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ORIGINAL CONTRIBUTION

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A pilot randomised controlled trial of oral doxycycline after endoscopic sinus surgery and its effects on the sinonasal microbiome*

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Abstract

Background: Oral antibiotics are commonly prescribed after endoscopic sinus surgery (ESS) despite minimal clinical data supporting this practice. We aim to assess the effect of post-ESS doxycycline on clinical outcomes and on the diversity and composition of the sinonasal microbiome.

Methods: Samples from the middle meatus were collected from twelve patients undergoing ESS to treat chronic rhinosinusitis. Patients were double-blind randomised to receive either oral doxycycline or placebo in the post-operative period. Further samples were collected at two weeks and three months post-operatively. The sinonasal microbiome was characterized using 16S ribosomal RNA (rRNA) gene amplicon sequencing. SNOT-22 scores, Lund Mackay scores, and Modified Lund Mackay Endoscopic Scores (MLMES) were collected.

Results: After ESS, bacterial diversity increased while SNOT-22 score decreased for both treatments. Microbiome composition diverged between treatments, and random forest analysis identified nine taxa that may distinguish treatment groups. There was no significant difference in SNOT-22 score, 3-month MLMES or bacterial diversity between the placebo and doxycycline groups. The trends for all of these measures favour placebo.

Conclusion: In this pilot study, we detected no significant difference between placebo and antibiotic treatments in clinical outcome. As patient symptoms improved after ESS, we detected a concurrent increase in the diversity of the sinonasal microbiome. Our data highlight the need for and facilitate the design of future larger studies to explore the relationship between prophylactic antibiotics and post-ESS recovery.

Key words: endoscopic sinus surgery, bacteriology, evidence-based medicine, post-operative, therapeutics

Introduction

Our current understanding of the pathogenesis of chronic rhinosinusitis (CRS) and the optimal treatment for this disease is inadequate. Previous studies have suggested that the sinonasal microbiome plays a role in the development or maintenance of this disease[1]. The current research suggests bacterial dysbiosis (deviations from the typical microbial community) and a decrease in bacterial diversity are associated with this disease[2-4]. There is currently level 1 evidence demonstrating the efficacy of macrolide and tetracycline antibiotics for the treatment of CRS[5,6]. However, it is noted that both of these classes of antibiotics have anti-inflammatory properties as well as antibacterial properties[7,8]. It has been suggested that antibiotic exposure may be harmful in the long term, increasing the risk of bacterial dysbiosis and reducing bacterial diversity[9]. Other questions relating to the microbiology of CRS remain unanswered, including

what impact oral antibiotics have on the sinonasal microbiome[10] and what the optimal collection method for sinonasal samples is[11, 12].

There is increasing evidence demonstrating the efficacy of endoscopic sinus surgery (ESS) in CRS management[13]. Staphylococcus aureus was found to be associated with worse post-operative outcomes in patients undergoing ESS[14], suggesting that the use of peri-operative anti-staphylococcal antibiotic may be beneficial. ESS, however, falls under the category of "clean-contaminated" surgery where there is generally considered to be no benefit in the use of post-operative antibiotics for the reduction in risk of surgical site infection[15]. A recent meta-analysis concluded that there is a lack of high-quality evidence to guide the decision around the use of antibiotics after ESS[16]. A further randomised trial that did not feature in the meta-analysis concluded that patients receiving placebo likely achieved equivalent results to patients provided with co-amoxiclav after ESS[17]. Significantly, however, in a 2015 study, 73.1% of surgeons reported routine use of antibiotics after ESS[18]. This is important given the frequency with which ESS is performed and the increasing need for antibiotic stewardship[19].

Given the lack of certainty around the need for antibiotics after ESS, the potential beneficial role of anti-staphylococcal treatment, and the proven efficacy of doxycycline in the treatment of CRS[5,20], this study aims to investigate the use of oral doxycycline after ESS. Particular focus is on the impact of doxycycline on overall clinical recovery from surgery and the impact doxycycline has on the sinonasal microbiome through the post-operative recovery period. This is intended as a pilot study to facilitate a larger, appropriately powered study allowing us to more definitively answer these questions. In so doing, we further describe the efficacy of ESS in treating CRS, the microflora of CRS both before and after ESS, and allow us to further investigate the optimal sampling method for sinonasal microbiome samples.

Materials and methods

Patients and sample collection

This study was a randomised, double-blind, placebo-controlled trial comparing prophylactic oral doxycycline with placebo after ESS in CRS patients. Ethical approval was gained from the New Zealand Health and Disability Ethics Committee (reference number: 19/NTA/64/AM01, Universal Trial Number: U1111-1229-8735). The study was registered with the Australian New Zealand Clinical Trials Registry (reference: ACTRN12619000505101). Written informed consent was gained from all participants.

Twelve patients fulfilling the EPOS criteria for CRS[1] undergoing ESS after failing medical treatment were prospectively recruited. Patients underwent a complete sphenoethmoidectomy and frontal recess dissection. Exclusion criteria included the following: prior ESS, underlying condition predisposing to CRS (e.g., vasculitis, cystic fibrosis, aspirin-exacerbated respiratory disease), unilateral CRS, Lund-Mackay[21] score (LMS) less than 10/24, any antibiotic usage in the 12 weeks prior to surgery, allergy to doxycycline, confirmed or possible pregnancy. On the day of surgery, demographic and clinical data was collected, including age, gender, presence or absence of nasal polyps, prior diagnosis of asthma, pre-operative Lund-Mackay score, SNOT-22 score, and completion of any adjunct procedures (e.g., septoplasty or inferior turbinoplasties) (Table 1).

During ESS, immediately after the induction of anaesthetic and before administration of any antibiotic prophylaxis, swab samples (Aluminium Applicator Rayon-Tipped Sterile Swab, Fort Richard Laboratories, Auckland, New Zealand) and tissue samples were collected from both the right and left middle meatus. Patients were then administered intravenous cefazolin. Swab and tissue samples were immediately placed into DNA/RNA Shield (catalogue no. R1100-250, Ngaio Diagnostics, Nelson) and transferred to the Thermophile Research Unit at the University of Waikato, where samples were stored at 4°C until processed within four days of collection.

Patients were randomised into two treatment groups using Microsoft Excel random number generation in a double-blind fashion. The first treatment group received 100 mg of oral doxycycline twice daily for 28 days, while the second treatment group received a matching placebo produced by a compounding pharmacy. Patients received the same routine care after surgery, including 20 mg prednisone for ten days, analgesia, saline nasal spray, high-volume saline lavage and topical corticosteroids in the post-operative period.

Patients underwent clinical review at 2 weeks post-operative and approximately 3 months post-operative, although the exact timing varied for the second check-up due to restrictions associated with COVID-19 lockdowns in New Zealand. For purposes of the analysis, the day of surgery was defined as pre-op, the 2-week post-operative check-up was defined as first post-op, and the 3-month post-operative check-up was defined as second post-op. Follow-up samples and data collected during the post-op check-ups included: SNOT-22 scores (first and second post-op), Modified Lund Mackay Endoscopic Score (MLMES) (22) (second post-op), medication side effects (first and second post-op), and mucus swab from the right and left middle meatus samples (first and second post-op).

Genomic DNA extraction and 16S rRNA gene PCR amplicons sequencing

Total DNA was extracted from tissue and swab samples using

Table 1. Clinical and demographic data collected from patients across 3 months during this study. Patients 1 and 4 did not significantly improve their symptoms after recovering from surgery according to their pre-op and second post-op 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) indicated better surgical outcomes.

Patient	Age	Gender	Adjunct procedure	Nasal Polyps	Asthma	Treatment	SNOT pre-op	SNOT first post-op	SNOT second post-op	LMS pre-op	MLMES second post-op
1	57	M	Septo	CRSsNP	Nil	Doxycycline	64	12	44	10	0
2	26	M	Septo	CRSwNP	Asthma	Placebo	81	14	20	21	45
3	27	F	ITs	CRSsNP	Asthma	Doxycycline	50	57	5	10	2
4	51	M	Nil	CRSwNP	Nil	Doxycycline	43	40	49	20	50
5	59	M	Nil	CRSsNP	Nil	Doxycycline	19	7	13	16	46
6	59	M	Nil	CRSwNP	Nil	Placebo	37	4	5	15	10
7	56	M	Nil	CRSwNP	Asthma	Placebo	65	12	21	14	4
8	45	M	Septo	CRSwNP	Nil	Placebo	62	43	14	14	5
9	30	F	Septo	CRSsNP	Nil	Placebo	46	31	20	12	6
10	34	M	Septo	CRSwNP	Asthma	Doxycycline	39	37	15	11	8
11	37	M	Septo/ITs	CRSsNP	Nil	Placebo	33	47	9	14	0
12	43	F	ITs	CRSwNP	Asthma	Doxycycline	44	32	20	14	2

the ZymoBIOMICS DNA Microprep Kit (catalogue no. D4301, Ngaio Diagnostics, Nelson). Procedural controls (i.e., extraction blanks consisting of only reagents from ZymoBIOMICS kit) were extracted alongside tissue and swab samples. A two-step polymerase chain reaction (PCR) was used to amplify the V4 hypervariable region of the 16S rRNA gene using the Earth Microbiome Project primers [supplementary methods][23, 24]. A PCR negative control was created using ultrapure water (catalogue no. 10977015, Thermo Fisher Scientific, Auckland), which was processed along with the samples. Non-target amplification products of the human mitochondrial DNA were removed 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 [supplementary methods]. The 16S rRNA gene PCR amplicons underwent barcode addition using the Quick-16S NGS Library Prep Kit (catalogue no. D6400, Ngaio Diagnostics, Nelson) following the non-quantitative protocol. Barcoded PCR amplicons were pooled, and a normalised DNA library was created using SequelPrep Normalization Plate Kit (catalogue no. A1051001, Thermo Fisher Scientific, Auckland). The library was sent to GENEWIZ in Suzhou, China and sequenced using an Illumina MiSeq DNA sequencer in a 2 x 250 bp paired-end configuration using the MiSeq Reagent Kit v2. Sequences are deposited under the accession number PRJEB46412

Bioinformatics and statistical analysis

Bioinformatic and statistical analyses are described in detail in the supplementary methods. Briefly, the raw FASTQ files were imported into R (v4.0.2) using 'DADA2' package (v.1.16.0)[25] for amplicon sequence variant (ASV) inference. Taxonomy was assigned with the native implementation of the naive Bayesian classifier and a DADA2-formatted reference database for the SILVA v138 database[26].

The taxonomic data and ASV data were imported in R with phyloseq (v1.32.0)[27]. Contaminant taxa were identified with the decontam pipeline (v1.8.0)[28] (Supplementary Figure 1) using procedural control samples and removed from the dataset (Supplementary Figure 2 and Supplementary Table 1). Bacterial community composition was transformed with a centred log-ratio transformation. Euclidean distances were calculated between samples and a Principal Coordinates Analysis (PCoA) was performed. After confirming that samples did not differ significantly in their dispersion using an analysis of multivariate homogeneity (PERMDISP), a PERMANOVA was performed to check for differences over time and between treatments, both performed in vegan (v2.5-6)[29]. A procrustes analysis, also performed in vegan, was used to correlate datasets obtained by tissue or swab sampling. A random forest analysis was conducted to model ASV potentially associated with each treatment group (random forest (v4.6-14)[30]). Pearson correlation coefficients were employed to assess the correlation between the centred log-ratio transformed ASV abundance and time. ggplot2 (v3.3.3)[31] was used to visualise the data.

Clinical data analysis was conducted in Microsoft Excel. Pre-op variables were compared to the second post-op visit using a two-tailed t-test. Paired t-test was employed to compare across timepoints, while unpaired t-tests were used to compare treatments.

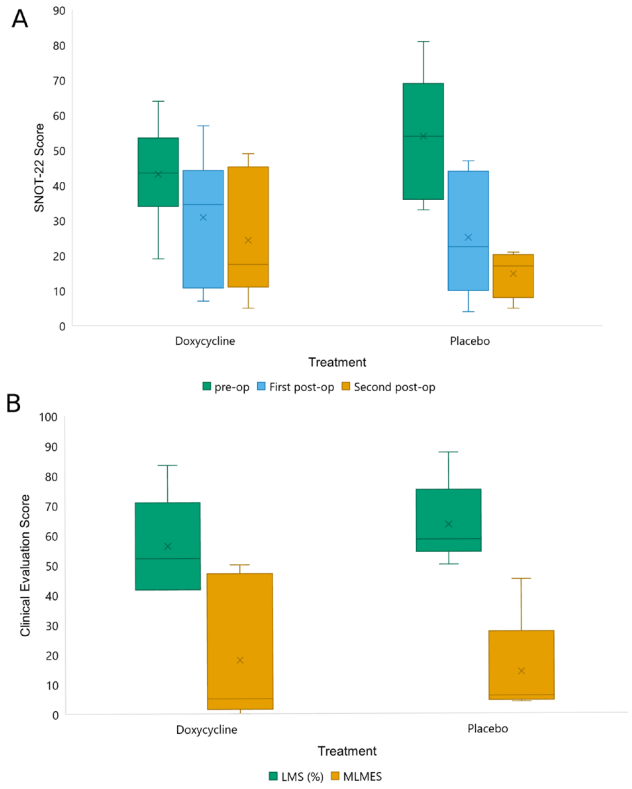

Figure 1. (a) SNOT-22 scores at pre-op, first post-op, and second post-op for each patient sorted according to treatment group. (b) Post-op [LMS] and second post-op [MLMES] clinical evaluation scores for each patient, sorted according to treatment group. LMS data transformed into a percentage.

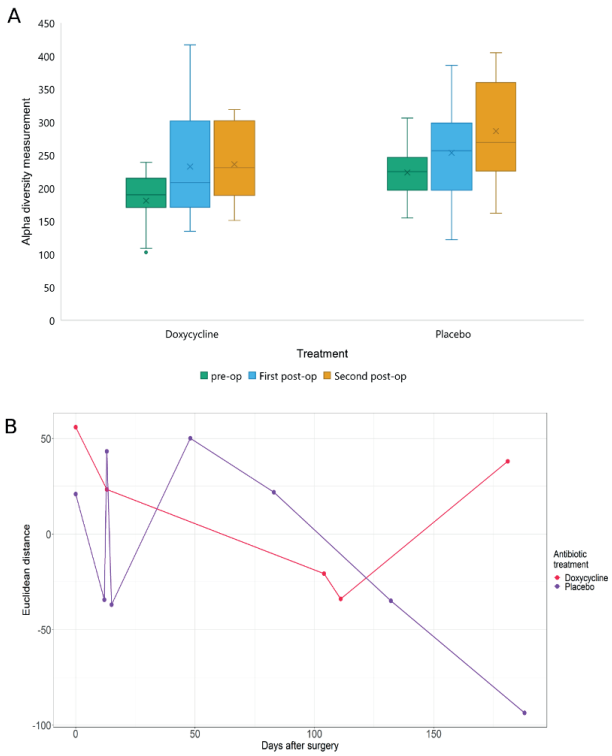

Figure 2. (a) Difference over time in bacterial diversity for patients receiving doxycycline or placebo treatment. Bacterial diversity was calculated using the number of observed ASVs. A paired t-test comparing diversity at each timepoint for each treatment ranged from p-value = 0.637 to 0.05 and was generally not considered significant. A t-test analysis [unpaired] comparing the diversity between treatments at the post-operative timepoints (p-value = 0.604 and 0.156) and pre-op timepoint (p-value = 0.035) found that there was no significant difference after ESS between treatment groups.
(b) Difference over time in bacterial community composition after ESS for patients receiving doxycycline or placebo treatment. The euclidean distances were obtained on centered log ratio transformed bacterial community abundance data obtained on the procedure day (pre-op), on the first post-op visit, and on the second post-op visit (PERMANOVA, F= 1.505; p-value = 0.016; variance at the post-op timepoints between treatment groups).

Results

General description of data

A total of 94 samples were collected between November 2019 and November 2020. Each patient had two swab and two tissue samples collected pre-operatively at the time of ESS (pre-op), two swab samples collected at the first post-op check-up, 13 days after the surgery (except for patient 2 samples, which were collected after 15 days), and two swab samples collected at the second post-op check-up, after an average of 105 days (41-188 days).

The sequencing data underwent de-noising and quality filtering using the DADA2 pipeline. This process yielded 11,340 amplicon sequence variants (ASVs, broadly equivalent to bacterial species) across 94 samples, 22 extraction blanks, and one PCR negative control. Quality filtering reduced the number of samples with useful sequencing data down to 78 samples [16 samples removed, Supplementary Table 1] composed of 3,272 ASVs. Left and right samples from the same patient at the same timepoint were shown to have similar bacterial community composition and were treated as replicates (Supplementary Figures 3 and 4).

Clinical and demographic data for included patients are shown in Table 1. Patient 11 was excluded from the dataset throughout this analysis since this patient received Augmentin as a treatment for another illness during this study (Table 2).

Clinical outcomes

Both treatment groups showed improved quality of life based on the patient SNOT-22 scores (pre-op v second post-op, p-value = 0.0002) (Figure 1a and Table 1). When the pre-op and second post-op SNOT-22 scores were compared, the mean reduction in SNOT-22 score was 18.8 for the doxycycline group compared to

Table 2. Side effects reported by patients across the study. Three patients undergoing doxycycline treatment reported side-effects while no patient undergoing the placebo treatment reported any side effects. *Patient 11 was removed from analysis as they were given augmentin by their GP to treat another disease.

Patient	Treatment	Side effects
1	Doxycycline	None
3	Doxycycline	vomiting - patient elected to stop study medication at day 11
4	Doxycycline	dizziness, tiredness, diarrhoea
5	Doxycycline	None
10	Doxycycline	None
12	Doxycycline	facial rash - day 1 post-op
2	Placebo	None
6	Placebo	None
7	Placebo	None
8	Placebo	None
9	Placebo	None
11*	Placebo	None

39.2 for the placebo group (p-value = 0.052). Objective clinical data related to disease severity, namely pre-operative LMS and second post-op MLMES, are shown in Figure 1b. The mean pre-op LMS was 13.5 (56%) for the doxycycline group and 15 (63%) for the placebo group (doxycycline v placebo, p-value = 0.753). The mean MLMES at the second post-op was 18 for the doxycycline group and 11.7 for the placebo group (doxycycline v placebo, p-value = 0.466).

Three patients reported side-effects from the medication (Table 2), and they were all receiving doxycycline. No side-effects were reported for the placebo group. Reported side-effects correspond to known side-effects of the medication[32].

Microbiome analysis

Diversity of the bacterial communities was calculated using the observed number of ASVs, which is a widely accepted proxy for delineating bacterial species based on 16S rRNA gene PCR amplicon sequencing (Figure 2a). On average, the placebo group had a higher diversity at all timepoints compared to the doxycycline group; however, this was not significant for the post-operative timepoints (p-values = 0.604 [first post-op] and 0.157 [second post-op]). Within each treatment group, there was no significant difference in the observed diversity across timepoints for the doxycycline treatment (Supplementary Table 2), but a significantly higher diversity was observed between the pre-op and second post-op timepoints for the placebo treatment (p-value = 0.045). There was no significant difference between the remaining timepoint comparisons for the placebo treatment (Supplementary Table 3).

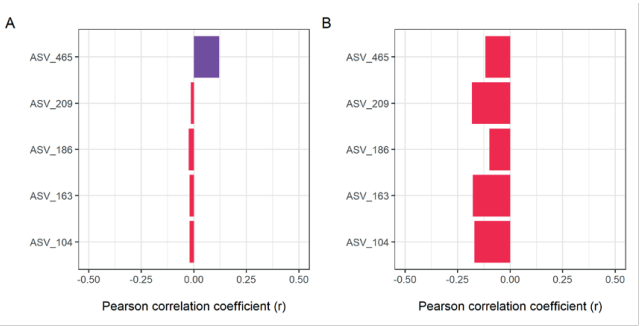

Figure 3. Pearson correlation of Corynebacterium sp. ASVs abundance over time for the (a) antibiotic treatment and the (b) placebo treatment. Correlations were obtained on a centered log ratio transformed biological dataset selected using a random forest model.

The bacterial community composition was analysed using a PCoA based on Euclidean distances, which is an acceptable proxy for identifying differences/similarities in the community composition for different groups. A PCoA analysis where the first PCoA axis was plotted against the days after surgery suggested a difference in the bacterial community composition between the treatment groups overtime (Figure 2b). PERMANOVA analysis showed that the variance at the post-op timepoints differed for treatment groups (F= 1.505; p-value = 0.016). Data dispersions in these groups were not significant (p-values = 0.964 and 0.518 for time and doxycycline treatment, respectively), supporting the assumption that the PERMANOVA results reflected differences in bacterial communities.

A random forest (RF) model was constructed to assess whether any ASVs were associated with a specific treatment (Supplementary Figure 5). Nine ASV were shown to be more indicative of the sinonasal microbiome for the doxycycline group than the placebo group. Five of these ASVs are affiliated with the *Corynebacterium* genus, while the remaining were not classifiable at the genus level but mostly belonged to class *Clostridia*, in the phylum *Firmicutes*. The RF model exhibited an accuracy - area under the receiver operating characteristic curve - of 63% (95% confidence interval = 46-78%). Pearson correlation analysis of *Corynebacterium* sp. ASVs identified by the RF model showed that they decreased in abundance over post-op timepoints, particularly for the placebo treatment group (Figure 3). The only exception was ASV_465, which displayed a positive correlation with time in the doxycycline treatment group. However, the correlations were weak for all of the identified ASVs ($r < 0.2$).

The effect of the specimen collection method was investigated by comparing tissue and swab samples collected during

the surgery (pre-op). Patient 12 was excluded due to the low number of samples for this comparison. Bacterial diversity was calculated using the observed number of ASVs (Supplementary Figure 6). On average, the tissue samples had a significantly higher diversity (246) compared to the swab samples (211) (p-value = 0.0114). The bacterial community composition for the different collection methods was compared using a PCoA based on euclidean distances (Supplementary Figure 7). The PCoA ordination obtained with the swab samples was correlated to the one obtained with the tissue samples (Procrustes analysis r = 0.6), indicating that the collection method had some overlap between the bacterial communities (tissue and swab); however, this was not extensive. The PERMANOVA analysis showed the bacterial community composition to be similar between the collection methods (F = 1.3849, p-value = 0.131).

Discussion

Several studies have investigated whether post-operative antibiotics improve outcomes in CRS patients undergoing ESS but the answer remains unclear[17, 33-35]. In this pilot study, we investigated the use of doxycycline after ESS and the impacts this medication had on the clinical outcomes and sinonasal microbiome. The clinical data (with the exception of side-effects) were not statistically different between patients who received doxycycline versus placebo. In contrast, bacterial diversity and composition differed according to treatment groups, suggesting that doxycycline may have a harmful impact on the patients' sinonasal microbiome, post-ESS. It is noted that previous research has shown that a more diverse microbiome is associated with improved health[36, 37].

Clinical outcomes

Our study showed a non-significant trend favouring placebo with less reduction in SNOT-22 scores (p-value = 0.052) and a higher 3-month endoscopic score in the doxycycline group (Figure 1). Acknowledging the small sample size, our data do raise the possibility that doxycycline may result in a worse outcome than placebo. This is especially relevant when taking into account the sinonasal microbiome data. There was also a noted difference between treatment groups in regards to side-effects suffered by patients. The difference suggested by this study is concordant with the potentially deleterious impact of antibiotics on other mucosal sites, including on the gastro-intestinal and urinary tract[38, 39].

Sinonasal microbiome outcomes

A higher bacterial diversity was observed when comparing the pre-op and second post-op samples (Figure 2a). This trend, however, was only statistically significant in the placebo treatment group (p-value = 0.046). Previous observations have indicated that antibiotic therapy reduces bacterial diversity[40], noting that a decrease in bacterial diversity may be associated with CRS[2]. The bacterial community composition also appeared to differ between the treatment groups over time (Figure 2b). Bacterial composition was similar between treatment groups at the pre-op stage but differed over time post-ESS. This difference may be attributed to doxycycline's impact on the patient's microbiome, preventing some bacteria from repopulating the sinuses[38]. As such, we postulate that antibiotics in this context may have a deleterious effect on the sinonasal microbiome and may impact the recovery of the bacterial diversity and community composition in patients.

Random forest analysis provided some insight into whether ASVs could be used to differentiate between the two treatment groups and indicated which bacteria may have been impacted by the treatment mechanisms and potentially play a role in the different responses seen between the treatment groups. Our study found that five ASVs from the Corynebacterium genus (Supplementary Figure 3) were important in differentiating between the treatment groups. Further analysis using Pearson correlation coefficient showed that the Corynebacterium ASV identified in the random forest analysis decreased over time post-ESS. Previous research had found an association between CRS and Corynebacterium, suggesting a potential pathogenic role of this genus in CRS[41-43]. The stronger decrease in potentially pathogenic bacteria in the placebo group also provides some evidence towards doxycycline not having a superior outcome. It should, however, be noted that our results showed a low Pearson correlation coefficient, and that other studies have found Corynebacterium to be associated with healthy patients and this genus may not play a pathogenic role but rather a beneficial role in CRS[3, 44].

Collection methods

This study provided further evidence that tissue and swab samples may have distinct microbiomes[11, 12]. Our results found some similarities between the bacterial community composition when comparing the collection method. We also note a correlation between the ordination of the tissue and swab sample communities (r=0.6), indicating overlap between these communities; however, this was not extensive (Supplementary Figure 6). In contrast to bacterial composition, bacterial diversity differed significantly between collection methods (p-value = 0.0114). On average, tissue samples had higher diversity than swab samples. This difference may be due to tissue samples also representing the bacteria present in biofilms and/or bacteria within the epithelium[11]. Our study indicates that tissue samples capture a greater bacterial diversity, but it should be noted that tissue sample collection is invasive and logistically challenging. Our study did not find a significant difference between the position of the samples (right v left) for bilateral CRS patients (p-value =

0.298), suggesting that the nostril of the sample did not impact the sinonasal microbiome significantly.

Strengths

A particular strength of this study was the molecular assessment that occurred alongside the clinical assessment, as this provided some suggestions as to why antibiotics may generate worse outcomes for the patient. A further strength of this study was that patients were excluded if they had had any antibiotics 12 weeks prior to enrolment to allow the microbiome to recover from the impacts of previously consumed antibiotics[45]. This study has also provided an opportunity to further describe the microbial ecology of CRS in patients at the time of ESS without the influence of antibiotics or prior surgery.

Limitations and further research

Patients in our study were not a priori separated into groups according to their clinical state, e.g., CRSwNP, asthma, etc. Although these clinical factors, as well as host genetics, may influence the microbiome, our study design did not specifically target them, and our observations were limited by small sample sizes. Comparison of our findings to other studies of the CRS microbiome is further complicated since analysis of results can be affected by experimental conditions (e.g. nucleic acid extraction and amplification) and decisions made during data analysis (e.g. quality control, statistical analyses). Another limitation was inconsistent timing for collecting the second post-op samples and clinical data, which varied significantly (41-188 days; average of 105 days) due to the COVID-19 lockdown restrictions. This variation may have impacted taxonomic composition and bacterial 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, single surgeon study. It does not account for revision surgery, nor does it account for the risk of antibiotic-induced dysbiosis worsening the CRS over time.

Conclusion

Our pilot study has found no significant difference between the clinical outcomes for patients who received the placebo and patients who received oral doxycycline. Patients showed improved symptoms in parallel to an increase in bacterial diversity of the sinonasal microbiome. This increase in diversity was shown to be significant in the placebo group, which could indicate a deleterious impact of doxycycline on the sinonasal microbiome. Our data highlight the need for further larger studies to explore the relationship between prophylactic antibiotic use and the recovery of the sinonasal microbiome after ESS. We intend to complete such a study.

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Authorship contribution

JMC: study design, laboratory analysis, data analysis, manuscript drafting. MSB: data analysis, manuscript review, RR: study design, randomisation, manuscript review, CKL: study design, laboratory analysis, data analysis, manuscript drafting, AJW: study design, clinical data collection, manuscript drafting.

Conflict of interest

Nil.

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This manuscript contains supplementary material

SUPPLEMENTARY MATERIAL

Supplementary methods

Genomic DNA extraction and 16S rRNA gene sequencing

16S rRNA Earth Microbiome Project primers:

Primer ID	Primer Sequence with Adapter
FWD (515FB)	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA
REV (806RB)	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACNVGGGTWTCTAAT

Underlined primer sequence corresponds to the V4 Earth Microbiome Project primer sequence. The sequence that is not underlined corresponds to the adapter sequence which will bind to the barcode primers from the Quick-16S NGS Library Prep Kit.

E.Z.N.A. Gel Extraction Kit 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 [60^{o}C] before being added to the column, and the elution buffer was incubated on the column for longer [additional 3 minutes].

Bioinformatics and statistical 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)[1]. Briefly, the forward and reverse reads' quality profiles were visually examined, and it was determined that truncLen trimming was unnecessary. Reads were filtered to remove "N" nucleotides [parameters: maxN=0, (DADA2 requires no Ns) truncQ=2, rm.phix=TRUE, maxEE=2 (maximum number of "expected errors" allowed in a read)] and then truncated and trimmed [parameters: filterAndTrim(fnFs, filtFs, fnRs, filtRs), truncLen=c(240,160), maxN=0, maxEE=c(2,2), truncQ=2]. Forward and reverse reads were merged and denoised, and the amplicon sequence variants (ASVs) were infered. Chimeras were removed with the removeBimeraDenovo function using the method "consensus".

Contaminant taxa were identified with decontam from extraction blanks and the PCR negative control using the decontam package (Supplementary Figure 1). Contaminated taxa were identified according to the prevalence-based method. This method used the prevalence of ASV present in the extraction blanks to calculate the prevalence of known contaminants in clinical samples[2]. This method accounts for competing template DNA (from the ASV present in the sinonasal microbiome) in the clinical samples, which would reduce the abundance or prevent some contaminants from present in these samples[2]. 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[2]. 'decontam' identified 889 taxa as contaminants at a 0.5 threshold (higher sensitivity compared with the default threshold of 0.1[2]. 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 and removed (Supplementary Figure 2 and Table 1).

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1. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods. 2016;13(7):581-583.
2. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome. 2018;6(1):226.

Supplementary Table 1. Samples removed during the trimming process due to low read count (<25,000 reads).

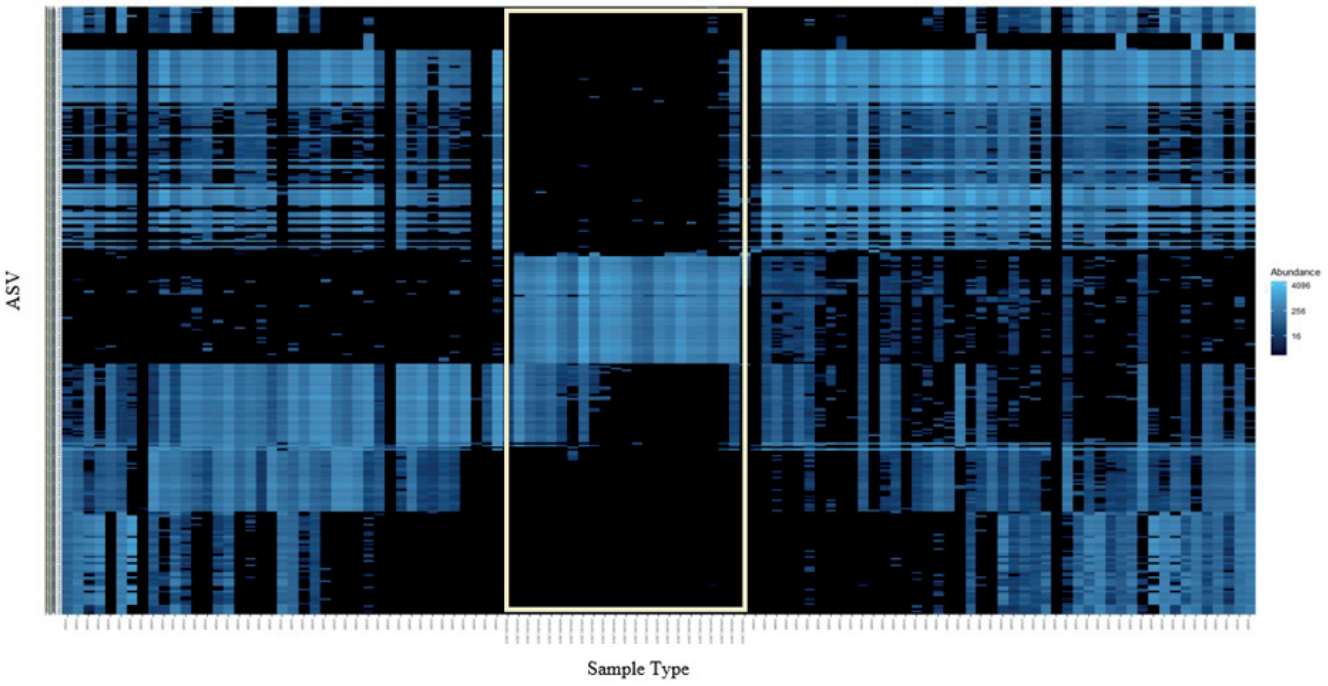
Sample	Read count
001_2_SR	9829
003_1_SR	21769
003_3_SL	6879
010_3_SR	2103
010_3_SL	87
011_1_TR	212
011_1_TL	147
011_2_SR	24077
011_2_SL	894
011_3_SR	7
011_3_SL	0
012_1_TR	0
012_1_TL	0
012_2_SL	0
012_3_SR	0
012_3_SL	0

Supplementary Table 2. Paired t-test comparing alpha diversity of Doxycycline samples at each timepoint. There was no significant difference for bacterial diversity when comparing timepoints for patients treated with doxycycline.

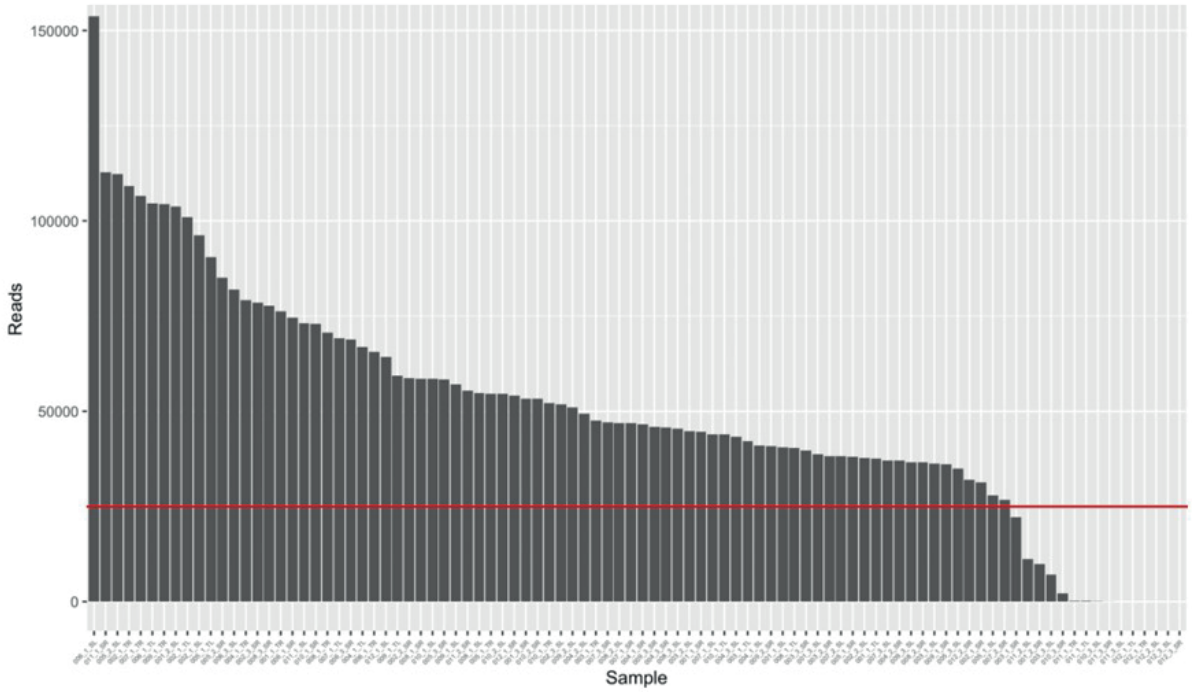
Timepoint comparison	p-value
pre-op v first post-op	0.104
pre-op v second post-op	0.159
first post-op v second post-op	0.516

Supplementary Table 3. Paired t-test comparing alpha diversity of placebo samples at each timepoint. Results were only significant (*) for pre-op v second post op in the placebo treatment group.

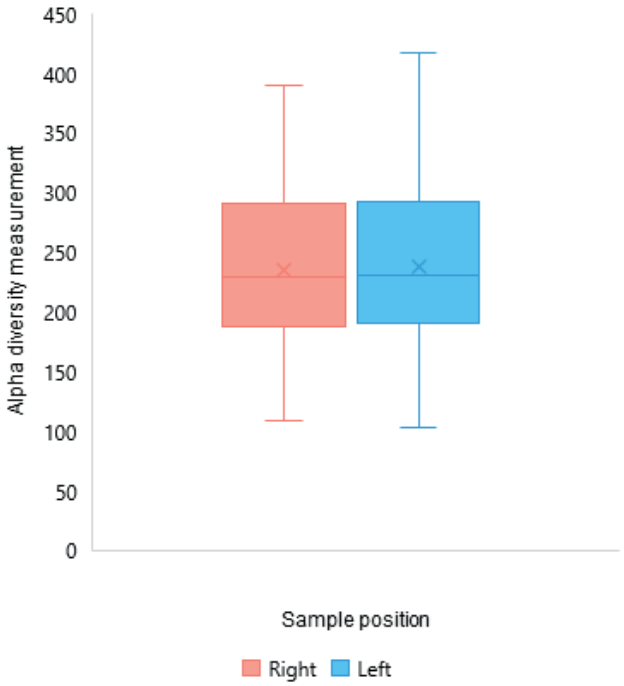
Timepoint comparison	p-value
pre-op v first post-op	0.632
pre-op v second post-op	0.045 *
first post-op v second post-op	0.524



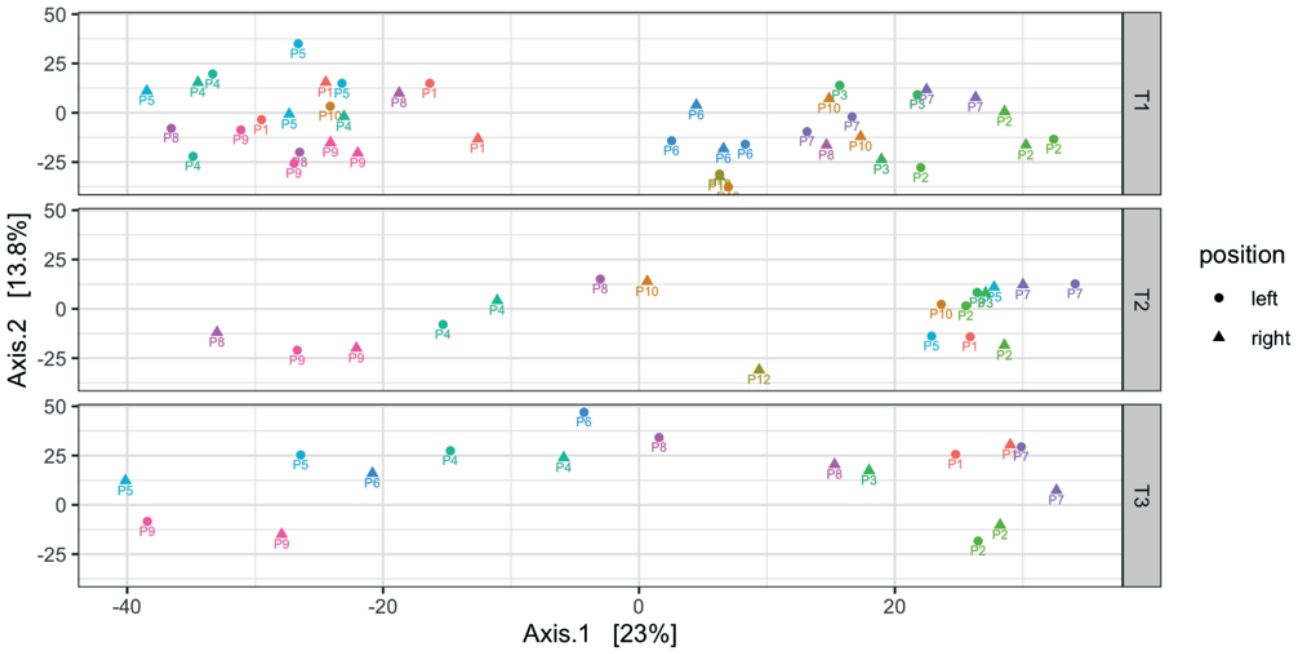
Supplementary Figure 1. Heatmap of ASV present in extraction blanks and patient samples. The white box shows the ASV present in the extraction blanks. ASV outside the white box corresponds to patient samples.



Supplementary Figure 2. 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 and were removed from subsequent analysis.

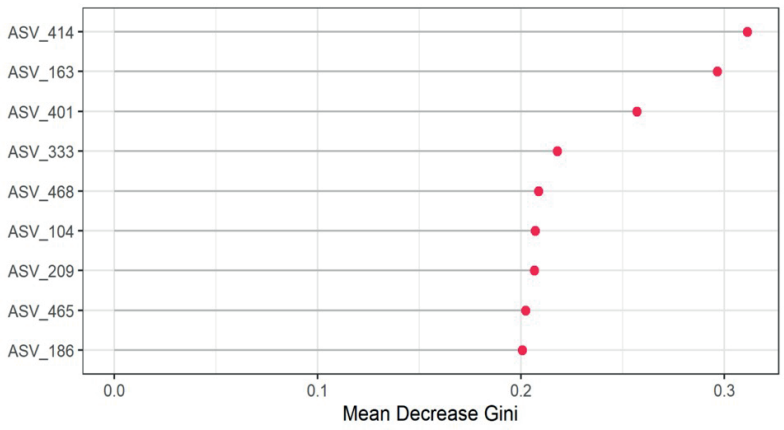


Supplementary Figure 3. Alpha diversity of samples sorted according to sample position using observed richness. Alpha diversity showed similar diversity between left and right samples (paired t-test, p-value =0.477).

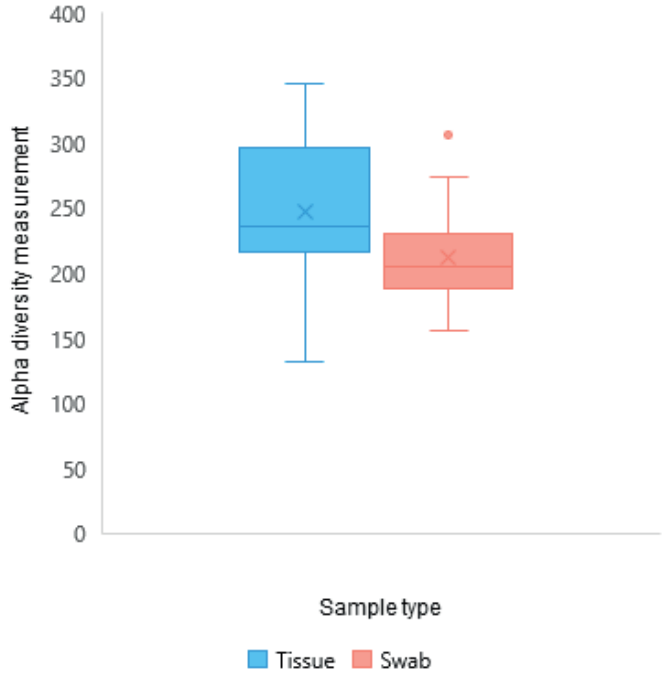


Supplementary Figure 4. PCoA plot of samples, labelled according to sampling on left or right nostril. No apparent clustering was observed between left and right samples and A PERMANOVA analysis showed no significant differences between both (p-value = 0.298).T1 = pre-op, T2= first post-op, T3= second post-op.

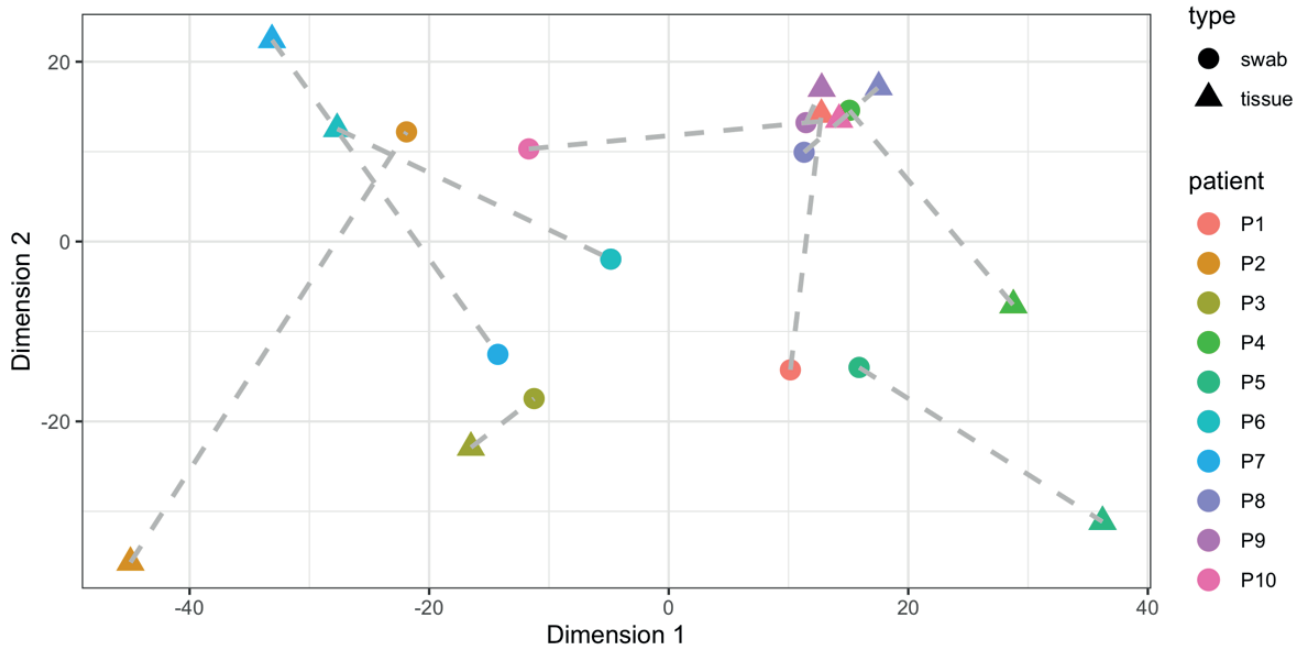
Phylum	Class	Order	Family	Genus	ASV
Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptoniphilus	unassigned	ASV_414
Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	ASV_163
Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Finegoldia	unassigned	ASV_401
Proteobacteria	unassigned	unassigned	unassigned	unassigned	ASV_333
Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Finegoldia	unassigned	ASV_468
Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	ASV_104
Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	ASV_209
Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	ASV_465
Actinobacteriota	Actinobacteria	Corynebacteriales	Corynebacteriaceae	Corynebacterium	ASV_186



Supplementary Figure 5. ASV identified using a Random Forest model that may be associated with the different treatment groups. Nine ASV identified, with five belonging to the Corynebacterium genus while the remaining ASV were unassigned at a genus level. Model accuracy =63.2%. Sensitivity = 61.1% and specificity = 65.0%. Antibiotic treatment was the positive class.



Supplementary Figure 6. Alpha diversity of samples sorted according to sample type using observed richness. Alpha diversity showed a higher diversity within tissue samples compared to swab samples (paired t-test, p-value = 0.0114).



Supplementary Figure 7. Procrustes analysis for pre-op swab and tissue samples. Patient samples (left and right) were merged for each patient. Patient 12 was removed from data as there were no tissue samples for this patient. Ordination shows that the sample type had some overlap between communities however this was not extensive (r=0.6). PERMANOVA analysis showed similarities in bacteria community composition between sample types (PERMANOVA, F = 1.3849, p-value = 0.131).