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Differential Nasal Recolonization and Microbial Profiles in Chronic Rhinosinusitis With Nasal Polyps Patients After Endoscopic Sinus Surgery or Dupilumab Treatment: A Prospective Observational Study

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ABSTRACT

Introduction: The role of microbial profiles in Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) pathogenesis is increasingly recognised, with microbial imbalances perpetuating inflammation. We performed this study to associate the different nasal microbiological profile changes with the response to surgical or monoclonal treatment.

Methods: This prospective observational study evaluated changes in the nasal microbial profiles of 44 patients (22 dupilumab, 22 surgery) over 6 months. Clinical assessments were performed at baseline and follow-ups, including Sino-Nasal Outcome Test-22 (SNOT-22) scores and Sniffin Sticks-Identification (SS-I) olfactory testing. Microbial profiling of nasal swabs was carried out by microbial culture and subsequent molecular identification by Polymerase chain reaction (PCR) and sequencing.

Results: Baseline characteristics of 44 patients (22 dupilumab, 22 surgery) enrolled in this study were similar between groups. In the dupilumab group, *Staphylococcus epidermidis* prevalence rose from 37.03% to 59.25%, while *Pseudomonas aeruginosa* was eradicated. Moreover, dupilumab stabilised *Staphylococcus aureus* at 63.64%, while its prevalence increased in the surgery group (from 22.72% to 50%). When bacterial groups were associated with clinical scores, *P. aeruginosa* carriers had worse SNOT-22 (21.00 ± 1.41) and SS-I (5.50 ± 0.71) scores. Instead, *S. epidermidis*-colonised patients exhibited significantly lower mean SNOT-22 (15.39 ± 8.54) and greater SS-I scores (8.39 ± 3.77). The best outcomes were found in the subgroup of *S. epidermidis* carriers undergoing the dupilumab treatment.

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Conclusion: The two treatments modulated the microbial profiles differently, and, most importantly, clinical responses might depend on the association between treatment and the dominant bacterial species colonising the nasal cavity. Further investigation into microbial-restorative strategies could enhance outcomes for better treatment of CRS.

Summary

- Nasal microbial profiles may change after 6 months of treatment with either dupilumab or surgery in patients with severe CRSwNP.
- Dupilumab treatment may eradicate *Pseudomonas aeruginosa* and stabilise *Staphylococcus aureus*, while surgery could lead to increased *S. aureus* colonisation.
- Patients colonised with *P. aeruginosa* may present worse symptom scores, while *S. epidermidis* carriers may report better outcomes.
- Microbial profiles may influence CRS treatment outcomes, highlighting the potential for personalised, microbiota-targeted therapies.

1 | Introduction

The human nasal microbiota is vital for maintaining health [1] and influences disease states [2, 3]. Imbalances of the nasal microbiota, defined as dysbiosis, can perpetuate inflammation and contribute to the persistence of Chronic Rhinosinusitis (CRS) symptoms [4–6]. Nowadays, there is a growing interest in novel restorative treatments to reestablish a healthy microbiota [7, 8]. Grasping how treatments affect nasal microbial colonisation may significantly shape CRS management, outcomes, and therapeutic effectiveness [9, 10]. Moreover, type 2 inflammation might be associated with different pathogens colonisation, such as *Staphylococcus aureus* or *Pseudomonas aeruginosa* [11, 12]. Dupilumab, an interleukin-4 receptor alpha antagonist, has emerged as a promising option [13], inhibiting the specific signalling of type 2 inflammation. This prospective observational study aimed to investigate and compare the changes in nasal repopulation in treatment-naive patients with severe Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) after either surgical or dupilumab therapy. Furthermore, our study wanted to link variations in microbial profiles with clinical outcomes, in order to better understand the important role of different bacterial species in CRSwNP.

2 | Materials And Methods

2.1 | Study Design

This study was a prospective, parallel-group observational study following the EQUATOR (Enhancing the QUAlity and Transparency Of health Research) guidelines (https://www.equator-network.org/) and conducted following the STROBE (Strengthening the Reporting of Observational studies in Epidemiology) checklist [14]. The study was approved by the Human Medical Research and Ethics Committee of the University of Catania and was conducted under the Declaration of Helsinki (code 24121-21/05/2021).

2.2 | Setting

All patients enrolled were recruited in our tertiary otolaryngological center from January 2021 to July 2023.

2.3 | Participants

Inclusion criteria were: (1) adults aged ≥18 years; (2) diagnosis of severe CRSwNP according to the guidelines of the latest European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) [3]; (3) failure of standard medical therapy, including intranasal corticosteroids (INCS) and short courses of oral corticosteroids (OCS); (4) no previous sinonasal surgery; (5) type 2 inflammatory profile with Serum IgE levels ≥150 IU/mL and increased blood eosinophil counts ≥300 cells/μL; and (6) able and willing to provide informed content. Exclusion criteria were autoimmune diseases genetic, congenital, systemic diseases affecting the respiratory tract, concurrent pregnancy or breastfeeding or acquired immunodeficiencies; active neoplasms; previous chemoradiation therapies; known previous or existing non-CRS related olfactory disorders; other ongoing biologic therapies. Patients who satisfied every inclusion requirement and none of the exclusion requirements could participate in the research. Eligible subjects were assigned to one of the two groups based on their treatment: a dupilumab group (treatment group) and a surgical group (comparison group). Patient recruitment and study flow are illustrated in Figure 1. A clinical and endoscopic nasal evaluation was performed in all eligible patients to confirm the diagnosis of CRSwNP and disease stage according to the latest EPOS guidelines [3]. The nasal microbial profile composition in surgery-naive patients at baseline and after the respective treatment (either dupilumab or surgery treatment) was assessed. All patients presented failure after medical therapy, including INCS and short courses of OCS [3]. The treatment group participants were treated with dupilumab at a dosage of 300 mg subcutaneously bi-weekly (utilising a safety syringe) for a period of 6 months. Conversely, surgical patients underwent Functional endoscopic sinus surgery (FESS) using the Messerklinger technique, preserving the middle turbinate, by one experienced rhinology surgeon. The surgery extent was determined based on the Computed tomography (CT) scan findings. After the procedure, expandable sponges (Merocel, Medtronic-XOMED, Jacksonville, FL) were packed and left in place for 24 to 48 h before removal. Following this, normal saline lavage was applied for 2 to 3 months.

2.4 | Variables

The primary objective of this research was to examine and compare changes in nasal microbial profiles in treatment-naive patients with severe CRSwNP over a 6-month period after endoscopic sinus surgery (ESS) or dupilumab therapy. We adopted as primary outcomes: (1) the variations from

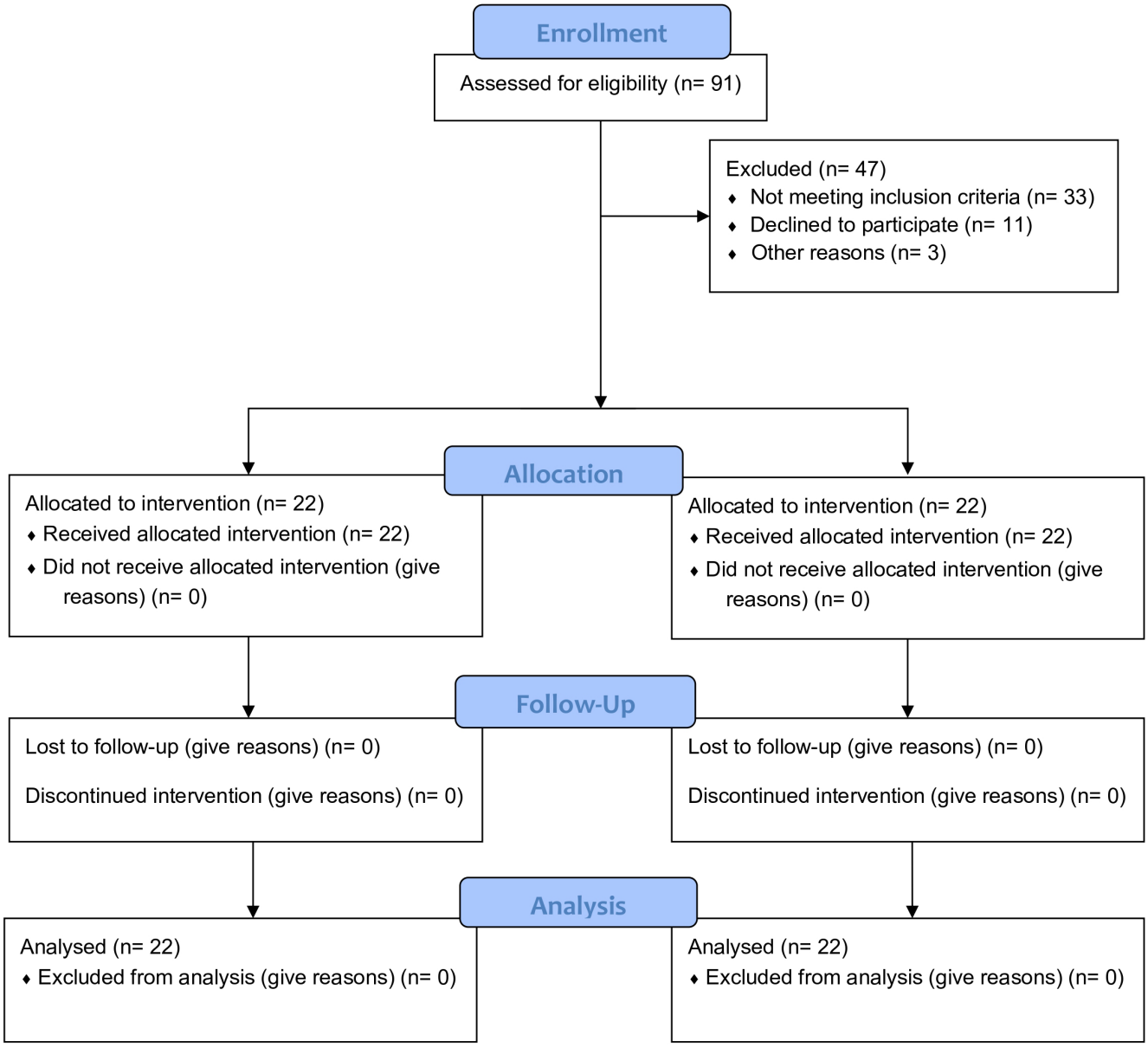

FIGURE 1 | Consort flow diagram for the study protocol.

baseline to 6-month post-treatment prevalence of key bacterial species (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*); (2) the association between variations in microbial profiles and clinical outcomes, as measured by: (a) Sinonasal Outcome Test (SNOT-22) scores; (b) Nasal Polyp Score (NPS); and (c) Sniffin' Sticks-16 Items (SS-I) olfactory test scores. Conversely, we considered as secondary outcomes: (1) the variations in the microbiological profile between the groups receiving dupilumab and surgery and (2) changes in additional clinical parameters, including Visual Analog Scale (VAS) scores for rhinorrhea, headache, and nasal obstruction. We assessed all outcomes at baseline and at 1-, 3-, and 6-months post-treatment.

2.5 | Data Sources/Measurement

We evaluated participants at baseline and follow-up visits set at 1, 3, and 6 months. Each patient underwent a pulmonological evaluation, and asthma diagnosis was made according to the latest guidelines [9]. We assessed symptoms according to the visual analog scale (VAS) from 0 (no symptoms) to 10 (most severe symptoms) for nasal obstruction, headache, and rhinorrhea. Type 2 inflammation was evaluated at baseline with laboratory tests for blood markers (eosinophils count (EOS) and immunoglobulin E (IgE), according to the EPOS 2020 guidelines) [3]. We evaluated nasal polyp size (NPS) [10] by nasal endoscopy with a 2.7-mm flexible endoscope (Olympus, Germany). We assessed the sense of smell through the identification subset of the Sniffin Sticks- 16 items (SS-I) (Burghart, Wedel, Germany) [15]. The Minimal Clinically Important Difference (MCID) for olfactory recovery was established as an increase of at least 3 points based on the findings of Gudziol et al. [16] The hyposmia cut-off was consequently normalised according to age-related values of the SS-I domain described by Oleszkiewicz et al. [17] The impact of CRSwNP on HRQoL was assessed with the Sinonasal Outcome Test (SNOT-22) [18].

Nasal swabs were gathered from January 2021 to July 2023, using CultureSwab (BD, Franklin Lakes, NJ). The swabs were endoscopically directed toward the ethmoid area, an adjacent sinus, or both when pus was detected [5]; measures were implemented to prevent contamination from the anterior nasal cavity during probing. After at least five complete cycles of rotation to complete saturation, the probes were placed in sterile Eppendorf tubes containing 2 mL of saline solution NaCl 0.9% and delivered for microbiological analysis. To reduce confounding variables for our microbiota research, a period of medication washout was implemented, during which topical nasal medicines were stopped 2 weeks before surgery and oral antibiotics were stopped 4 weeks beforehand. During the week before surgery, patients were told to only utilise saline nasal irrigations. We prepared the outer part of the nose with chlorhexidine, carefully avoiding the nasal vestibule to protect the native microbiota state. Before any surgical procedures or the application of anaesthetic, microbiota samples were taken. Following surgery, we standardised care by delaying regular antibiotic prescriptions and starting saline irrigations after 24 h.

Nasal swabs were processed for routine microbiological cultures. The collected sample was plated on Tryptic Soy Agar (Oxoid, Basingstoke, UK) with 5% horse blood (Thermo Scientific, Basingstoke, UK) and cultured overnight at 37°C in 5% CO_2, and on selective media Mannitol Salt Agar and MacConkey Agar (Oxoid, Basingstoke, UK) both incubated overnight at 37°C in aerobic conditions. For anaerobic conditions, plates were cultivated using anaerobic bags (Biomeriux, France) at 37°C for 48 h. Subsequently, each morphologically different colony was identified by biochemical tests using the API Biochemical Gallery system (Biomeriux, France): API Staph for *Staphylococcus* spp., API 20E for enteric Gram-negative bacteria, and API 20NE for non-enteric ones. The biochemical identification was corroborated by molecular identification by polymerase chain reaction (PCR) amplification and sequencing of the 16S rRNA [19], tuf (TUF-F/TUF-R) for *Staphylococcus* spp. [20] and 68d and DG74 for Gram-negative bacteria for accurate identification [21]. Genomic DNA was extracted using a PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions. All PCR products were purified using the QIAquick PCR gel extraction kit (Qiagen, Germany) and sequenced. Sequence analyses were performed using Gapped BLAST [22], identifying each strain at the species level with a sequence identity of > 99%.

2.6 | Bias

The microbiologist evaluating the nasal microbiological samples was unaware of the treatment group to minimise potential observer bias.

2.7 | Study Size

The study sample size was calculated assuming 95% confidence, a p value < 0.05, a power of 0.8, and a mean SNOT-22 difference set at 2.0. As a result, a minimum of 30 patients (15 for each group) were needed to be included. Additionally, a 30% dropout rate was factored in, reaching a total of 44 patients.

2.8 | Quantitative Variables

We utilised standard descriptive statistics, presenting the mean and standard deviation for continuous variables and percentages for categorical variables.

2.9 | Statistical Methods

The independent t-test was used for values with a normal distribution, while the Mann–Whitney U test was conducted for values with a non-normal distribution. The chi-square test was used to assess the divergence between the observed and expected data. Violin plots were subsequently produced using SS-I, NPS, and SNOT-22 scores at baseline and follow-up, and the Kruscall–Wallis test was performed to assess intergroup differences. A p value < 0.05 was deemed statistically significant. All analyses were conducted using statistical software for the social sciences (IBM SPSS Statistics for Windows, IBM Corp., Version 29.0, Armonk, NY: IBM Corp).

TABLE 1 | Demographic features and clinical parameters of patients enrolled in this study.

	Dupilumab ($n = 22$)	Surgery ($n = 22$)	p
Age	52.09 ± 8.55	48.68 ± 12.08	0.262
Sex	17 M/5F (77.27)	12 M/10F (54.54%)	0.469
BMI	27.04 ± 2.1	27.63 ± 1.89	0.405
Blood eosinophilia	521.87 ± 74.58	502.13 ± 62.41	0.445
IgE total (kU/L)	399.75 ± 120.23	378.23 ± 117.67	0.103
Aspirin Intolerance	4/22 (18.18%)	3/22 (13.63%)	0.725
N-ERD	3/22 (13.63%)	2/22 (9.09%)	0.671
Comorbidities			
Atopy	14/22 (63.63%)	16/22 (72.72%)	0.778
Asthma	9/22 (40.9%)	8/22 (36.36%)	0.836

Abbreviations: BMI, body mass index; N-ERD, NSAIDs Exacerbated Respiratory Disease.

3 | Results

3.1 | Participants

We enrolled a total of 44 patients, with 22 in the dupilumab group and 22 in the surgery group.

3.2 | Descriptive Data

At baseline, surgery group was comparable to dupilumab in terms of age (48.68 ± 12.08 vs. 52.09 ± 8.55; $p = 0.262$), sex (77.27% vs. 54.54%; $p = 0.469$), and BMI (27.04 ± 2.1 vs. 27.63 ± 1.89; $p = 0.405$) (Table 1). Furthermore, no significant differences were observed in clinical symptoms ($p > 0.05$ for all) or comorbidities as atopy ($p = 0.778$) and asthma ($p = 0.836$).

3.3 | Outcome Data

At the 6-month follow-up, both dupilumab and surgery groups significantly reduced all clinical parameters during intragroup analysis (Table 2).

3.4 | Main Results

3.4.1 | Changes in Clinical Outcomes

The surgery group reported superior improvements than dupilumab at intergroup analysis for NPS (1.23 ± 1.52 vs. 0.45 ± 0.59; $p = 0.198$). Conversely, scores for SNOT-22 (13.36 ± 9.46 vs. 19.13 ± 6.01; $p = 0.007$), SSIT (10.45 ± 3.67 vs. 6.36 ± 1.39; $p < 0.001$), VAS Obstruction (1.27 ± 0.63 vs. 1.95 ± 1.25; $p = 0.012$) and VAS Rinorrhea (0.77 ± 0.68 vs. 1.31 ± 0.89; $p = 0.014$) were better in the dupilumab group.

3.4.2 | Changes in Bacterial Species Prevalence

First of all, the microbiological analysis of nasal swabs underlined the prevalence of *Staphylococcus aureus* (approximately 10^6 CFU/mL) and *Staphylococcus epidermidis* (approximately 10^6 CFU/mL), while for Gram negative bacteria *Pseudomonas aeruginosa* was more prevalent (10^4 CFU/mL) (Table 2). Overall, the presence of *S. aureus* rose modestly from 43.18% to 56.82% after 6 months ($p = 0.200$), while *P. aeruginosa* decreased from 15.91% to 4.55% ($p = 0.078$). Interestingly, a striking increase in *S. epidermidis* occurred after 6 months, nearly doubling from 36.36% to 75% ($p < 0.001$). Other bacterial species were grouped together as they were less prevalent than the three key species (Data S1), and their prevalence showed only minor fluctuations through time, shifting from 68.18% to 65.91% ($p = 0.820$). At subgroup analysis, the prevalence of *S. aureus* in the dupilumab group remained steady at the 6-months follow-up (63.64%). Conversely, the surgery group showed an increase from 22.72% to 50% after 6 months ($p = 0.097$). Interestingly, *P. aeruginosa* was completely eradicated in the dupilumab group, from an initial 27.27% to 0%. In contrast, the surgery group experienced an increase from 4.54% at baseline to 9.09% ($p = 0.576$). The prevalence of *S. epidermidis* increased in both groups: with dupilumab it rose from 45.45% to

TABLE 2 | Subgroup outcomes comparison.

	Total ($n = 44$)			Dupilumab ($n = 22$)			Surgery ($n = 22$)		
	Baseline	6 months	*p*	Baseline	6 months	*p*	Baseline	6 months	*p*
Blood eosinophilia	512.45 ± 78.42	543.26 ± 84.15	0.014	521.87 ± 74.58	556.14 ± 66.79	0.114	502.13 ± 62.41	446.31 ± 105.38	0.018
NPS	5.52 ± 0.99	1.23 ± 1.52	< 0.001***	5.77 ± 0.97	2 ± 1.79***	< 0.001	5.27 ± 0.98	0.45 ± 0.59***	< 0.001
SNOT 22	56.16 ± 15.50	16.14 ± 8.41	< 0.001***	55.22 ± 16.68	13.36 ± 9.46***	< 0.001	57.09 ± 14.93	19.13 ± 6.01***	< 0.001
SSIT Score	3.02 ± 1.95	8.41 ± 3.40	< 0.001***	2.9 ± 2.09	10.45 ± 3.67***	< 0.001	3.13 ± 1.88	6.36 ± 1.39***	< 0.001
VAS Obstruction	7.82 ± 1.27	1.61 ± 1.03	< 0.001***	7.95 ± 1.25	1.27 ± 0.63***	< 0.001	7.68 ± 1.32	1.95 ± 1.25***	< 0.001
VAS Rinorrhea	7.07 ± 1.14	1.05 ± 0.82	< 0.001***	7.18 ± 1.13	0.77 ± 0.68***	< 0.001	6.95 ± 1.17	1.31 ± 0.89***	< 0.001
VAS Headache	5.20 ± 0.97	1.27 ± 1.01	< 0.001***	5.36 ± 1.09	1.5 ± 1.05***	< 0.001	5.04 ± 0.84	1.04 ± 0.95***	< 0.001
S. aureus	19 (43.18%)	25 (56.82%)	0.200	14 (63.64%)	14 (63.64%)	—	5 (22.72%)	11 (50%)	0.097
P. aeruginosa	7 (15.91%)	2 (4.55%)	0.078	6 (27.27%)	0*	—	1 (4.54%)	2 (9.09%)	0.556
S. epidermidis	16 (36.36%)	33 (75%)	< 0.001***	10 (45.45%)	16 (72.73%)	0.05*	6 (27.27%)	17 (77.27%)	0.007**
Other aerobic species	30 (68.18%)	29 (65.91%)	0.820	13 (59.09%)	20 (90.90%)	< 0.001***	17 (77.27%)	9 (40.90%)	0.061

Note: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ at intragroup analysis for each follow up.
Abbreviations: NPS, nasal polyp score; SNOT-22, Sino-nasal outcome test; SSIT, Sniffin sticks identification test; VAS, visual analogue scale.

TABLE 3 | Subgroup species analysis of baseline and 6-months nasal outcomes.

	S. aureus	*P. aeruginosa*	*S. epidermidis*	Others
Baseline				
NPS	5.63 ± 1.16	6.00 ± 1.00	5.67 ± 0.97	5.50 ± 1.01
SNOT-22	56.74 ± 15.67	52.71 ± 18.45	57.17 ± 16.54	55.00 ± 15.34
SS-I	3.05 ± 2.19	2.71 ± 1.79	2.83 ± 2.06	3.20 ± 1.90
6-months				
NPS	1.32 ± 1.67	1.50 ± 0.71	1.33 ± 1.45	1.54 ± 1.63
SNOT-22	20.40 ± 5.61[a]	21.00 ± 1.41[b,c]	15.39 ± 8.54[a,b]	15.81 ± 9.02[c]
SS-I	7.52 ± 2.16[d]	5.50 ± 0.71[d,e,f]	8.39 ± 3.77[e]	8.65 ± 3.68[f]

Note: [a,b,c]$p < 0.05$; [d,e,f]$p < 0.001$.

72.73% ($p = 0.05$), while with surgery it escalated from 27.27% to 77.27% ($p = 0.007$). Other bacterial species had different trends in the two groups: for dupilumab, an increase from 59.09% at baseline to 90.90% ($p = 0.001$) was observed, whereas it decreased in the surgery group, going from 77.27% to 40.90% after 6 months ($p = 0.061$).

3.4.3 | Association Between Bacterial Species and Clinical Outcomes

Patients with *P. aeruginosa* had higher SNOT-22 scores (21.00 ± 1.41) and lower SS-I scores (5.50 ± 0.71) than other species analysed at follow-up (Table 3). Conversely, patients with *S. epidermidis* showed lower SNOT-22 scores among all groups and greater improvements in olfaction than *S. aureus* and *P. aeruginosa*. No significant differences were found in SS-I scores between the different groups at the Kruskal–Wallis test (Figure 2a–f).

3.4.4 | Other Analyses

Subgroup analysis for the dupilumab group showed that patients with *S. aureus* had significantly worse SS-I (8.21 ± 2.52 vs. 10.88 ± 3.96; $p = 0.024$, respectively) and SNOT-22 outcomes than *S. epidermidis* ones (18.57 ± 7.79 vs. 11.94 ± 9.07; $p = 0.037$, respectively). No significant differences were observed between groups for the NPS. In the surgical group sub-analysis, only the SNOT-22 scores indicated a significant difference between *S. aureus* and *S. epidermidis* patients (22.73 ± 4.13 vs. 19.36 ± 5.08; $p = 0.034$), while SS-I scores were not significantly different (6.64 ± 1.21 vs. 6.18 ± 1.08; $p = 0.360$).

4 | Discussion

4.1 | Key Results

This study contributes to the growing research literature on the relationship between nasal microbiota and CRS treatments by providing a direct comparison between surgical intervention and dupilumab therapy. While the significance of nasal microbiota in CRS patients has been studied in the past, our findings provide new insights into how these two different treatment approaches may change microbial profiles differently over time [23–26]. Our study observed precise shifts in microbial profiles over 6 months linked to surgical and dupilumab treatments. Overall, the colonisation of *S. aureus* moderately increased from 43.18% to 56.82% ($p = 0.200$), *S. epidermidis* colonisation went from 36.36% to 75%, and *P. aeruginosa* decreased from 15.91% to 4.55% ($p = 0.078$). The rate of *S. epidermidis* colonisation increased in both groups; moreover, our findings reveal a novel insight into the positive role of *Staphylococcus epidermidis* on nasal outcomes. In contrast, other bacterial species showed a different trend (increased with dupilumab, decreased with surgery treatment). Despite dividing the participants into two selected groups, with dupilumab the colonisation rate for *S. aureus* remained constant at the 6-month follow-up, while it increased notably in the surgery group. As previously described [24], this increase might be explained by surgical impact on nasal microbial populations. Jain et al. reported changes in bacterial composition and abundance in the middle meatus after ESS, with increased bacterial richness for the *Staphylococcus* genus ($p = 0.002$) compared to other taxa [25]. *S. aureus* colonisation has also been linked to worse patient outcomes after ESS [23]. Our data suggests that ESS might lead to a significant shift toward *S. aureus* in the nasal cavity repopulation. Contrarily, dupilumab treatment appears to stabilise the different bacterial populations. Additionally, the dupilumab treatment showed a complete eradication of *P. aeruginosa* from 27.27% (6 patients) to 0%, which might be involved in better outcomes for CRS patients [24]. Various factors, such as the environmental context and individual characteristics like immune responses [13], could shape this dynamic response. Probably, dupilumab treatment can trigger a more immediate shift in the nasal microbiota to a healthier repopulation, inhibiting type 2 inflammation. Nevertheless, the precise influence of dupilumab on microbial profiles remains unclear and necessitates further exploration.

Our results have substantial implications for the treatment of CRS. Both dupilumab and surgery groups had better outcomes in SNOT-22, NPS, and SS-I scores compared to baseline, confirming the success of these treatments. Nonetheless, although when analysed together no bacterial species significantly impacted NPS and SNOT over the 6 months, different results were found in the treatment sub-analysis. The surgical group showed a significant trend only in the SNOT-22 scores, with worse outcomes for *S.*

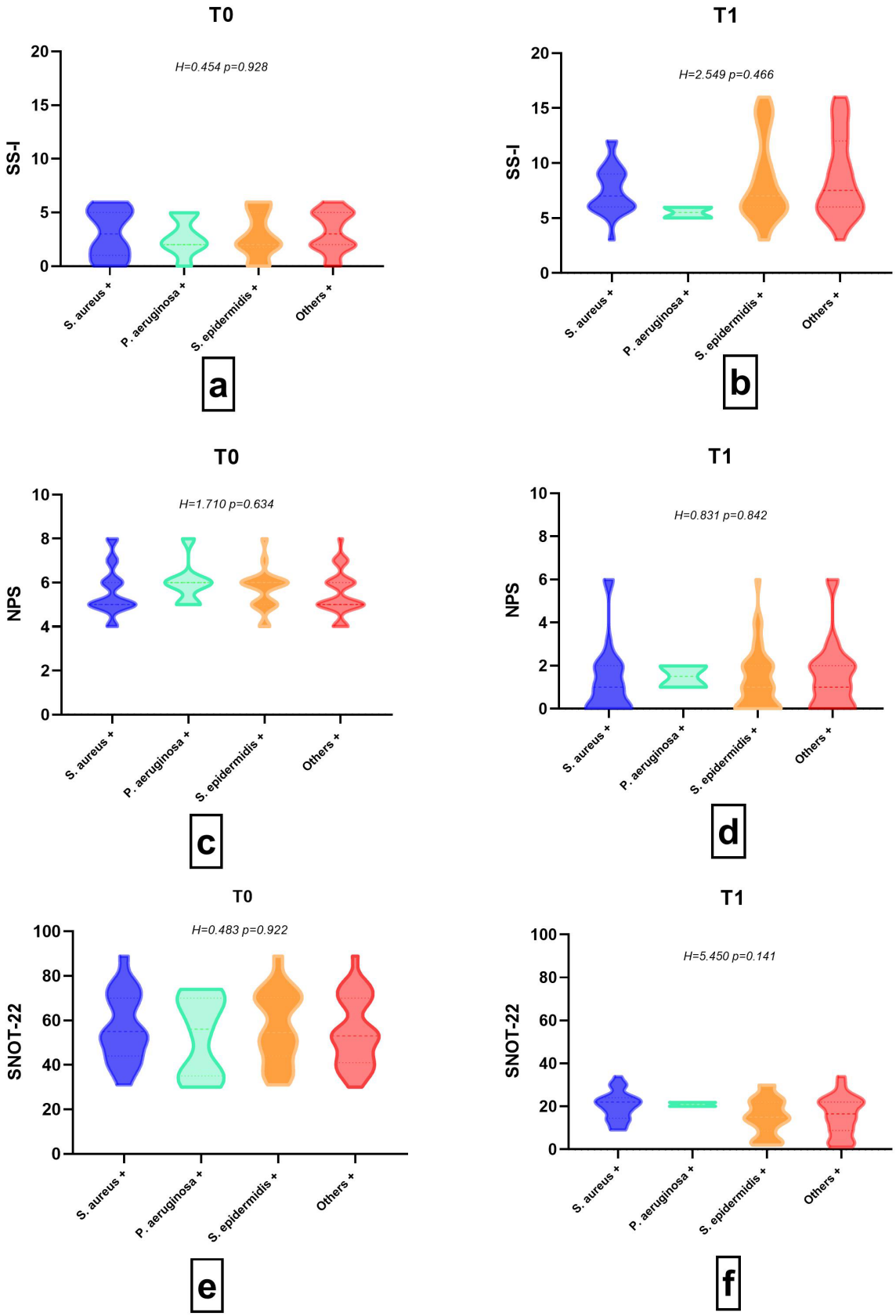

FIGURE 2 | Baseline vs. 6-months outcomes comparison divided by bacterial species, represented by Violin Plot. (a) SS-I baseline; (b) SS-I after 6 months; (c) NPS baseline; (d) NPS after 6 months; (e) SNOT-22 baseline; and (f) SNOT-22 after 6-months.

aureus than *S. epidermidis* and other bacterial species ($p < 0.05$ for both). Moreover, in the dupilumab group, *S. aureus* colonisation was also linked to worse results in SNOT-22 and SS-I scores compared to other bacterial groups like *S. epidermidis* ($p = 0.037$ and $p = 0.024$, respectively) and other bacterial species ($p = 0.498$ and $p = 0.202$, respectively).

4.2 | Interpretation

As previously described, *S. aureus* produces proinflammatory substances connected to type 2 host inflammation. Kanemitsu et al. [27] have analysed the correlation between sensitization to mould and/or *S. aureus* enterotoxins and CRS surgical outcomes. All type 2 biomarkers were found to be significantly higher in patients with moulds or *S. aureus* sensitization compared to the comparison group ($p < 0.05$). Notably, in the dupilumab group our study found a link between the presence of *S. epidermidis* and improved nasal perspective outcomes (SNOT-22 and SS-I) compared to the *S. aureus* group ($p < 0.05$ for both). This suggests that *S. epidermidis* may play a beneficial role in protecting the nasal environment and preventing the colonisation of harmful bacteria. Lastly, no significant differences were observed for the Nasal Polyp Score (NPS), with excellent results at follow-up regardless of treatment or species identified.

4.3 | Limitations

Our 6-month longitudinal study faced challenges, including insufficient duration to capture long-term microbial changes or the full impact of treatments. We sought to capture stable changes in the nasal microbiota after intervention by focusing on 6-month outcomes. We do acknowledge that, in the early post-intervention period, this approach does not allow us to characterise the trajectory of changes in the microbial community. These extra time points could be analysed in future research with more resources to give a more complete picture of the dynamics of the microbial population after CRSwNP treatments. In addition, it's crucial to remember that a large number of the comparisons in this study were not statistically significant. Moreover, the study's observational design could be the cause of this issue. The stringent selection criteria resulted in a small cohort, limiting the statistical power for detailed microbiological analyses and reducing subgroup sizes. Additionally, the culture method may not have effectively isolated fastidious or low-abundance microbes. The variable response to CRS and dupilumab treatments further complicated microbial profile comparisons. Uncontrolled variables, such as patient health, lifestyle, and comorbidities, may also skew outcomes, cautioning against broad generalisations of our findings. Despite our best efforts to account for confounding variables, our protocol might not accurately represent clinical situations in the real world, especially for surgical management with antiseptics or antibiotics. To measure the effect of conventional methods on nasal microbial populations, future research might compare this 'microbiota-preserving' technique with standard preoperative protocols. Although the observed trends offer insightful information about possible distinctions between surgical and dupilumab treatment, these results should be evaluated cautiously and confirmed in bigger, more rigorous trials. Lastly, our exploratory design aimed to enhance understanding rather than test specific hypotheses, highlighting the intricacies of prospective study design and interpretation.

4.4 | Generalizability

Despite these promising insights into the potential differences between surgical and dupilumab treatments for CRSwNP, additional research is required to validate these associations and explore the practicality and effectiveness of microbiota-focused therapies in managing CRS.

5 | Conclusion

This study shines a light on the potential influence of microbial profiles on the development and treatment outcomes of CRS. These findings suggest the potential benefits of personalised CRS treatments, which would be tailored to the patient's individual microbial profile.

More extensive research involving a wider range of patient populations is required to validate and enhance these results.

Author Contributions

G.V.A., M.S., and I.L.M. validation. A.M., G.V.A., M.S., and I.L.M. formal analysis. A.M., G.V.A., M.S., and I.L.M. investigation. A.M., G.V.A., M.S., and I.L.M. resources. A.M., G.V.A., M.S., and I.L.M. data curation. A.M., G.V.A., M.S., and I.L.M. writing – original draft preparation. A.M., G.V.A., M.S., and I.L.M. writing – review and editing. A.M., G.V.A., M.S., and I.L.M. visualization. A.M., G.V.A., M.S., and I.L.M. supervision. A.M., G.V.A., M.S., and I.L.M. project administration. A.M., G.V.A., M.S., and I.L.M. All authors have read and agreed to the published version of the manuscript.

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Consent

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.