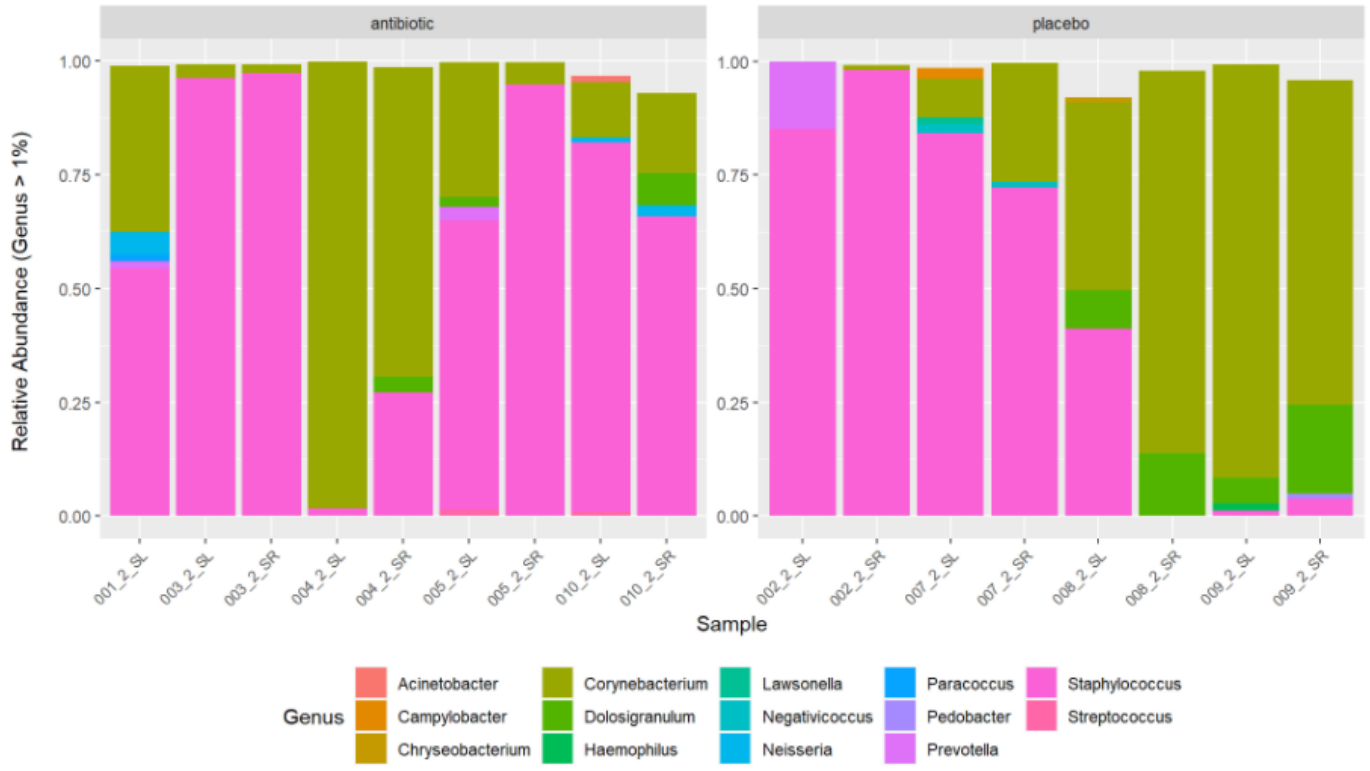

Figure 3.30: Relative abundance (>1%) of T2 samples, sorted according to treatment.

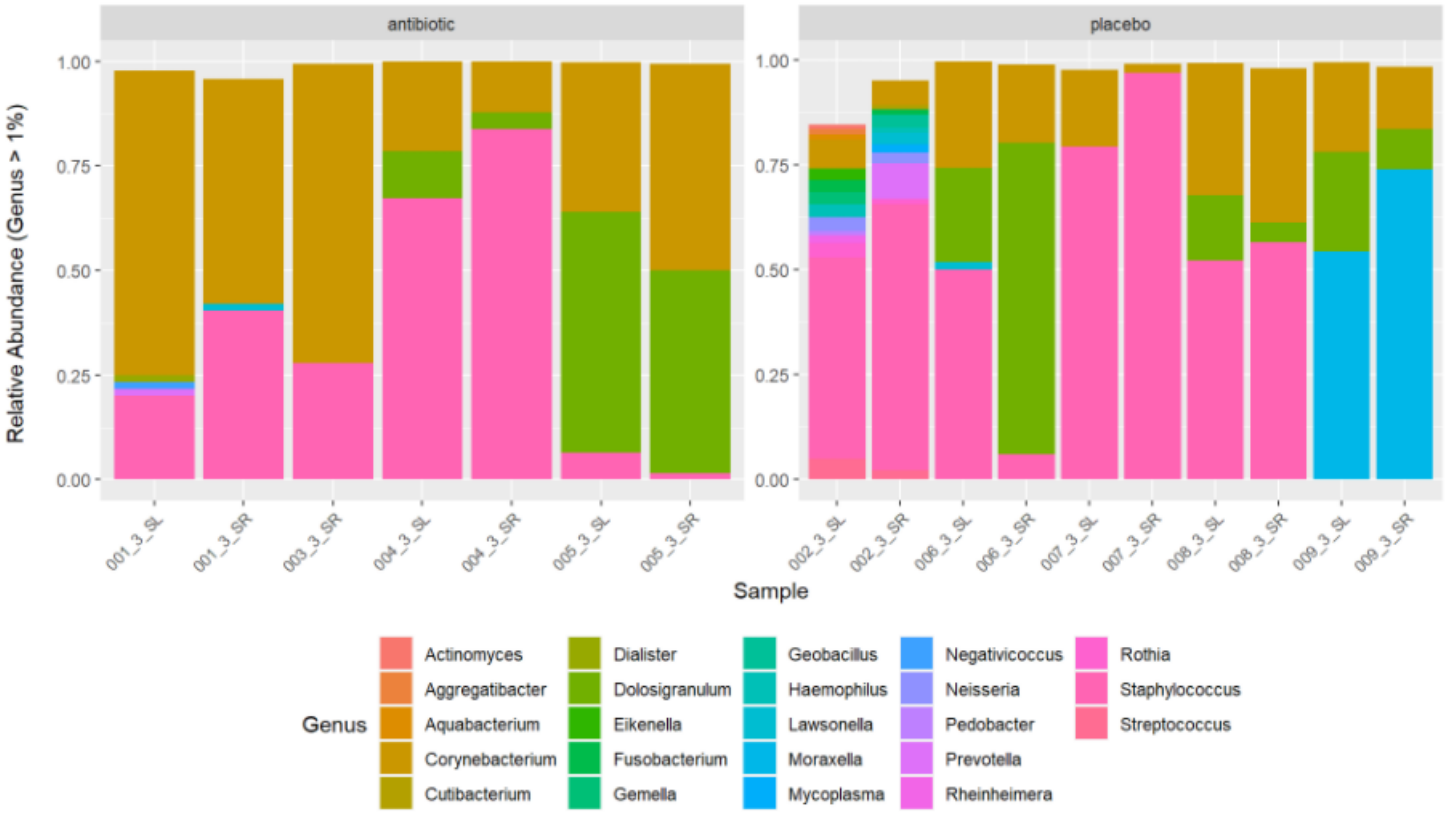

Figure 3.31: Relative abundance (>1%) of T3 samples, sorted according to treatment.

3.6.3 Comparing timepoints across antibiotic treatment samples

Antibiotic samples were compared across timepoints to determine the effect antibiotics had on the sinonasal microbiome over time. The observed richness and breakaway estimate indices show that the T1 antibiotic samples had the lowest diversity of the timepoints while the T3 had the highest average diversity (Figure 3.32). The Shannon index showed that the T1 antibiotic treatment had a similar richness for the T2 and T3. A paired t-test using breakaway estimates showed no statistical significance between these timepoints (Table 3-5). The NMDS plot of the different timepoints for the antibiotic samples did not show any distinct clustering and all three timepoints intermixed throughout the graph (Figure 3.33). Pairwise Adonis showed that the three timepoints were not statistically significant (Table 3-6).

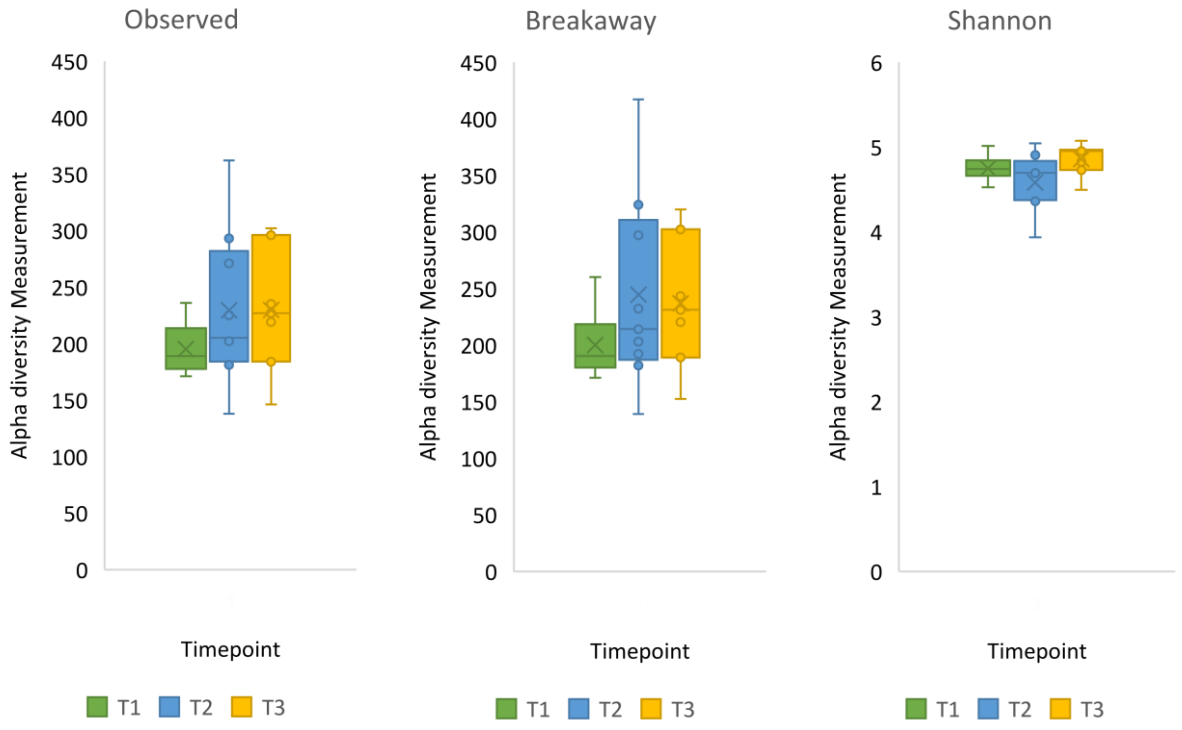

Figure 3.32: Alpha diversity of antibiotic samples sorted according to the timepoint using observed richness, breakaway estimate, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

Timepoint	*p*-value
T1 vs T2	0.150
T1 vs T3	0.217
T2 vs T3	0.520

Table 3-5: Paired t-test p-values for patients who received the antibiotic treatment, sorted according to the timepoint. Calculated using the breakaway estimate index.

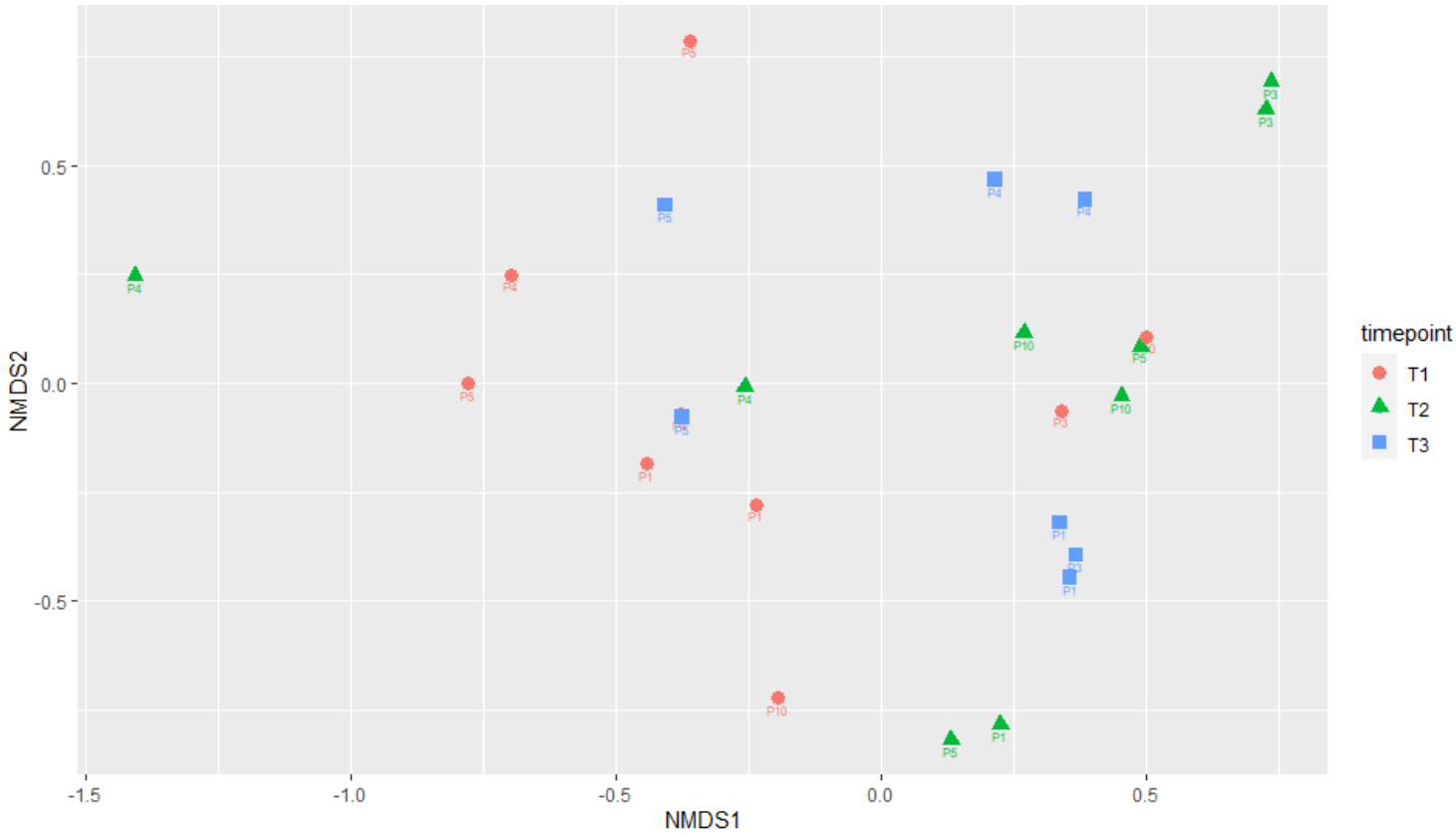

Figure 3.33: NMDS plot of antibiotic samples sorted according to the timepoint. Ordination based on Bray-Curtis dissimilarities (2D stress = 0.21, max residual = 0.0027).

Timepoint	R^2	*p*-value
T1 vs T2	0.14	0.150
T1 vs T3	0.10	0.573
T2 vs T3	0.16	0.096

Table 3-6: Pairwise Adonis analysis comparing timepoints for patients who received the antibiotic.

3.6.4 Comparing timepoints across placebo samples

The different timepoints were compared for the placebo samples to determine what effect this treatment had on the sinonasal microbiome overtime. The alpha diversity using observed richness, breakaway estimates, and Shannon indices showed that the T1 placebo samples had the lowest diversity (Figure 3.34). The Shannon index showed that the T2 samples had the highest diversity, whereas the observed and breakaway estimate indices showed that the T2 and T3 had similar richness. A paired t-test using breakaway estimates showed that T1 placebo samples compared to T3 placebo samples were statistically significant while T1 compared to T2 and T2 compared to T3 placebo samples were not significant (Table 3-7). The

NMDS plot for patients who received the placebo did not show any distinct clustering between the different timepoints (Figure 3.35). Pairwise Adonis analysis of this data also showed no significant difference between the different timepoints (Table 3-8).

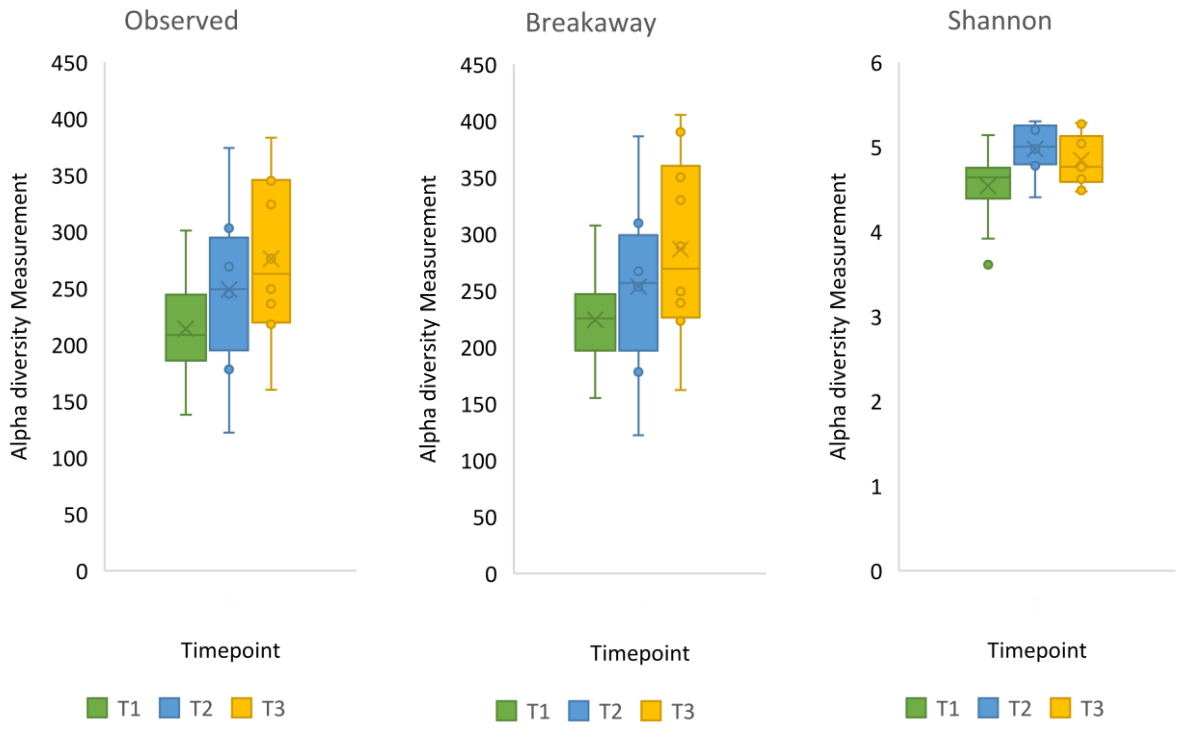

Figure 3.34: Alpha diversity of placebo samples sorted according to the timepoint using observed richness, breakaway estimates, and Shannon indices. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were calculated using unrarefied data.

Timepoint	*p*-value
T1 vs T2	0.637
T1 vs T3	0.046
T2 vs T3	0.530

Table 3-7: Paired t-test p-values for patients who received the placebo treatment, sorted according to the timepoints. Calculated using the breakaway estimates index.

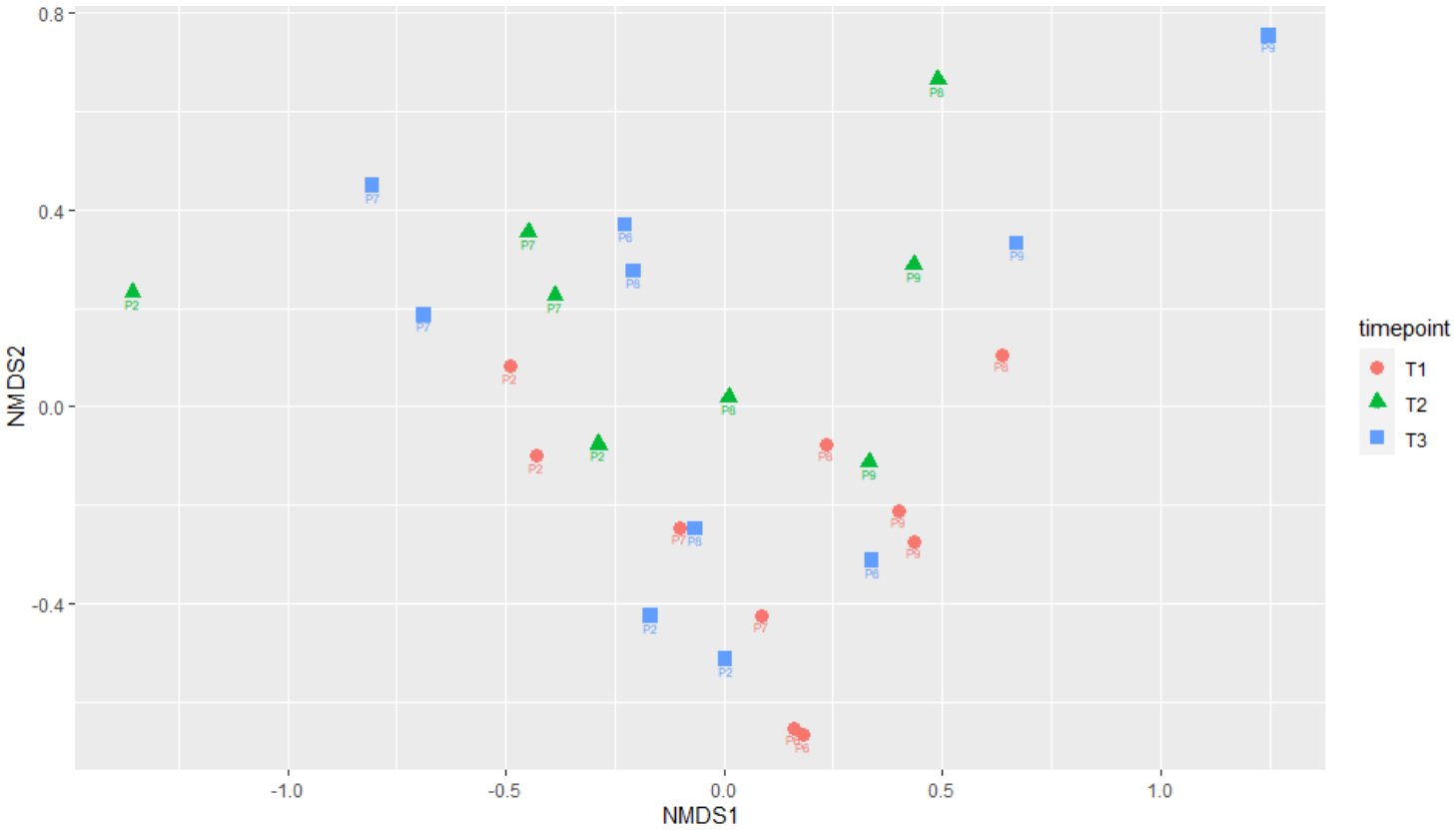

Figure 3.35: NMDS plot of placebo samples sorted according to the timepoint. Ordination based on Bray-Curtis dissimilarities (2D stress = 0.12, max residual = 0.0010).

Timepoint	R^2	*p*-value
T1 vs T2	0.158	0.084
T1 vs T3	0.086	0.477
T2 vs T3	0.073	0.849

Table 3-8: Pairwise Adonis analysis comparing timepoints for patients who received the placebo medication.

Chapter Four: Discussion

The composition of the sinonasal microbiome in CRS patients has received growing interest in recent years with the development of cultivation-independent techniques. The majority of the research in this area has been dedicated to comparing CRS patients' sinonasal microbiome to healthy patients. Few studies have investigated the sinonasal microbiome of patients undergoing ESS and the effect post-operative prophylactic antibiotics have on the microbial composition (Hauser et al., 2016). To our knowledge, this study was the first placebo-controlled double-blinded clinical trial investigating the relationship between the clinical outcomes of ESS and the post-operative treatment used with the sinonasal microbiome. Our study also investigated whether the microbial composition of swab samples usefully represents the microbial community in tissue samples.

4.1 Tissue and swab samples have similar but distinct microbiomes

Swab samples of the nasal mucosa are commonly used to study CRS patients' sinonasal microbiome; however, this sampling method's reliability has been questioned (Bassiouni et al., 2015; Kim et al., 2015). Our study provided evidence that swab and tissue samples have distinct microbiomes. According to breakaway estimates and observed richness, tissue samples had higher richness than their swab counterparts (Figure 3.8). The Shannon indices, in contrast, indicated that swab samples had a higher richness. This difference could be explained by the fact that the Shannon index also accounts for evenness (Morris et al., 2014), indicating that swab samples have more evenly structured communities. The diversity patterns in our results contradicts the findings from Kim et al. (2015), which found no significant difference between swab and tissue diversity indices. Similar to our study, Bassiouni et al. (2015) found that tissue samples had higher richness than swab samples; however, this relationship was not significant in their study. Our results also showed that the beta diversity of swab and tissue samples was statistically different. The NMDS plot and Adonis2 analysis indicated that tissue samples were dissimilar to swab samples (p-value = 0.009). The difference in richness and dissimilarity between tissue and swab samples could be due to biofilms and mucosa tissue acting as barriers, preventing the swabs from collecting a representative sample (Kim et al., 2015). Another factor is that the tissue samples theoretically include bacteria in the tissue epithelium and sinus

mucosa (Kim et al., 2015). After ESS, patients were encouraged to use nasal irrigation. This irrigation may have impacted the swab samples' composition as it might wash away bacteria on the mucosa that were not contained in a biofilm.

Unlike alpha and beta diversity, tissue and swab samples' taxonomic compositions were similar for most patients (Figure 3.10). Both sample types were composed primarily of the same genera (*Staphylococcus, Dolosigranulum,* and *Corynebacterium)* with some variation in relative abundance. Tissue and swab samples exhibited different compositional patterns in some patients, such as patient 10, where the relative abundance of *Staphylococcus* and *Moraxella* differed between swab and tissue samples. Swab and tissue samples for patient 2 also differed as there was a wider diversity of genera present in the tissue samples. Patient 2's sinonasal microbiome was composed primarily of *Staphylococcus,* which could have formed a biofilm, protecting the other genus observed in the tissue (Hayes et al., 2015; Kim et al., 2015). Interestingly, *Staphylococcus* was typically found in higher relative abundance in swab samples. Bassiouni et al. (2015) also found *Staphylococcus* at a higher relative abundance in swab samples; however, the cause of this difference is unknown.

Overall, this study showed that the microbiome of swab and tissue samples were broadly similar but distinct. It should be noted that although tissue samples appeared to capture a greater bacterial diversity, this type of sample collection is invasive and logistically challenging. Our study, along with Bassiouni et al. (2015) and Kim et al. (2015), collected tissue samples during ESS; however, swab samples can be easily collected in a clinic rather than during surgery. This makes swab samples less invasive and therefore more viable for studies not involving surgery. Future studies may wish to investigate alternative, less invasive methods to collect samples from the sinuses that also collects cells from the epithelium. This type of sampling would allow the biofilms and bacteria present in the epithelium to be sampled, along with the microorganisms present in the nasal mucosa. This is important due to the current hypothesis that biofilms may play a role in the maintenance or pathogenesis of CRS (Fokkens et al., 2012; Hayes et al., 2015; Wood & Douglas, 2010).

4.2 The placebo treatment had better clinical outcomes than the antibiotic treatment

Over the past few years, there has been a push in the medical community to reduce the use of unnecessary antibiotics. In response, several studies have been conducted to investigate

whether post-operative antibiotics improve surgical outcomes in CRS patients undergoing ESS (Albu & Lucaciu, 2010; Jiang et al., 2008; Lehmann et al., 2020; Liang et al., 2011). Albu and Lucaciu (2010) investigated the effects of post-operative antibiotics after ESS on 75 patients in a randomised, double-blind, placebo-control study. This study found that patients who received antibiotics had better endoscopic outcomes during the blood crust healing phase (days 5-12), along with better patient scores for nasal obstruction and drainage (Albu & Lucaciu, 2010). Jiang et al. (2008) investigated the efficacy of the post-operative use of amoxicillin/clavulanate after ESS for 71 patients in a randomised placebo-control study and found that amoxicillin/clavulanate did not improve the short-term outcomes of ESS for endoscopic or symptom scores (Jiang et al., 2008). Lehmann et al. (2020) assessed the impact of post-operative use of amoxicillin/clavulanate after ESS on 77 patients in a randomised, double-blind, placebo-control, noninferiority trial. No significant difference in the quality of life, endoscopic scores, or infection rate were observed, but the authors did observe a higher percentage of side effects in patients who received antibiotics (Lehmann et al., 2020). Liang et al. (2011) examined the efficacy of Chinese herbal medicine, amoxicillin, and a placebo, on 97 patients in a randomised, double-blind, placebo-control study. This study observed no benefit for patients' symptoms or endoscopic scores when using Chinese herbal medicine or amoxicillin, as these treatments recorded similar results to the placebo group (Liang et al., 2011). Corroborating these earlier studies, our results provided additional clinical evidence to show that there is no significant difference in short-term surgical outcomes between patients who receive a placebo or antibiotics.

In our study, all but one patient reported improved symptoms according to the pre-operative and three-month post-operative SNOT-22 scores (Figure 3.1). The difference between the SNOT-22 scores pre- and post-operatively was significant (p-value = 0.0002). The radiological and endoscopic scores also showed an improvement in the sinuses' appearance after the surgery (Figure 3.3). Our results were similar to previous research, indicating that ESS successfully treated CRS short-term for most patients (Jain et al., 2018; Terris & Davidson, 1994).

Interestingly, although most patients show a difference between the pre- and post-operative clinical results, our results showed no significant difference in the clinical outcomes between antibiotic and placebo treatment groups. There was a weak trend that may indicate better SNOT-22 scores after ESS for the placebo treatment (Figure 3.2), noting that our study had a modest sample size and was statistically underpowered. Both the radiological and endoscopic scores for patients receiving antibiotics or placebos were similar. These findings

corroborate Jiang et al. (2008), Lehmann et al. (2020), and Liang et al. (2011), which all found no significant difference between antibiotic and placebo treatment for CRS patients undergoing ESS. In contrast, Albu and Lucaciu (2010) found that antibiotics were associated with better surgical outcomes, specifically for discharge and nasal obstruction symptoms, and endoscopic score results.

Although this study did not find any significant difference in clinical outcomes between treatment groups , there was a difference regarding patients' reported side effects (Table 3-2). Half of the patients who received antibiotics reported side effects, while one patient who received the placebo medication reported side effects. This side effect appeared to be due to this patient having a throat infection (unlikely to be related to ESS). This increase in reported side effects corroborates results in Lehmann et al. (2020), which also showed a higher percentage of patients receiving antibiotics reporting side effects. The increase in side effects suffered by patients receiving antibiotics should be considered when determining if post-operative antibiotics should be encouraged. Previous studies, except Lehmann et al. (2020), did not consider the side effects (Albu & Lucaciu, 2010; Jiang et al., 2008; Liang et al., 2011).

4.3 ESS surgery alters the sinonasal microbiome over time

The bacterial communities examined in this study were composed primarily of *Staphylococcus*, *Dolosigranulum*, and *Corynebacterium* (Figure 3.29, Figure 3.30, and Figure 3.31). *Staphylococcus* and *Corynebacterium* were identified in previous studies as the dominant genera in the sinonasal microbiome, while *Dolosigranulum* is only identified in some studies (Cope et al., 2017; Hoggard, Biswas, et al., 2017; Jain et al., 2017). Although the reported bacterial compositions in our study are similar to previous research, our data showed a less diverse microbiome for most samples (Cope et al., 2017; Hoggard, Biswas, et al., 2017; Jain et al., 2017). Some patients in previous studies had a similar diversity to this study, but the inter-individual variation for the microbial composition is more evident in previous research (Hoggard, Biswas, et al., 2017; Jain et al., 2018). The phylum-level composition was similar to previous studies [data not shown] (Cope et al., 2017; Hoggard, Biswas, et al., 2017; Jain et al., 2017). This difference in taxonomic composition may have been due to our modest sample size or differences in the PCR primers used in our study. Cope et al. (2017) and our study sequenced the V4 region of the 16S rRNA gene, whereas Jain et al. (2017) and Hoggard, Biswas, et al. (2017) sequenced the V3-V4 regions of the 16S rRNA gene.

There was no statistically significant difference for alpha and beta diversity between treatments (Figure 3.21 and Figure 3.22). These results indicated that the antibiotics did not significantly impact the microbial diversity. Interestingly, although there was no statistical difference between the treatment groups after surgery (T2 and T3), Adonis2 analysis showed a significant difference between the two treatment groups prior to surgery and medication (T1) (*p*-value = 0.008). The difference in alpha diversity between the treatment groups at T1, however, was not significant. At T1, placebo samples had a higher relative abundance of *Staphylococcus*, whereas the antibiotic samples had a higher abundance of *Corynebacterium* (Figure 3.29). This shift in diversity after T1 is most likely due to the surgery itself rather than any post-operative medication since there was no statistically meaningful difference in beta diversity between treatments at T2 and T3. Intravenous antibiotics administered during the ESS in all patients may account for the observed change in diversity. A previous study investigating the post-operative microbiome of CRS patients indicated that post-operative antibiotics were ineffective at eliminating microbes in the sinonasal microbiome as the local flora were still present in the microbiome of patients treated with post-operative antibiotics (Hauser et al., 2016).

Both treatments showed an increase in alpha diversity over time (Figure 3.32 and Figure 3.34). The alpha diversity of the placebo treatment showed a significant difference between the T1 and T3 time points (*p*-value = 0.046). In contrast, the antibiotic treatment did not show a significant relationship (*p*-value = 0.217). This suggests that the placebo treatment had a more significant increase in richness compared to the antibiotic treatment. Previous studies found that ESS was associated with an increase in richness (Hauser et al., 2016; Jain et al., 2017), but it is unknown whether this increase in diversity is associated with improved clinical outcomes. However, evidence generally suggests that a more diverse microbiome is associated with better health (Pflughoeft & Versalovic, 2012; Ramakrishnan et al., 2016). This suggests that ESS may help improve clinical outcomes through facilitating a more diverse microbiome; however, the mechanics behind this increase in diversity is unknown. Our findings indicate that antibiotics may inhibit an increase in diversity and that patients who received the placebo had better outcomes on the basis of sinonasal microbial community richness.

The taxonomic composition for both treatments showed an increase in *Staphylococcus* and a decrease in *Corynebacterium* in most samples after the surgery (Figure 3.29, Figure 3.30, and Figure 3.31). This trend has previously been reported by Jain et al. (2017). Previous research linked *S. aureus* to an increased risk in revision surgery and a more recalcitrant disease (Cleland et al., 2013). The increase in the relative abundance of *Staphylococcus* did not

significantly link to worse surgical outcomes in Jain et al. (2017) and our study. It should be noted, however, that the one patient who had worse clinical outcomes (patient 4) also showed a significant increase of *Staphylococcus* at the T3. *Corynebacterium,* which decreased in the T3 samples, is associated with a diseased state and worse symptom scores (Anderson et al., 2016; Jain et al., 2017). This decrease in *Corynebacterium* could be linked to improved clinical outcomes in this study; however, further research into the relationship between clinical outcomes and these genera is needed before conclusive statements can be made.

Our study provides evidence that ESS can change patients' microbial composition and may lead to an increase in richness. The exact reason for these changes is unknown; however, antibiotics administered during ESS or sinus structure may play a role. This study found no significant difference between patients who did/did not receive antibiotics, so post-operative antibiotics use is unlikely to play a significant role in changing the microbiome. It should be noted that there was a general trend of increased richness in the placebo group compared to the antibiotic group (Figure 3.23, Figure 3.24, and Figure 3.25); however, our study was underpowered due to the modest sample size. Other factors that may account for this change in the sinonasal microbial composition include physical change in the sinus structure and associated increased airflow. Increased airflow may lead to a change in the nasal conditions leading to a microbiome change. Our findings potentially indicate that it is more beneficial not to have post-operative antibiotics.

4.4 Relationship between clinical variables and the sinonasal microbiome

Asthma appeared to affect the pre-ESS sinonasal microbial composition (Figure 3.14). The alpha diversity in swab samples for patients with/without asthma was significantly different (p-value = 0.026), and Adonis2 analysis showed notable differences for both tissue and swab samples (p-value = 0.005 and 0.056, respectively). *Corynebacterium* or *Dolosigranulum* dominated the sinonasal microbiome of patients without asthma, whereas *Staphylococcus* appeared associated with asthma (Figure 3.14 and Figure 3.15). Asthma has been shown to influence the lung microbiome (Mahdavinia et al., 2016) and has previously been associated with the variability of the microbiome in CRS patients undergoing ESS (Jain et al., 2017). Analysis of CRS patients with/without asthma has shown a significant difference in the sinonasal microbial composition (Ramakrishnan et al., 2015). Asthma is a disease

characterised by chronic inflammation of the respiratory system (Mahdavinia et al., 2016). This inflammatory response may be associated with the microbiome, which may explain some differences observed between the microbiome of patients with/without asthma (Mahdavinia et al., 2016). Although this disease may influence the microbiome, it is unknown what impact this has on clinical outcomes, and due to our modest sample size, this could not be investigated in greater detail.

Nasal polyps were also investigated; however, they were not found to impact the sinonasal microbiome significantly. Hauser et al. (2016) and Ramakrishnan et al. (2015) also found that nasal polyps' presence did not significantly correlate with microbial community composition. This result was interesting since there is some evidence that CRS with/without nasal polyps are different CRS subtypes (Yang et al., 2015), and there has been evidence suggesting that CRSwNP has a different inflammatory response (Yang et al., 2015). Nevertheless, our results indicate that the microbiome for patients with/without nasal polyps were similar in both tissue and swab samples.

Both asthma and nasal polyps are associated with inflammatory conditions but have different aetiology. Asthma was the only recorded clinical condition to significantly correlate with differences in the microbial communities in our study; this may be due to CRSwNP/CRSsNP having some overlapping features (Lam et al., 2015; Yang et al., 2015). Further analysis of the effects of these conditions on the microbiome and the effect on the clinical outcomes of ESS should be investigated using a more targeted sampling design and large sample sizes.

4.5 Limitation of this study

There were some limitations to our study. First, our study is preliminary and consequently has a modest sample size. This means that there is very limited statistical power for many of the analyses. A large study is needed to strengthen and further investigate the observations made in this study. Patients in our study were not *a priori* separated into groups according to their clinical state, i.e., CRSwNP, asthma, etc. Although these clinical factors may influence the microbiome, as seen in asthma patients, they were not specifically targeted by our study design, and our observations were limited by small sample sizes. Another limitation was inconsistent timing of the T3 samples due to the COVID-19 lockdown, which led to some patients having their T3 samples collected after six months, compared with six weeks for other patients. This may have impacted taxonomic composition and alpha diversity since some

patients had longer for their sinonasal microbiome to recover from the surgery or antibiotics. Finally, this study is only a short-term study and does not account for revision surgery, nor does it account for the risk of antibiotic-induced dysbiosis worsening the CRS overtime.

Chapter Five: Conclusions and Future Directions

The aim of this study was to investigate the effects of post-operative antibiotics on the sinonasal microbiome and clinical outcomes in CRS patients undergoing ESS. Our study also compared the microbial composition of swab and tissue samples collected from the sinuses to determine whether swab samples had similar microbial compositions to tissue samples. The findings of our study revealed that tissue and swab samples had distinct but broadly similar sinonasal microbiomes. Our study found no significant difference between patients who received the antibiotic treatment and those who received the placebo. The clinical results (SNOT-22 and LMS/MLMES) showed no significant difference between the treatments before or after ESS; however, patients who received the antibiotic treatment had a higher risk of side effects. The placebo treatment also showed a lower SNOT-22 and MLMES on average; however, this difference was not statistically significant. There was no significant difference in the post-ESS sinonasal microbiome of antibiotic and placebo treatment groups. The results of our study found that the alpha diversity significantly increased over time for placebo treatments, while the beta diversity and composition for both treatments did not significantly differ across the T2 and T3 samples. The T1 samples showed significant differences between the treatment groups according to the beta diversity analyses. Overall, our results showed that, in the short term, there is no significant clinical or sinonasal microbial difference between patients who received placebo and antibiotics. This may indicate that there is no demonstrable need for post-operative antibiotics unless the patient shows symptoms of post-operative infection. Furthermore, post-operative antibiotics may be associated with negative outcomes such as reduced alpha diversity and increased risk of side effects. It should be noted that our study is underpowered due to the modest sample size, and a more extensive study could expand on the hypothesis that antibiotics have a detrimental impact on CRS patients recovering from ESS.

A more extensive study could account for clinical variables such as asthma, which our study was unable to do due to its modest sample size. A larger study could further analyse the relationship between the treatment groups and expand on the difference observed in alpha diversity between the treatment groups. Along with further antibiotic trials, another study investigating sampling techniques could give further insight into what sampling method is ideal for sinonasal microbiomes studies and allow for standardised protocols in this field. A study comparing cytology brush samples to tissue and swab samples should be considered as the use of a cytology brush as a sampling method may allow for the first layers of epithelium and

biofilms to be collected. A cytology brush might exhibit a similar microbial ecology to a tissue sample, however, be less invasive to collect as it would not require surgery. Finding the best sampling method for sinonasal microbiome studies would allow for more comparable results between the different studies and reduces the variables needed to be considered when making conclusions about the results of a study.

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Appendix

6.1 Supplementary material for chapter two

Sample	input	filtered	denoisedF	denoisedR	merged	nonchim
001_1_TR	147193	121964	118219	118262	104249	76219
001_1_TL	98510	79999	79312	79336	75927	37540
001_1_SR	100213	81094	80442	80453	75405	44612
001_1_SL	91844	78987	78299	78379	74322	40465
001_2_SR	28753	17572	16934	17102	14085	9829
001_2_SL	190668	161898	156751	156986	142743	103714
001_3_SR	125660	101370	99939	100092	93290	53354
001_3_SL	94821	75051	73289	73597	68260	38744
002_1_TR	197362	166213	162302	161904	146674	109135
002_1_TL	178750	153213	148804	148484	136189	100943
002_1_SR	74181	57587	54994	55251	48393	31345
002_1_SL	175356	150326	148296	148401	139320	96164
002_2_SR	108092	91497	90004	90513	83010	58713
002_2_SL	95656	79791	79323	79511	78112	37784
002_3_SR	148222	126083	121565	120551	108428	78515
002_3_SL	113552	91490	86496	85749	74610	51869
003_1_TR	95574	76248	71793	72564	61580	47561
003_1_TL	87509	71974	70740	70784	65245	42216
003_1_SR	51436	38426	37022	37160	34205	22216
003_1_SL	76805	63417	62573	62629	58205	36213
003_2_SR	79321	64456	63738	63929	61106	38253
003_2_SL	90661	75407	74216	74369	71399	44739
003_3_SR	82141	68174	67688	67740	65159	39691
003_3_SL	14079	11710	11511	11606	10754	7070
004_1_TR	142195	119638	119367	119347	110261	79110
004_1_TL	139693	118383	116444	116855	107554	66886
004_1_SR	111708	89060	88792	88911	84818	46563
004_1_SL	96159	81351	80987	81112	78132	41019
004_2_SR	74105	61914	61383	61335	56371	36994
004_2_SL	98658	85945	85581	85524	83698	49335
004_3_SR	93786	78211	77002	76767	72997	45702
004_3_SL	99129	81434	80581	80572	77274	43263
005_1_TR	112161	94757	94387	94391	91429	54658
005_1_TL	195958	167838	166750	166801	157484	90476
005_1_SR	100269	82123	81730	81961	79516	37978
005_1_SL	102661	86580	86059	86306	82308	27933
005_2_SR	167985	139476	138765	138717	131340	85031
005_2_SL	206311	177492	174453	174970	159422	112287
005_3_SR	128356	112320	111668	111703	107026	58281
005_3_SL	85951	74353	74039	73992	72126	34942

006_1_TR	133400	108827	106485	106664	94691	65563
006_1_TL	187282	161163	159634	160036	146030	104513
006_1_SR	137091	115429	113769	113862	103072	74535
006_1_SL	254034	227155	226033	226370	211719	153792
006_3_SR	132160	112504	111220	111380	105308	68789
006_3_SL	194840	162541	161589	161808	154332	81971
007_1_TR	192886	167060	165748	165934	152882	106611
007_1_TL	147137	126518	125688	125685	116805	69201
007_1_SR	90764	73957	72821	73221	66640	46866
007_1_SL	115257	92665	90693	91180	82072	44007
007_2_SR	63369	48218	46886	47128	42075	26774
007_2_SL	91438	72118	70349	70065	60945	38134
007_3_SR	96842	78692	77254	77522	70457	47113
007_3_SL	86658	69640	68831	68979	63012	37043
008_1_TR	130932	111669	108363	107543	94686	70637
008_1_TL	85539	72088	70610	70632	62907	40360
008_1_SR	125466	104618	103363	103546	95098	58590
008_1_SL	114442	98231	97069	97218	91633	54754
008_2_SR	84003	67844	64420	64684	57418	36515
008_2_SL	95737	80516	78130	78188	69560	46912
008_3_SR	145689	123169	122267	122192	114073	77687
008_3_SL	106000	86668	85383	85363	78346	45433
009_1_TR	181347	158665	155890	155988	143595	104381
009_1_TL	111407	97301	94063	94352	85805	59313
009_1_SR	97406	73628	72554	72899	68011	36111
009_1_SL	110272	95426	93638	94096	87255	57013
009_2_SR	81434	67966	65086	65387	57251	40775
009_2_SL	98177	85221	82970	83296	76059	51035
009_3_SR	95228	83569	82348	82441	77355	45970
009_3_SL	85371	75828	74813	74946	72366	36636
010_1_TR	95371	83141	80858	81061	73663	52180
010_1_TL	77351	68232	66586	67080	60045	43916
010_1_SR	137523	117218	116468	116471	109747	73000
010_1_SL	123311	108082	107568	107437	101363	58570
010_2_SR	117443	93396	91735	91868	82870	53245
010_2_SL	106463	90153	88663	88925	80901	54561
010_3_SR	4155	3418	3390	3389	3360	2103
010_3_SL	1866	719	629	646	109	87
011_1_TR	712	256	213	217	212	212
011_1_TL	1199	232	156	163	147	147
011_1_SR	190691	165676	162723	162983	150706	112825
011_1_SL	145283	122453	121462	121168	115073	73033
011_2_SR	110669	90533	90322	90270	86564	55378
011_2_SL	24122	20312	20196	20243	19263	11252
011_3_SR	315	166	146	116	22	22
011_3_SL	293	138	113	90	22	0

012_1_TR	263	142	117	100	14	0
012_1_TL	209	107	92	63	0	0
012_1_SR	112156	89605	88888	89207	81712	54050
012_1_SL	167093	130062	129229	129469	111933	64232
012_2_SR	62172	52235	50078	50358	43459	32006
012_2_SL	915	235	189	177	0	0
012_3_SR	193	66	48	42	0	0
012_3_SL	207	72	44	37	0	0
C_21/11/19	122987	87975	87515	87259	82443	39596
C_05/12/19	185622	146128	145493	145258	140047	70983
C_06/12/19	65499	40242	39958	39758	37116	18641
C_19/12/19	87365	62376	61940	61952	59132	33397
C_21/12/19	124806	91657	91072	90623	85606	38653
C_11/01/20	199524	162303	161192	161756	155266	91592
C_24/01/20	128095	101970	101648	101230	98591	57475
C_12/03/20	57825	42462	42368	42080	40800	17508
C_21/03/20	50949	34535	34324	33721	32117	20244
C_05/06/20	27763	21286	20778	20767	19693	11279
C_12/06/20	88069	59414	59153	59138	56991	36855
C_26/06/20	127127	101703	100999	100390	94541	45080
C_10/07/20	146023	113234	112805	112357	108538	56618
C_17/07/20	124475	102073	101773	101533	98515	56803
C_31/07/20	110563	90412	89916	89426	83793	40747
C_14/08/20	66805	53140	52905	52491	49965	26486
C_18/09/20	130943	99380	99030	98699	95774	50917
C_02/10/20	108429	84298	83634	83661	78901	43203
C_09/10/20	75181	54272	54072	53931	52650	24159
C_16/10/20	125426	98282	97740	97572	93072	46562
C_23/10/20	134929	106389	105957	105117	100842	52878
C_11/11/20	161103	130617	128124	128221	114622	64798
PCR_pos	145425	53697	52476	52651	51256	35150
PCR_neg	44443	32617	31967	32007	22892	7068

Table 6-1: Sequencing data and DADA2 analysis for each sample.

6.2 Supplementary material for chapter three

Sample	Observed Richness	Shannon	Breakaway Estimates
001_1_TR	299	4.54	326.07
001_1_TL	262	4.71	274.09
001_1_SR	214	4.77	215.00
001_1_SL	187	4.68	188.07
001_2_SL	362	4.91	417.12
001_3_SR	302	5.07	319.76
001_3_SL	296	4.97	302.22
002_1_TR	287	4.32	345.09
002_1_TL	230	4.08	291.20
002_1_SR	301	5.14	307.35
002_1_SL	198	4.59	227.26
002_2_SR	178	4.78	178.01
002_2_SL	122	4.41	122.00
002_3_SR	345	4.49	390.07
002_3_SL	383	5.08	405.13
003_1_TR	188	4.17	207.11
003_1_TL	225	4.92	234.13
003_1_SL	236	4.90	260.09
003_2_SR	181	4.36	182.03
003_2_SL	187	4.38	192.08
003_3_SR	146	4.49	152.46
004_1_TR	148	3.76	152.00
004_1_TL	202	4.81	215.05
004_1_SR	171	4.74	172.00
004_1_SL	189	4.78	190.01
004_2_SR	202	4.75	203.02
004_2_SL	138	3.93	139.00
004_3_SR	227	4.73	231.03
004_3_SL	235	4.96	243.06
005_1_TR	136	4.33	139.02
005_1_TL	288	4.96	296.15
005_1_SR	171	4.64	171.00
005_1_SL	192	5.01	192.25
005_2_SR	205	4.70	214.02
005_2_SL	271	4.42	323.94
005_3_SR	184	4.82	189.03
005_3_SL	219	4.95	220.25
006_1_TR	292	4.51	310.09
006_1_TL	215	4.12	225.03
006_1_SR	191	3.92	204.06
006_1_SL	138	3.61	155.02
006_3_SR	218	4.47	223.04
006_3_SL	347	5.28	350.01

007_1_TR	320	4.68	329.03
007_1_TL	226	4.33	229.00
007_1_SR	219	4.55	230.70
007_1_SL	236	4.69	238.04
007_2_SR	269	5.20	267.01
007_2_SL	303	5.30	309.55
007_3_SR	236	4.77	239.02
007_3_SL	249	5.04	249.00
008_1_TR	215	4.19	231.09
008_1_TL	173	4.65	174.01
008_1_SR	269	4.93	273.01
008_1_SL	170	4.66	178.04
008_2_SR	246	5.03	253.03
008_2_SL	374	5.27	386.10
008_3_SR	276	4.62	289.07
008_3_SL	324	5.27	330.02
009_1_TR	241	4.15	259.08
009_1_TL	217	4.57	236.11
009_1_SR	200	4.63	203.01
009_1_SL	217	4.65	223.01
009_2_SR	252	4.84	259.06
009_2_SL	245	4.97	254.05
009_3_SR	220	4.76	227.06
009_3_SL	160	4.65	162.03
010_1_TR	274	4.95	294.10
010_1_TL	126	4.12	132.05
010_1_SR	213	4.69	222.04
010_1_SL	184	4.53	190.25
010_2_SR	293	5.04	297.00
010_2_SL	225	4.76	232.07

Table 6-2: Alpha diversity data for each sample. Observed richness and Shannon indices were calculated using rarefied data while breakaway estimates were made using unrarefied data.