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ORIGINAL ARTICLE

Chitogel following endoscopic sinus surgery promotes a healthy microbiome and reduces postoperative infections

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Abstract

Background: Postoperative infections following endoscopic sinus surgery (ESS) impair wound healing and lead to poor outcomes. The aim of this study is to assess the effectiveness of Chitogel to reduce postoperative infections and restore a healthy microbiome following ESS.

Methods: In this double-blinded randomized control trial, 25 patients undergoing ESS were prospectively recruited. At the end of surgery, patients were randomized to receive Chitogel to one side of the sinuses (allowing the other side to serve as control). Patients underwent routine follow-up with nasoendoscopies performed at 2, 6, and 12 weeks postoperatively. Sinus ostial measurements, microbiology, and microbiome swabs from bilateral sides were collected intraoperatively and at 12 weeks postoperatively. Additional swabs were collected if infection was present.

Results: Improved endoscopic appearance of the sinuses ($p = 0.03$) and ostial patency were noted on the Chitogel side compared with control at 12 weeks ($p < 0.001$). A significant decrease in infections on the Chitogel side (12.0%) compared with control (52.0%) ($p = 0.005$) was evident. Following the use of Chitogel, there was a significant increase in the combined relative abundance of commensals *Corynebacterium* and *Cutibacterium* (*Propionibacterium*) from 30.15% at baseline to 46.62% at 12 weeks compared with control (47.18% to 40.79%) (p.adj = 0.01).

Conclusion: Chitogel significantly improved both the nasoendoscopic appearance of the sinuses and sinus ostial patency at 12 weeks postoperatively. Chitogel used following ESS helps restore an improved microbiome resulting in an increase in the relative abundance of commensals *Corynebacterium* and *Cutibacterium* (*Propionibacterium*). A significant decrease in postoperative infections was noted following use of Chitogel.

KEYWORDS
bacteriology, chronic rhinosinusitis, endoscopic sinus surgery, postoperative, topical therapy for chronic rhinosinusitis

1 | INTRODUCTION

Postoperative infections following endoscopic sinus surgery (ESS) have been shown to lead to poor outcomes. Presence of bacterial biofilms at the time of ESS is associated with persisting postoperative sinonasal symptoms and worsened nasoendoscopic appearance including significantly worse inflammation. It also causes patients to present for extra postoperative visits and requires multiple courses of antibiotics.[1–3] *Staphylococcus aureus* intracellularly has also been shown to cause ongoing sinonasal symptoms, inflammation, and infection post ESS.[3,4]

Increasing research is emerging about the sinonasal microbiome and the role that dysbiosis plays in disease. In an international sinonasal microbiome study, in which samples taken from 410 healthy control and chronic rhinosinusitis (CRS) patients were sequenced, *Staphylococcus* and *Corynebacterium* were found to be the two most prevalent and abundant bacteria.[5] Mean relative abundance amongst all patients was 44.02% for *Corynebacterium* and 27.34% for *Staphylococcus*. Interestingly, among patients with the severest phenotype of CRS, CRS with nasal polyps (CRSwNP), a significant reduction in relative abundance of *Corynebacterium* was found when compared with healthy control patients.[5]

The antimicrobial activity of *Corynebacterium accolens* against *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in planktonic and biofilm form has been investigated by Menberu et al.[6] Cultured *C. accolens* extracted from healthy sinuses showed antimicrobial activity against *S. aureus* and MRSA clinical isolates from patients with CRS.[6] Cell-free culture supernatants from *C. accolens* also significantly reduced *S. aureus* and MRSA biofilm metabolic activity and mass.[6]

Paramasivan et al.[7] demonstrated in vitro the antibiofilm and pro-wound-healing properties of chitosan-dextran gel. In a fibroblast model, with fibroblasts sourced from human nasal tissue, chitosan-dextran gel at 50% (v/v) concentration and dextran alone at 1.25% (w/v) had anti-*Staphylococcus* biofilm properties.[7] In addition, chitosan-dextran gel was shown to decrease fibroblast proliferation and wound healing time.[7] Chitosan on its own was able to reduce IL-8 levels in a fibroblast model in which cells were challenged with superantigens *S. aureus* enterotoxin B and toxic shock syndrome toxin.[7]

A prospective, blinded, randomized control trial using Chitogel in one side of the sinonasal tract following ESS in 26 patients was performed by Ha et al. In this trial, Chitogel was shown to significantly reduce ostial stenosis of the frontal, sphenoid, and maxillary sinuses compared with control.[8] This finding was replicated in another randomized control study, which showed that increased sinus ostial area patency was maintained on the Chitogel side when compared with control.[9]

The effect, however, that the absorbable nasal dressing Chitogel has on the sinonasal microbiome has yet to be explored. The aim of this trial is to explore the effect that Chitogel has on the sinonasal microbiome following ESS and to assess its impact on postoperative infections and ability to improve wound healing. Chitogel has three components; the effect of chitosan and dextran on bacterial growth has already been investigated,[7] but the third component, glycerol, has yet to be established. To further understand the effect that Chitogel plays in postoperative infections, we assessed the growth of bacterial isolates from the nasal cavity treated with 20% glycerol (concentration in Chitogel).

2 | MATERIALS AND METHODS

2.1 | Study design and participants

The double-blinded, randomized control study was conducted between October 2019 and November 2021. Twenty-five patients with CRS undergoing primary bilateral full-house functional endoscopic sinus surgery (FH-FESS) were prospectively recruited to receive Chitogel to one side of the sinuses and nothing to the other side (control) at the end of surgery. Patients received follow-up at 2, 6, and 12 weeks postoperatively. Additionally, "long-term" follow-up was performed when available in patients presenting after 12 months. Approval for this clinical trial was granted by a tertiary teaching hospital's Human Research Ethics Committee in South Australia (HREC reference number HREC/17/TQEH/245. ACTRN12618000577213). Patients over 18 years and able to give informed consent were included in the study. Patients with a shellfish allergy who were pregnant or breast feeding were excluded from the trial.

To better understand the antibacterial properties of Chitogel, an in vitro study was also performed to assess the

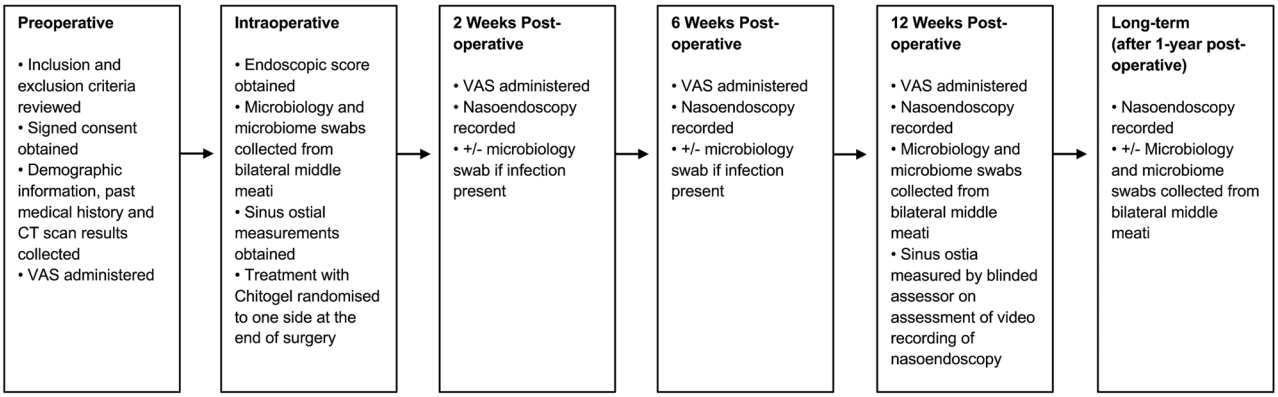

FIGURE 1 Flow diagram describing the study protocol at all timepoints

antimicrobial effects of 20% glycerol (a component of Chitogel), as the antimicrobial effects of the other components of Chitogel (chitosan and dextran) had been previously reported[7,10]. The growth of various nasal bacterial isolates treated with 20% glycerol was assessed and compared with control.

2.2 | Outcomes

Subjective and objective measures were used to assess postoperative outcomes following use of Chitogel. Postoperative sinus ostial area was the primary outcome, for which sample size was calculated. Secondary outcomes included: visual analogue scale (VAS) symptom scores, endoscopic appearance scores, infection rates, and microbiome data. See Figure 1 detailing the study protocol and collection of subjective and objective data at each time point.

The outcome of the in vitro work was to assess the growth of nasal bacterial isolates when treated with 20% glycerol compared with control.

2.3 | Statistical power analysis

Power calculations were performed based on effects assessed at 5% alpha level with 80% statistical power. The primary outcome for sample size calculation was centered on a significant difference in frontal ostial size. Hosemann et al. showed the average diameter of the frontal sinus neoostia postoperatively was 3.5 mm (range 0–11 mm).[11] A difference of 3 mm^2 area in the frontal sinus ostia at 12 weeks with a standard deviation based on half of the magnitude of the mean difference (i.e., 1.5 mm^2) was therefore chosen. Sample size was thus determined to be 20 patients. To account for dropout of patients, five additional patients were recruited.

2.4 | Collection of preoperative data

Prior to surgery, all participants gave informed consent. Demographic information (including age and gender) was collected and patients completed a VAS[12] to assess the severity of sinonasal symptoms on each side of the sinonasal tract. Preoperative computed tomography (CT) scans of the sinuses were assessed, and the severity of disease was graded as per the Lund–Mackay score.[13] Patients were classified into CRSwNP and chronic rhinosinusitis without nasal polyps (CRSsNP) based on the European Position Paper on Rhinosinusitis and Nasal Polyps 2020 (EPOS).[14] Past medical history of asthma, gastroesophageal reflux disease, diabetes mellitus, and smoking status were collected from patient medical records.

2.5 | Surgery and collection of intraoperative data

All patients underwent primary bilateral FH-FESS performed with cold steel and powered instruments with particular attention given to conserving mucosa. Septoplasty was performed concurrently as indicated. No patient had inferior turbinoplasty. No patient underwent an extended approach such as a frontal drill-out or medial maxillectomy. Surgery was performed by one of two operating surgeons. Microbiology and microbiome swabs were taken by the surgeon from the middle meatus on each side of the sinonasal tract under endoscopic guidance at the beginning of surgery. Baseline scoring of the endoscopic appearance of the sinuses on either side was performed assessing for adhesions, evidence of infection, oedema, crusting, and granulation tissue. At the end of surgery, the frontal, maxillary, and sphenoid sinus ostial areas were determined by measuring height and width of the sinus ostia with a standardized 5-mm measuring probe.

2.6 | Randomization and intervention

Small block randomization was performed using GraphPad Quickcalcs software (GraphPad Software, San Diego, United States). At the end of the operation, the surgeon was informed which side the patient had randomly been assigned to receive Chitogel, thereby preventing any difference between the two sides during surgery. The other side received nothing. The only difference in the treatment between both sides of the sinuses was presence or absence of Chitogel at the end of surgery; therefore, the side that received nothing served as an internal control.

Chitogel was supplied by Chitogel Pty Ltd (Wellington, New Zealand). Up to 20 ml of Chitogel was applied using the supplied malleable cannula at the end of surgery under endoscopic guidance filling the floor of the frontal sinus, frontal ostium, frontoethmoidal recess, ethmoid cavity, and sphenoid and maxillary sinuses. Chitogel was placed in the middle meatus to support the middle turbinate. The nasal tract was not filled with gel to allow for an unobstructed nasal airway. Patients were blinded to which side received treatment.

2.7 | Postoperative care and follow-up

Patients received standard postoperative care and follow-up at 2 weeks, 6 weeks, and 12 weeks postoperatively. If patients presented for follow-up after 1 year postoperatively, data from the patient's medical records (including microbiology results and nasoendoscopic recordings) were collected; results collected from this timepoint were collectively termed "long-term." Figure 1 shows the details of the study protocol at each timepoint.

Following surgery all patients received a course of oral antibiotics (amoxicillin/clavulanic acid 875/125 mg twice daily for 7 days) and those with nasal polyps had a 3-week tapering course of prednisolone (25 mg daily for 7 days, 12.5 mg daily for the next 7 days, and 12.5 mg alternate days for the final 7 days). Patients were directed to commence 240 ml saline nasal douches bilaterally four times a day, starting the day after surgery. The first postoperative visit was performed at 2 weeks. There was minimal or no Chitogel seen in the sinus cavity that received the gel at that visit. At each visit patients had endoscopic debridement of the sinuses as required. Topical steroid, budesonide 1 mg/2 ml (Pulmicort Respules 1 mg/2 ml), added to one of the daily saline nasal douches was commenced at two weeks postoperatively. If a patient presented with infection, it was managed as per standard care with a microbiology swab and culture-directed antibiotics.

2.8 | VAS symptom score collection

At the 2-, 6-, and 12-week postoperative visits, patients completed additional VAS questionnaires to assess severity of sinonasal symptoms on each side of the sinonasal cavity compared with preoperative baseline. The VAS was adapted from commonly used rhinological VAS questionnaires[15] to assess any differences in symptoms between Chitogel-treated and control sides of the nose. Patients were asked to score on a scale of 0–10 (where 0 indicated absence of symptom and 10 severe) symptoms of facial pain or discomfort, bleeding, nasal obstruction, nasal secretions, postnasal drip, and sense of smell. The individual scores for each symptom were combined to give a total VAS score for each side of the sinonasal tract at preoperative baseline and 2, 6, and 12 weeks postoperatively. Quality of life questionnaires that included general symptom questions could not be used in this study as each patient had a Chitogel-treated and control side.

2.9 | Endoscopic appearance data collection

Nasoendoscopies performed at 2, 6, and 12 weeks postoperatively were recorded and sent for blinded assessment. Additional videos were included for assessment if available at the long-term timepoint. All video-recorded nasoendoscopies were given in random order to a blinded assessor not involved in the care of the patient, who scored each side of the sinonasal tract for adhesions, evidence of infection, oedema, crusting, and granulation tissue. A total endoscopic score was collated for each side of the sinonasal tract at 2, 6, and 12 weeks postoperatively as well as long term and compared with scores obtained intraoperatively prior to randomization of treatment.

2.10 | Postoperative assessment of sinus ostia

At 12 weeks postoperatively, the frontal, maxillary, and sphenoidal sinus ostia were remeasured using a standardized 5-mm measuring probe to measure height and width of the ostia under endoscopic guidance. This nasoendoscopy was recorded, and the final area was determined by a blinded assessor viewing the video recording. The percentage of sinus ostial area maintained at 12 weeks from intraoperative baseline for each sinus (frontal, maxillary, and sphenoid) was compared between control and Chitogel-treated sides. If the percentage maintained was

above 100% (i.e., the area measured at 12 weeks was larger than the intraoperative baseline area), it was rounded down to 100%, as it was evident that no postoperative ostial stenosis had occurred.

2.11 | Microbiological samples

One swab (Sigma Transwab, MWE Medical Wire, Corsham, United Kingdom) was collected from each side of the middle meatus intraoperatively and at 12 weeks postoperatively under endoscopic guidance. Additional swabs were sent as per standard care if patients presented with infection up to the long-term follow-up. Identification of bacterial isolates was performed at a diagnostic laboratory (Clinpath Pathology, Adelaide, Australia). Samples were sent for microscopy, culture, and sensitivity. Growth on culture was quantified as "scant," "light," "moderate," or "heavy."

2.12 | Definition of infection

An infection was defined as a score of at least mild mucopurulent discharge on review of the video recording of the nasoendoscopy (as assessed by a blinded reviewer) in conjunction with a "moderate" or "heavy" growth of pathogenic bacteria on microbiology swab taken from the middle meatus as determined by a diagnostic laboratory (Clinpath Pathology, Adelaide, Australia). Although infections are associated with worsening of symptoms, in order to ensure objectivity, we did not include symptom data for the purpose of defining infection in this study.

2.13 | Microbiome collection and DNA extraction

Standardized collection of microbiome samples was performed intraoperatively and at 12 weeks postoperatively. Additional samples were available in some patients who presented for follow-up at the long-term timepoint. One guarded Copan flocked swab (Copan, Brescia, Italy) was collected from the middle meatus on each side under endoscopic guidance. The guard on the swab prevented accidental touching of the swab before the middle meatus was reached. Once the swab was taken, it was again guarded on removal. Swab tips were removed and stored in individual sterile cryotubes which were transported on ice and stored at −80°C.

DNA extractions were performed as per manufacturer's instructions using The Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany).

2.14 | Microbiome sequencing and analysis

Bacterial samples underwent sequencing at the Australian Genome Research Facility (AGRF) (Westmead, Australia). Libraries were generated by amplifying the 341F primer against the V3-V4 hypervariable region of the 16S rRNA gene (CCTAYGGGRBGCASCAG forward primer; GGACTACNNGGGTATCTAAT reverse primer). Sequencing was performed using the Illumina MiSeq platform (Illumina Inc, San Diego, United States).

2.15 | Bioinformatics

Paired-end fastq files were processed using QIIME2 version 2021.11.[16] First, the sequences were denoised and amplicon sequence variants (ASV) were formed using dada2 with the QIIME 2 plugin q2-dada2.[17] Taxonomy assigned was conducted against the Silva reference database (99% clustered similarity sequences) using a pretrained Naïve Bayes classifier as part of the q2-feature-classifier plugin.[18] The SATé-enabled phylogenetic placement (SEPP) technique was then used for insertion of the ASVs into the high-quality tree generated from the Silva database.[19] A rarefaction cutoff of 500 was chosen as quality control for downstream diversity and taxonomic relative abundance analysis. Furthermore, only patients with all four samples (Chitogel-treated and control sinuses, at baseline and 12-week timepoints) satisfying this cutoff were retained for downstream statistical analysis. In total, the microbiomes of 17 patients were retained (68 samples). Eight patients also had "long-term" microbiomes, where the swabs were taken from a follow-up appointment after 1 year postoperatively. The range of time after surgery from these eight long-term microbiomes was 350–735 days. Taxa were compared at the genus level. A relative abundance threshold of 1% and a prevalence threshold of 5% was chosen, as prefilters improved the performance of differential abundance detection.[20] All relative abundances of genera below these thresholds were aggregated into the catchall "Other" genus for each sample. Faith's phylogenetic diversity index,[21] Shannon's entropy, and the number of observed features were used as measures of alpha diversity, while unweighted and weighted Unifrac[22] and

Jaccard's distance matrices were calculated as measures of beta diversity analyses. All diversity metrics were calculated using the qiime2-diversity plugin with a sampling depth of 500.

2.16 | Experimental design to test the effect of glycerol on growth of nasal bacterial isolates

Bacterial isolates were retrieved from the nasal cavity and identified using standard microbiological assays as specified by Menberu et al.[6] Bacteria were treated with 20% (v/v) concentration of glycerol (Sigma-Aldrich, St. Louis, United States) to determine its effect on planktonic growth using nutrient-poor media, nutrient broth (NB) (obtained from Oxoid, Basingstoke, United Kingdom).

A total of 22 nasal clinical isolates including *C. accolens* ($n = 4$), *Corynebacterium propinquum* ($n = 3$), *Corynebacterium pseudodiptheriticum* ($n = 3$), *Staphylococcus epidermidis* ($n = 4$), *S. aureus* ($n = 4$), and *Pseudomonas aeruginosa* ($n = 4$) were used in this experiment. Prior to starting experiments, isolates were grown in tryptic soya agar (TSA) (Oxoid, Basingstoke, United Kingdom) and incubated at 37°C under aerobic conditions for 24 h, with the exception of *C. accolens* which was incubated for 48 h.

Glycerol at 20% (v/v) concentration was prepared by dilution in an NB media. In brief, 20% (v/v) glycerol treatment concentration was prepared in a 96-well microtitration plate containing 50 μl NB. Following this, a single colony of bacterial isolates such as *C. accolens, C. propinquum, C. pseudodiptheriticum, S. epidermidis, S. aureus*, and *P. aeruginosa* from overnight culture was suspended with 0.9% (w/v) NaCl (physiological saline) to McFarland standard of 0.5 and then diluted 1:100 in NB. Next, 50 μl of the diluted bacterial suspension was added to the treatment in each well and incubated aerobically for 24 h at 37°C. A negative control (NB without bacteria and treatment) and untreated growth control (NB with bacteria but no treatment) were used in this assay. After incubation, the planktonic bacterial growth was determined by measuring the optical density (OD) at 595 nm using microplate absorbance reader (iMark, Bio-Rad Laboratories Inc, Hercules, United States). For each bacterial strain, six replicate experiments were performed to assess bacterial viability.

2.17 | Statistical analysis

VAS symptom and endoscopic score data were analyzed using a linear mixed-effects modelling to test for differences between the Chitogel-treated and control sinuses over time with interaction terms, coding the patient as a random effect. A Box–Cox transformation was applied to the data before running the models where appropriate. To compare sinus ostial area difference at 12 weeks postoperatively between Chitogel-treated and control sinuses, an additive linear mixed-effects modelling was used, also coding the patient as a random effect.

Differences in the number of infections between the Chitogel-treated and control sides were assessed with Fisher's exact test. The effect of 20% glycerol treatment on planktonic growth was analyzed by means of one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test using GraphPad Prism version 8.0 (GraphPad Software, San Diego, United States).

For microbiome data, downstream statistical analysis was conducted using R v 4.1.0.[23] Differential relative abundance for each genus between intraoperative baseline and 12-week timepoints was tested using the metamicrobiomeR package,[24] which implements generalized additive models for location, scale, and shape (GAMLSS) with a zero-inflated beta (BEZI) family (GAMLSS-BEZI) model. The GAMLSS-BEZI models were specified to handle longitudinal data, with the main comparison variable being "Treated," adjusted with "Time." All p values were adjusted using a Benjimani–Hochberg false discovery rate of 5%.[25] Genera with insufficient non-zero datapoints to calculate GAMLSS-BEZI models (*Saccharimonadales* and *Rothia*) were aggregated into the catchall "Other" genus. Differences in alpha diversity metrics between timepoints and treatment groups were tested using linear mixed-effects models implemented in the lme4 R package, where the patient was coded as a random effect.[26]

3 | RESULTS

3.1 | Patient cohort

Twenty five patients were recruited, and there was heterogenicity in the baseline characteristics of the group. The median age was 55 years (range from 22 to 75 years), with the group consisting of 12 females (48%) and 13 males (52%). Twelve patients (48%) had CRSwNP and 13 patients (52%) had CRSsNP. Preoperative CT sinus scans showed a median Lund–Mackay score[13] of 12 (range 4–22). Eight patients (32%) had asthma, eight (32%) had gastroesophageal reflux disease, one (4%) had type 2 diabetes mellitus, and there was one (4%) smoker. Septoplasty at the time of surgery was performed in 19 (76%) patients (see Table 1). As each patient had a Chitogel-treated and control side, there was no difference in baseline characteristics between control and Chitogel-treated groups.

Twenty two patients completed follow-up at timepoints 2, 6, and 12 weeks postoperatively. Two patients failed

TABLE 1 Baseline characteristics of clinical trial patients

Patient characteristics	
Age, median years (range)	55 (22–75)
Female, no. (%)	12 (48%)
Male, no. (%)	13 (52%)
CRS with nasal polyps, no. (%)	12 (48%)
CRS without nasal polyps, no. (%)	13 (52%)
Lund–Mackay score, median (range)	12 (4–22)
Asthma, no. (%)	8 (32%)
Gastroesophageal reflux disease, no. (%)	8 (32%)
Type 2 diabetes mellitus, no. (%)	1 (4%)
Smoker, no. (%)	1 (4%)
Septoplasty performed at time of surgery, no. (%)	19 (76%)

Abbreviation: CRS, chronic rhinosinusitis.

to attend follow-up at 12 weeks, and one patient failed to attend follow-up at 6 weeks postoperatively; data collected from the follow-up timepoints attended by these patients were included in the analysis. Of the 20 patients that reached the 1-year postoperative follow-up period (collectively termed "long-term"), 14 patients attended follow-up. Median follow-up time in the long-term group was 15 months (range from 1 to 2 years).

Thirteen patients (52%) received Chitogel on the left and the other 12 (48%) on the right. There were no adverse outcomes following use of Chitogel and it was extremely well tolerated by all patients.

3.2 | VAS symptom scores

All patients had an improvement in total VAS scores following surgery compared with preoperative baseline, with decreasing scores noted over time at 2, 6, and 12 weeks postoperatively. When comparing total VAS scores between Chitogel-treated and control sides there was a trend for improved scores on the Chitogel-treated side across all postoperative timepoints; however, this difference was not statistically significant.

3.3 | Endoscopic appearance

All patients had improvement in the endoscopic appearance of the sinuses compared with preoperative baseline, with decreasing scores noted over time in both the Chitogel-treated and control groups.

The total endoscopic scores between the Chitogel-treated and control sides at baseline are similar. However, over time, a difference between the two groups emerges, with Chitogel-treated sinuses having improved endoscopic scores compared with the control group across all timepoints including the long-term timepoint. Total endoscopic scores were significantly lower in the Chitogel-treated sinuses after 12 weeks compared with the control side ($p = 0.03$) (Figure 2).

3.4 | Ostial measurements

To assess the effects Chitogel had on sinus ostial patency, the baseline ostial area (measured intraoperatively) was compared with ostial area measured at 12 weeks postoperatively by a blinded assessor. The percentage of the baseline area maintained at 12 weeks postoperatively was compared in both control and Chitogel-treated groups (Figure 3).

Taking into account data from all three sinuses (frontal, maxillary, and sphenoid) using an additive linear mixed-effects model, at 12 weeks postoperatively there was a statistically significant improvement in the percentage of ostial area maintained in the Chitogel-treated sinuses compared with control ($p < 0.001$).

3.5 | Rates of infection

Thirteen patients developed infections during follow-up: 10 at various time points in the initial 12-week follow-up period and three at the long-term follow-up period. Bilateral sinus infections were noted in three patients and unilateral infections (all on the control side only) in 10 patients. No patient developed a unilateral infection on the Chitogel-treated side. Therefore, there was significant decrease in infections noted in the Chitogel-treated sinuses compared with control, with three (12.0%) of the sinuses treated with Chitogel having developed infections compared with 13 (52.0%) of the control sinuses ($p = 0.005$) (Figure 4).

Staphylococcus aureus was the most common cause of infection as identified by a diagnostic laboratory; all three of the sinus infections on the Chitogel-treated side and eight of the sinus infections on the control side were caused by *S. aureus* (including one with MRSA). A further four of the control sinuses had an infection caused by *P. aeruginosa* and one caused by *Klebsiella aerogenes*.

3.6 | Microbiome

Eighteen unique genera passed the thresholds described in the methods: *Acinetobacter, Anaerococcus, Chloroplast, Corynebacterium, Cutibacterium, Dolosigranulum, Finegoldia, Flavobacterium, Lawsonella, Peptoniphilus,*

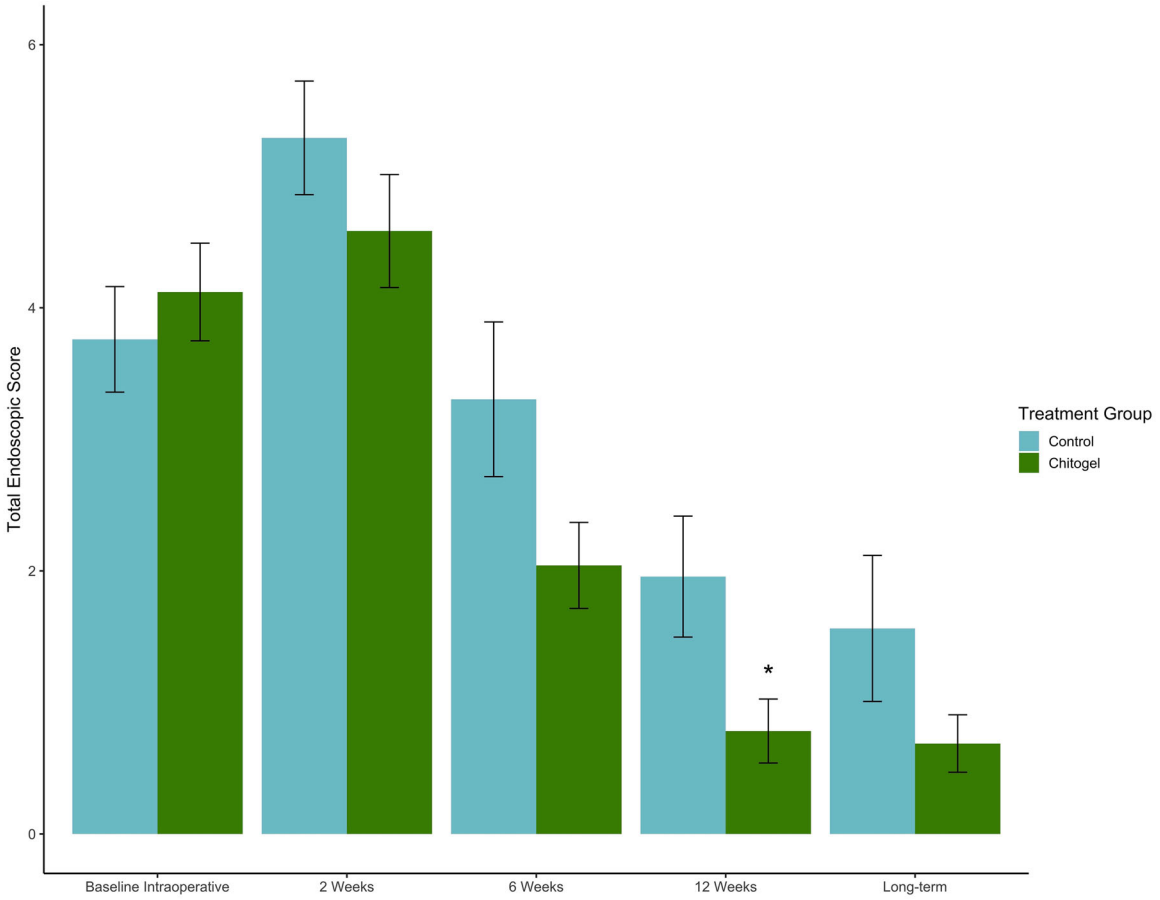

FIGURE 2 Total endoscopic scores at intraoperative baseline, 2 weeks, 6 weeks, 12 weeks postoperatively, and long term in control and Chitogel-treated sides. Data represent the mean ± SEM. * $p < 0.03$, linear mixed-effects model. SEM, standard error of the mean

Pseudomonas, Rothia, Saccharimonadales, Staphylococcus, Streptococcus, Enterobacteriaceae, unknown, and other (Figure 5 and Table 2).

Generally, the most abundant genera detected over all three timepoints were *Corynebacterium*, *Staphylococcus*, and *Cutibacterium*. *Flavobacterium* was only detected at intraoperative baseline, while *Enterobacteriaceae* and *Pseudomonas* were only detected following surgery.

Of the three most abundant genera, the proportion of *Corynebacterium* increased over time, particularly in the Chitogel-treated sinuses. In the control sinuses, the relative abundance increased from 24.76% at baseline to 33.40% after 12 weeks and plateaus at 31.72% long term. In the Chitogel-treated sinuses, the baseline proportion was lower at 17.45%, increasing to 35.02% at 12 weeks and 49.89% long term. According to the GAMLSS-BEZI model, the increase of *Corynebacterium* in the Chitogel-treated sinuses at the long-term point was statistically significant (p.adj < 0.05). The increase in the Chitogel-treated sinuses at the 12-week timepoint had a *p* value of 0.13 (Figure 6A).

With *Cutibacterium*, a beneficial commensal previously known as *Propionibacterium* (most commonly *Propionibacterium acnes*), there is evidence of a decrease in mean relative abundance over time following surgery; however, the decrease is smaller in the Chitogel-treated sinuses than the control. In the control group the mean relative abundance decreases from 22.42% at baseline to 7.39% at 12 weeks, whereas in the Chitogel-treated group the proportion remains stable, decreasing from 12.70% to 11.60% (p.adj = 0.07). In the long term, mean relative abundance in the control is 7.89% and in the Chitogel-treated group 7.49% (p.adj = 0.16).

There were no differences between time and treatment groups for *Staphylococcus* (p.adj > 0.05). It should be noted that at baseline, the Chitogel-treated sinuses had a higher proportion of *Staphylococcus* than control, which normalized following surgery, following an increase in *Staphylococcus* in the control group following surgery.

A further GAMLSS-BEZI model was also fitted with combined proportions for the beneficial commensals, *Corynebacterium* and *Cutibacterium* (*P. acnes*). The combined relative abundances of these two genera increased significantly in the Chitogel-treated sinuses following surgery, whereas it decreased in the control sinuses. In

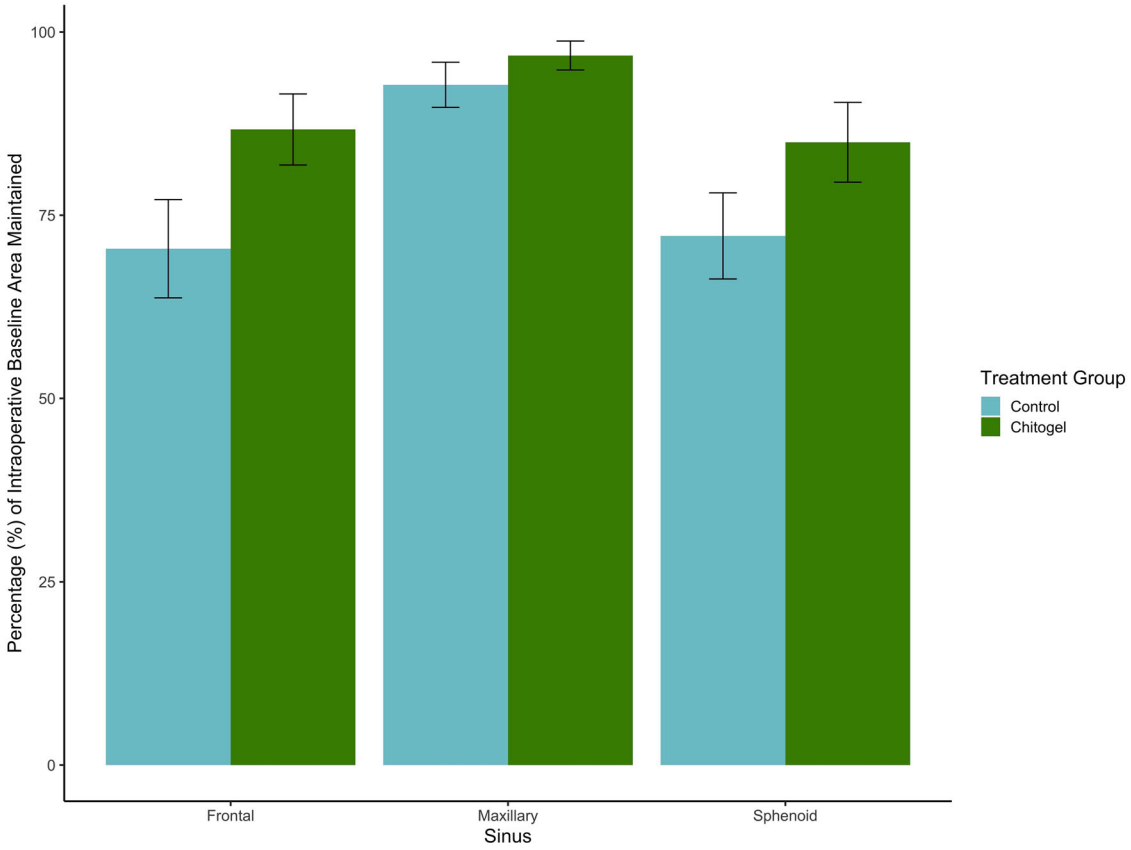

FIGURE 3 Percentage (%) of intraoperative baseline ostial area maintained at 12 weeks postoperatively in the control and Chitogel-treated sides for frontal, maxillary, and sphenoid sinuses. Data represent the mean ± SEM. SEM, standard error of the mean

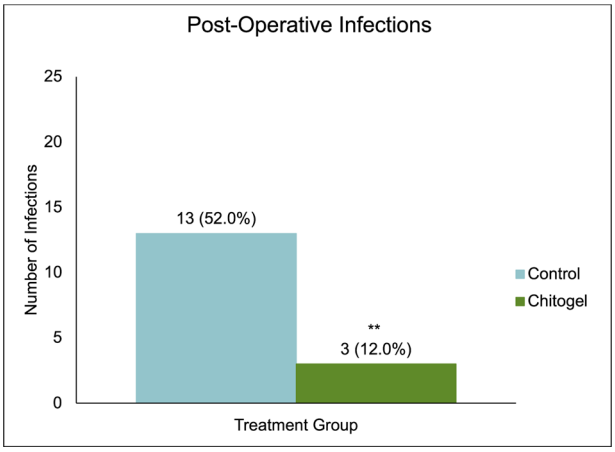

FIGURE 4 Postoperative infections on the control side compared with the Chitogel-treated side. ** $p = 0.005$, Fisher's exact test

the control group the mean relative abundance of the two genera was 47.18% at baseline, decreasing to 40.79% at 12 weeks and to 39.61% in the long term. In the Chitogel-treated group, the mean relative abundance was 30.15% at baseline, increasing to 46.62% at 12 weeks and to 57.58% in the long term. The comparative increase in the Chitogel-treated sinuses at 12 weeks and long term was statistically significant (p.adj = 0.01 and p.adj = 0.02, respectively) (Figure 6B).

There are no statistically significant differences in the alpha diversity metrics between Chitogel-treated and control sinuses ($p > 0.05$). However, all alpha diversity metrics significantly increased following surgery over time by the long-term timepoints compared with baseline ($p < 0.01$).

3.7 | Effect of glycerol on growth of nasal bacterial isolates

Treatment of glycerol at 20% (v/v) concentration, significantly reduced the growth of various nasal bacteria such as *C. propinquum, C. pseudodiptheriticum, S. epidermidis, S. aureus*, and *P. aeruginosa* ($p < 0.01$) in a nutrient-poor environment, compared with untreated growth controls. However, 20% (v/v) glycerol did not impact the growth of the most predominant nasal commensal bacteria, *C. accolens* ($p > 0.05$), compared with untreated growth controls (Figure 7).

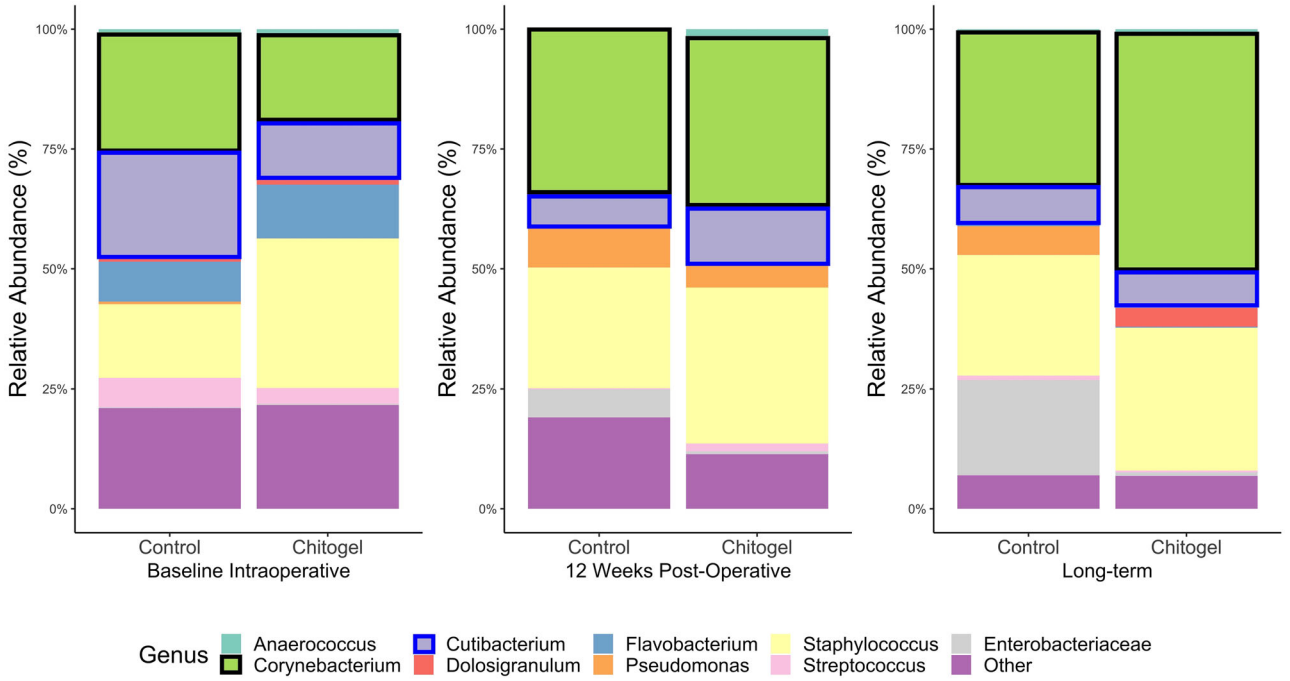

FIGURE 5 Mean relative abundance (%) of microbiota at intraoperative baseline, 12 weeks postoperatively, and long term in control and Chitogel-treated groups. The mean relative abundance of *Corynebacterium* is outlined in black and *Cutibacterium* in blue

TABLE 2 The mean relative abundance (%) of the most common genera found in control and Chitogel-treated groups at intraoperative baseline, 12 weeks postoperatively, and long term

	Mean relative abundance (%)					
Genera	**Control baseline**	**Control 12 weekspostoperative**	**Control long term**	**Chitogelbaseline**	**Chitogel12 weekspostoperative**	**Chitogellong term**
Anaerococcus	0.62	0.38	0.64.	1.29	2.01	0.78
Corynebacterium	24.76	33.40	31.72	17.45	35.02	49.89
Cutibacterium (Propionibacterium acnes)	22.42	7.39	7.89	12.70	11.60	7.49
Dolosigranulum	0.71	0.21	0.64	0.98	0.12	3.92
Flavobacterium	8.32	0.10	0.22	11.23	0.46	0.20
Pseudomonas	0.54	8.25	6.00	0.02	4.70	0
Staphylococcus	15.32	25.11	25.10	31.15	32.47	29.74
Streptococcus	6.08	0.19	0.90	3.27	1.63	0.34
Enterobacteriaceae	0.21	5.91	19.90	0.27	0.60	0.79

4 | DISCUSSION

In this study, where the patient is used as their own control, the application of Chitogel significantly decreased infections. Chitogel led to a significant increase in the mean relative abundance of *Corynebacterium* and combined *Corynebacterium* and *Cutibacterium (P. acnes)* forming the microbiome. In addition, an improved endoscopic appearance and significantly improved frontal and sphenoid ostial patency was noted following use of Chitogel.

It is of interest that there was an increase in mean relative abundance of *Corynebacterium*, and combined *Corynebacterium* and *Cutibacterium* (*P. acnes*) in the Chitogel-treated side at the long-term timepoint (after 12 months). There appeared to be a trend for improvement in endoscopic appearance at the long-term timepoint on the

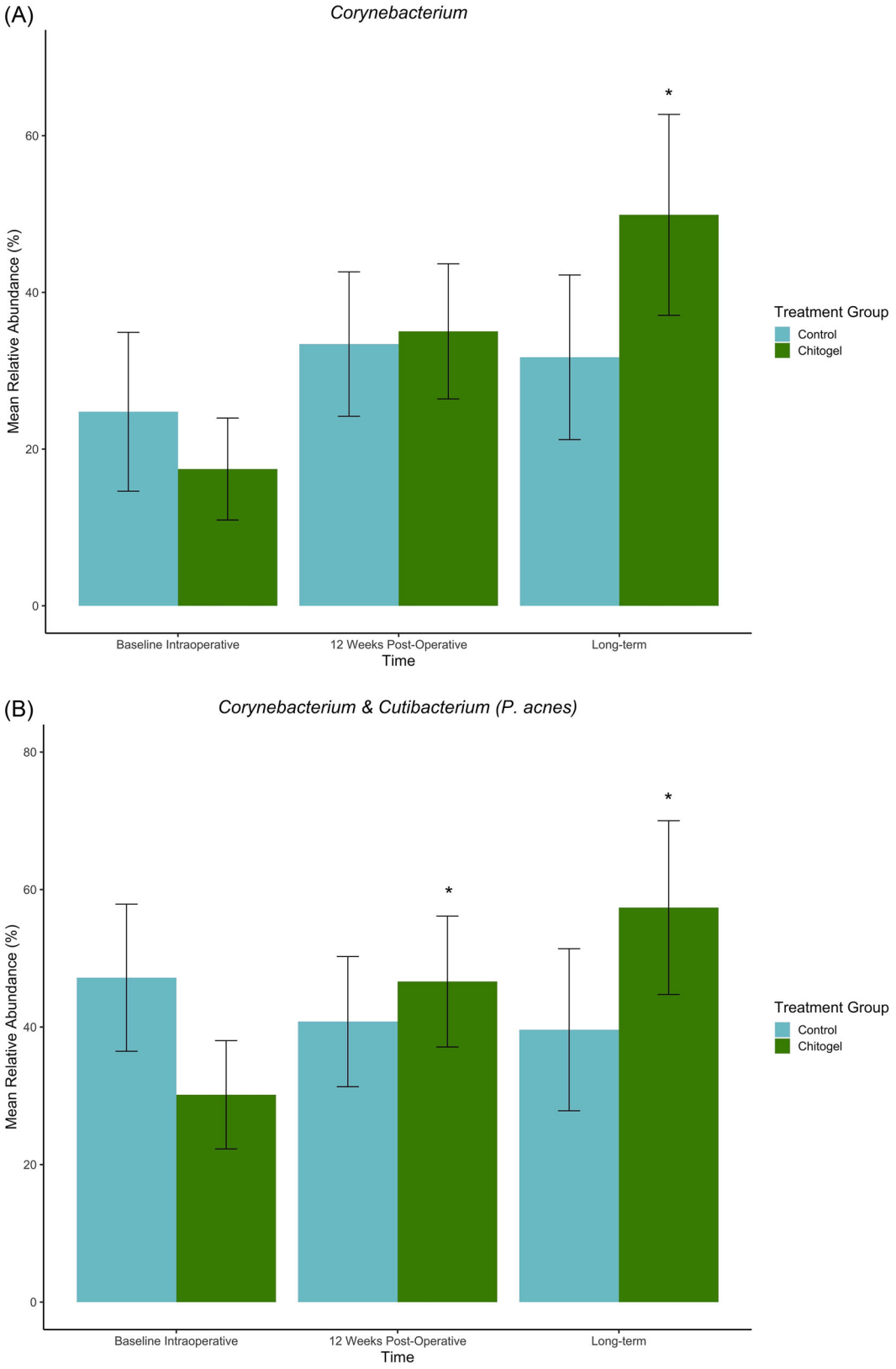

FIGURE 6 (A) Mean relative abundance (%) of *Corynebacterium* at intraoperative baseline, 12 weeks postoperatively, and long term in control and Chitogel-treated groups. Data represent the mean ± SEM. *p.adj = 0.048, GAMLSS-BEZI model. SEM, standard error of the mean. (B) Mean relative abundance (%) of combined *Corynebacterium* and *Cutibacterium (Propionibacterium acnes)* at intraoperative baseline, 12 weeks postoperatively, and long term in control and Chitogel-treated groups. Data represent the mean ± SEM. At 12 weeks postoperatively *p.adj = 0.010, and at long term *p.adj = 0.015, GAMLSS-BEZI model. GAMLSS-BEZI, generalized additive models for location, scale, and shape with a zero-inflated beta; SEM, standard error of the mean

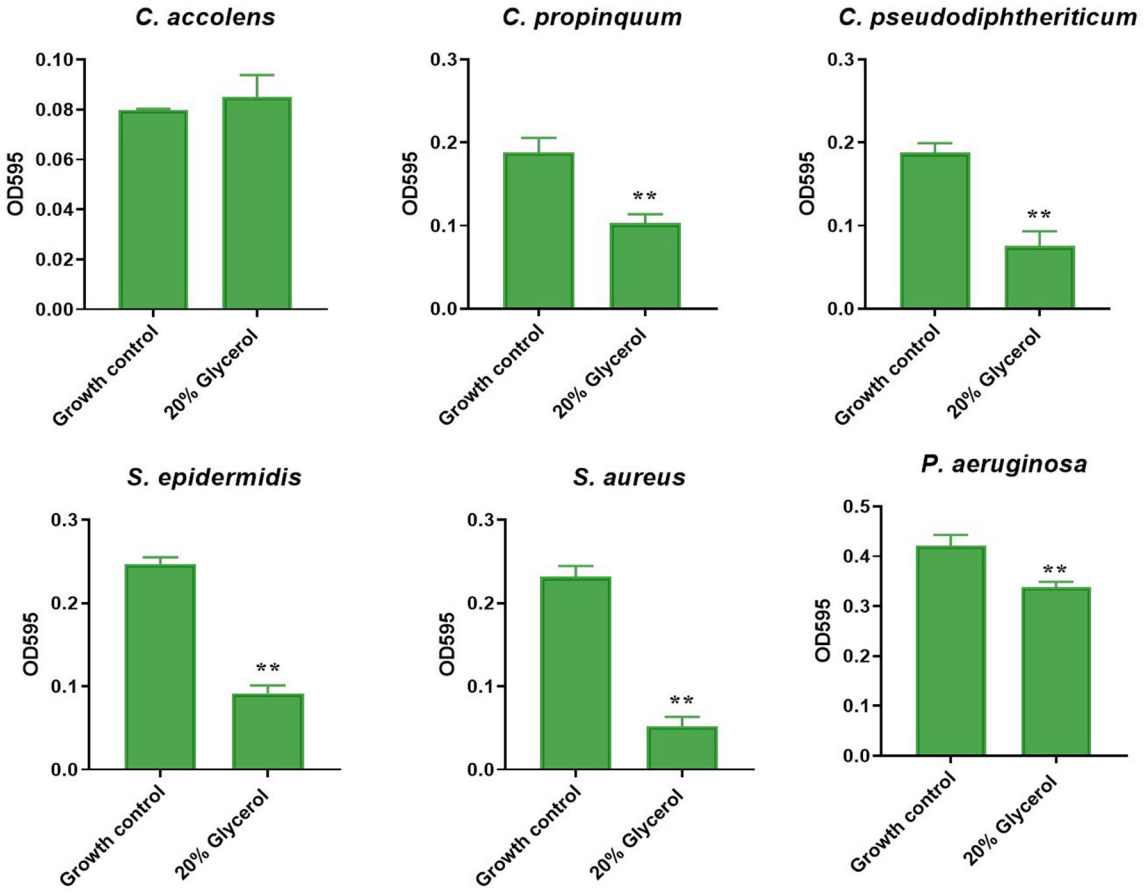

FIGURE 7 Effect of 20% (v/v) glycerol on bacterial planktonic growth after 24 h incubation in nutrient broth media. Data represent the mean ± SEM of each bacterial strains; *Corynebacterium accolens* ($n = 4$), *Corynebacterium propinquum* ($n = 3$), *Corynebacterium pseudodiptheriticum* ($n = 3$), *Staphylococcus epidermidis* ($n = 4$), *Staphylococcus aureus* ($n = 4$), and *Pseudomonas aeruginosa* ($n = 4$). ** $p <$ 0.01, one-way ANOVA; SEM, standard error of the mean

Chitogel-treated side, but this was not statistically significant. Further studies looking at long-term outcomes are warranted.

Although there was no statistically significant difference in the VAS symptom scores between control and Chitogel-treated sides; there was a trend for improved scores on the Chitogel-treated side. A limitation of the VAS symptom score data was that patients had difficulty recalling differences in symptom severity between the two sides of the nose. Ryan et al. showed that there is a poor correlation between symptom scores and nasal endoscopic appearance in post-ESS CRS patients,[27] which may explain why there was no significant difference in the symptom scores despite improved endoscopic appearance on the Chitogel-treated side.

This study further reinforces the potential antimicrobial role that Chitogel has when administered in the sinuses. The in vitro work shows that in a nutrient-poor environment, similar to that of sinonasal environment, the 20% glycerol component of Chitogel negatively impacts the growth of bacterial nasal isolates commonly implicated in infection, such as *S. aureus*, *P. aeruginosa*, *C. propinquum, C. pseudodiptheriticum*, and *S. epidermidis*, while it does not impact the growth of beneficial commensal bacteria, such as *C. accolens*. It is known that *Corynebacterium* is a lipophilic bacterium that requires a lipid such as glycerol for growth.[28] In addition, glycerol is an important nutrient for *P. acnes*, and its presence has been shown to increase the production of antibacterial short-chain fatty acids.[29] The antimicrobial benefits of the other components of Chitogel have already been described. Paramasivan et al. demonstrated the anti-*S. aureus* biofilm properties of chitosan and the dextran component of the gel,[7] and Aziz et al. showed that chitosan-dextran gel had antibacterial action against *S. aureus* and *Streptococcus pyogenes*.[10]

Sample size was calculated based on a significant difference in sinus ostial size at 12 weeks, and although three patients missed one of their follow-up appointments, this study accounted for a dropout of five participants. In terms of nonprimary outcome measures, including VAS symptom scores, endoscopic scores, and microbiome data,

additional studies with a larger sample size would be warranted to further explore the effect of Chitogel.

There was some heterogenicity in the baseline characteristics of the patient population (e.g., history of diabetes mellitus, presence of polyps, smoking, steroid use); by using each patient as their own control and only comparing the difference between Chitogel and control sides within each patient, we attempted to account for the heterogenicity in the group. There were also two operating surgeons involved in this study, which could contribute to heterogenicity; this again was accounted for by ensuring each patient had an internal control side. Surgeons were only told at the end of surgery which side was randomized to receive treatment thereby preventing any difference between the two sides during surgery. There was variation in culture-directed antibiotics used postoperatively for infections, which also contributes to heterogenicity in the group; however, any antibiotic used would have had effect on both sides (Chitogel-treated and control) and would therefore be unlikely to be the cause of differences noted in the microbiome between sides.

Although a strength of the study design is that participants each had an internal control side, which allowed for excellent matching, a limitation of this design is that quality of life questionnaires with general symptom questions could not be used. Further studies looking at quality of life outcomes are warranted.

A potential cause of bias is the filler effect of Chitogel within the sinuses causing subtle differences in sensation of airflow up to 2 weeks postoperatively, after which any residual gel was toileted. However, when asked, patients were unable to identify which side Chitogel had been placed, so this is unlikely to be a significant cause of partiality.

Sinonasal packing is commonly used at the end of ESS; however, this study only compared Chitogel to nothing (no packing). Although it could be argued that improved postoperative outcomes are a result of the packing function of Chitogel, the in vitro work in this study showing the antimicrobial effect of the 20% glycerol component in Chitogel, as well as previous research showing benefits of the chitosan and dextran components,[7,10] would suggest otherwise. It is important that future studies compare postoperative outcomes following Chitogel to other sinonasal packing material.

Despite appropriate medical and surgical management, recalcitrant disease continues to be an ongoing problem for some patients with CRS. The surgical process creates a barren environment knocking out important beneficial commensals such as *C. accolens*. Recolonization of pathogenic bacteria after surgery has been associated with poorer outcomes and recurrence of disease. Patients requiring revision surgery have been shown to be significantly more likely to culture *S. aureus* and *P. aeruginosa* from their nasal cavity.[30]

In environments with limited resources, such as that of the sinonasal cavity, bacteria have been shown to compete with each other for nutrients and space and release substances that are antagonistic to their competitors.[31] The predominant nasal commensal *Corynebacterium* has been shown to disrupt growth of pathogenic bacteria and confer benefit to the host. *Corynebacterium accolens* is known to interfere with the growth of *Streptococcus pneumoniae* by releasing fatty acids into the environment through the breakdown of triacylglycerol by a secreted enzyme, triacylglycerol lipase.[28] Menberu et al. demonstrated that *C. accolens* cultured from healthy sinuses and its secreted products also interfere with the growth of *S. aureus* and MRSA cultured from patients with CRS.[6]

Shu et al. showed that *Cutibacterium* (*P. acnes*), which is an important skin commensal, has antimicrobial properties against MRSA in the presence of glycerol.[29] *P. acnes* is able to ferment glycerol into short-chain fatty acids which act destructively against MRSA.[29] It is possible that *Cutibacterium* (*P. acnes*) therefore has a beneficial role in the sinonasal microbiome and that its antimicrobial activity is increased in the presence of glycerol following use of Chitogel.

The importance of the microbiome in the sinonasal mucosal environment and the role that dysbiosis plays in disease have become increasingly recognized. What defines a healthy sinonasal microbiome is still to be explored; however, we propose that it would be composed of an increase in abundance of commensal microbiota and a decrease in pathogenic microbiota. This study shows that following the application of Chitogel, there was a significant large increase in mean relative abundance of *Corynebacterium* when compared with control. There was also a significant large increase in combined *Corynebacterium* and *Cutibacterium* (*P. acnes*) when compared with control. The lower mean relative abundance of *Staphylococcus* and *Pseudomonas* combined with the significantly reduced infection rate on the Chitogel-treated side further indicates a lower pathogenic microbiota abundance. This suggests that Chitogel plays a role in promoting a healthier sinonasal microbiome.

When comparing individual microbiome samples, it is important to consider that the microbiota abundance data are not neatly distributed around the mean, but are much more variable, as would be expected by the current knowledge of sinonasal microbiomes being composed of five core genera.[5] To account for this, a BEZI family (GAMLSS-BEZI) model was used to analyze the data, to factor in that "0" relative abundance is far more likely in sinonasal microbiome data than standard statistical distributions would assume. Therefore, the mean differences are a proxy

for a shift in the "types" of microbiomes; in other words, we have shown that there are more patients with predominant *Corynebacterium* microbiomes in the Chitogel groups. The power of this study is the microbiome data and the infection data combined.

Although increased diversity may be an indicator of a healthier microbiome in other areas, alpha diversity metrics for sinonasal microbiomes are less informative because they tend to be low.[5] We found no difference in alpha diversity between Chitogel and control microbiomes. This was similarly noted in a comprehensive international study in which the sinonasal microbiomes from 410 healthy and CRS patients were sequenced; the authors concluded that there were no significant differences in alpha diversity when comparing between disease states (healthy control, CRSsNP, and CRSwNP patients).[5]

There has been emerging therapeutic interest in promoting a beneficial microbiome in patients. Following the use of Chitogel, a significant increase in the mean relative abundance of *Corynebacterium* and *Cutibacterium* (*P. acnes*) in the microbiome was noted at 12 weeks post ESS up to median follow-up of 1 year. This was reinforced with a reduced infection rate on the Chitogel-treated side. Chitogel appears to promote an improved microbiome composition, further demonstrated by the antimicrobial effect that 20% glycerol has on pathogenic bacteria.

Many of the benefits of the absorbable nasal dressing Chitogel have been reported, such as decreased sinus ostia stenosis, decreased adhesions, and its use as a hemostatic agent.[8,32] Our study similarly has shown improved nasoendoscopic appearance and improved sinus ostial patency at 12 weeks postoperatively following use of Chitogel. This is the first clinical study looking at the effect of Chitogel on the microbiome and postoperative infections.

5 | CONCLUSION

Chitogel significantly improves both the nasoendoscopic appearance of the sinuses and sinus ostial patency at 12 weeks postoperatively. Chitogel used following ESS has shown to significantly increase the proportion of beneficial *Corynebacterium* and *Cutibacterium* (*P. acnes*) at 12 weeks postoperatively and up to a median follow-up of 1 year, likely resulting in a healthier microbiome. A significant decrease in postoperative infections is noted following use of Chitogel. In vitro work shows that the concentration of glycerol in Chitogel (20%) inhibits the growth of pathogenic bacterial isolates, while promoting the growth of healthy bacteria, which may play a role in strengthening the normal sinonasal microbiome.

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[Correction added on 16 May 2022, after first online publication: CAUL funding statement has been added.]

CONFLICTS OF INTEREST

PJW is a shareholder in Chitogel, receives royalties from Medtronic and Integra, and is a consultant for Neurent, NeilMed, and Stryker.

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