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Correlations between Postoperative Changes in Nasal Microbes and Recurrence After Chronic Rhinosinusitis

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ABSTRACT The researchers aimed to analyze the correlations of nasal microbiome variations with postoperative recurrence of patients with chronic rhinosinusitis (CRS). Fifty patients receiving surgery for CRS were included. The conditions of patients with and without postoperative recurrence were evaluated by visual analogue scale and Lund-Mackay scores. The nasal bacterial composition before and after operation was analyzed by 16srDNA technology. Preoperative and postoperative operational taxonomic units showed no significant differences. At the phylum level, no significant differences were found in the dominant bacteria before and after operation. At the genus level, Moraxella and Neisseria had significantly higher proportions after operation than those before operation. Bacteroides, Pseudomonas and Streptococcus in Proteobacteria, Firmicutes and Bacteroidetes had significant differences between patients with and without postoperative recurrence. Bacteroides, Pseudomonas and Streptococcus were correlated with postoperative recurrence. Bacteroides, Pseudomonas and Streptococcus may be implicated in the poor prognosis and postoperative recurrence, as the predictors of CRS.

INTRODUCTION

Chronic rhinosinusitis (CRS) is a common and frequently-occurring disease (Hopkins et al. 2022), and has an overall incidence rate of 8 percent in China (Shi et al. 2015). CRS with nasal polyps, as a chronic heterogeneous inflammatory disease of the sinuses, has a global incidence rate of 1-4 percent (Bachert et al. 2020). Such patients often suffer from common nasal symptoms, accompanied by headache, tiredness, listlessness, dizziness, memory decline and inattention (Aldajani et al. 2022). The symptoms easily relapse, posing serious threats to the quality of life of patients (Arancibia et al. 2022). CRS has various etiologies, among which bacterial infection is a main factor (Stevens et al. 2016). Nevertheless, the positive rate of bacterial culture is not high, which may be attributed to obscured lesions and numerous types of bacteria. Bacterial flora is abundant in the nasal cavity, and changes in CRS patients (Bankova et al. 2020). However, the influence of surgery on the bacterial flora in CRS patients remains unclear. Furthermore, the role of bacterial flora in the clinical diagnosis and treatment of CRS also needs further research.

Nucleic acid sequence analysis has been applied in bacterial identification, phylogeny and classification (Gorgannezhad et al. 2019). 16S ribosomal deoxyribonucleic acid (rDNA) sequences have both conservative and highly variable regions, providing an important molecular basis for species identification and phylogenetic relationship (Ursenbach et al. 2021). Molecular biology technology with 16S rDNA as the target is capable of precisely determining the species and genetic diversity of microbes (Copeland et al. 2018).

Objectives

In this study, therefore, 50 patients with CRS were enrolled, and 16S rDNA technology was utilized to analyze the distribution of pathogens in their nasal secretions before and after operation, as well as the correlations of pathogens with postoperative recurrence. The aim of this study was to improve the diagnosis, prevention and treatment of CRS patients.

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MATERIAL AND METHODS

Baseline Clinical Data

This study was conducted after the approval of the hospital ethics committee (approval No. PHA202001003). According to the *Chinese Guidelines for Diagnosis and Treatment of Chronic Rhinosinusitis (2018)*, 50 patients diagnosed as CRS by computed tomography (CT) imaging to undergo surgery from January 2020 to December 2021 were enrolled as the subjects. The patients had no medication such as systemic antibiotics and hormones within 8 weeks before operation. Following diagnosis and treatment based on the guidelines and 2-month follow-up, conventional bacterial culture and 16S rDNA sequence analysis were carried out to explore the species and alterations of bacterial spectrum after treatment. The degree of lesions was evaluated by preoperative and postoperative visual analogue scale (VAS) score on rhinosinusitis and paranasal sinus CT Lund-Mackay score. Informed consent form was signed voluntarily by all the enrolled patients.

Exclusion criteria: i) Patients with immune deficiency, benign or malignant tumors, fungal rhinosinusitis or cystic fibrosis; ii) those who were in pregnancy or lactation; iii) those who recently received medication such as systemic antibiotics or hormones before operation; or iv) those who refused to participate in this study.

Presence of following conditions during postoperative follow-up indicated recurrence: i) chief complaint: nasal foreign body, ii) symptoms such as runny nose and nasal congestion, iii) soft tissue shadows in the nasal cavity, as well as high density shadows and increased mucosa in nasal sinuses revealed by CT imaging.

Among the 50 patients, there were 26 males and 24 females aged 25-60 years old, average age 45.62±2.67 years. Their baseline clinical data showed no significant difference (P>0.05) (Table 1). 16S rDNA sequence analysis was performed before operation, and all the patients underwent "nasal polypectomy/correction of nasal septum + opening of affected sinus" under a nasal endoscope. Then standardized drug therapy (second-generation cephalosporin, local hormone, eucalyptus, etc.) was administered. Two months after operation, 16S rDNA sequence analysis was conducted once again to analyze the bacteria in nasal secretions.

Table 1: Baseline clinical data of patients

Baseline clinical data	*Case*
Age (mean)/Y	45.62 ± 2.67
Gender	
Male	26
Female	24
Smoking	
Yes	9
No	41
Asthma	
Yes	5
No	45
Preoperative VAS score	6.12 ± 1.63
Preoperative Lund-Mackay score	8.03 ± 2.05

VAS: Visual analogue scale

Sample Collection

Before and after operation, secretion specimens were randomly selected from the middle nasal tract or anterior ethmoidal area of each patient using a swab with a sheath. After rapid cooling with liquid nitrogen, the specimens were stored in a refrigerator at -80°C for subsequent DNA extraction.

DNA Extraction, PCR Amplification and Sequencing

DNA extraction and PCR were carried out in an HEPA-filtered and UV-purified laminar flow hood. Firstly, ammonium acetate (7.5 mol/L) and 100 percent isopropanol at a volume ratio of 0.5:1 were added to precipitate DNA, followed by incubation at -80°C for 10 min and centrifugation (>14,000 ×g) for 25 min. After washing with 250 mL of 70 percent ethanol solution and centrifugation (>14,000 ×g) for 5 min, the nucleic acid precipitate was lyophilized to dryness, resuspended in 30 mL of sterile Tris-EDTA (pH 8.0) and then stored at -80°C. The primer 515F-806R targeting bacterial 16SV4 region was utilized, and the PCR amplification products were purified. Finally, the 16S rRNA gene template in the specimen was analyzed by quantitative PCR (Feazel et al. 2012). Using Illumina-Miseq platform, library construction and sequencing were performed for amplicons.

Analysis of Sequencing Data

Sequencing data were subjected to operational taxonomic unit (OTU) clustering and species

classification analyses. An OTU was generated by clustering sequences with identical classification. Calculated by dividing the observed sequence count of each OTU by the total number of generated sequences, the relative abundance (RA) of OTUs for each subject was obtained. The sum of OTU counts of composition taxa was calculated and then the total sequence count of each subject was normalized to construct a higher-level OTU (phylum/class/order/family/genus/species). In addition, the mean relative abundance (MRA) of different species was also calculated. Species-level classification was inferred by BLAST from the sequence database, in which Silva111 was labeled as the isolate (Ramakrishnan et al. 2015), and at least 99 percent of sequence similarity in 95 percent of the sequence length was required.

Statistical Analysis

SPSS 21.0 software was employed for statistical analysis. Pearson's Chi-squared test was conducted to compare the categorical variables between the two groups before and after operation. The measurement data were expressed as mean ± standard deviation (mean ± SD), and the *t*-test was used for comparison between variables. Bacterial floras with significant differences in the secretion of patients with postoperative recurrence were obtained by comparing the sequencing data of microbial floras in the nasal lavage fluid between different groups. $P<0.05$ was considered to be statistically significant.

RESULTS

Number of OTUs Before and After Operation

The clustering analysis results of OTUs before and after operation are shown in Table 2. After operation, 1 patient quitted the study. OTUs had no significant differences between the two groups before and after operation.

Table 2: Number of OTUs before and after operation

OTU	*Before operation (n=50)*	*3 months after operation (n=49)*
Total	45506	45305
Mean	910. 1	924.6

OTU: Operational taxonomic unit

Distribution of Dominant Bacteria Before and After Operation

Among all the samples before and after operation, 39 phyla, 116 classes, 332 orders, 708 families and 1,758 genera of bacteria were detected. The species with relative abundance of top 10 at the phylum and genus levels were obtained, including Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Gemmatimonadetes, Acidobacteria, Spirochaetes, Cyanobacteria and Epsilonbacteraeota (at the phylum level), as well as Moraxella, Staphylococcus, Haemophilus, Neisseria, Bacteroides, Streptococcus, Pseudomonas, Fusobacterium, Corynebacterium and Klebsiella (at the genus level) (Fig. 1). For dominant bacteria at the genus level, Moraxella ($P=0.005$) and Neisseria ($P=0.034$) had significantly higher proportions before operation than those after operation.

Postoperative Recurrence of CRS Patients

Three months later, 11 of 49 patients had relapse, so the 3-month postoperative recurrence rate was 22.45 percent (11/49). Recurrence was not correlated with gender or age. Considering that VAS and Lund-Mackay scores showed no significant differences before and after operation ($P>0.05$) and were significantly higher in the recurrence group than in the non-recurrence group, these two indicators were able to represent postoperative recurrence (Table 3).

Distribution of Dominant Bacteria at the Phylum and Genus Levels Before Operation in CRS Patients with Postoperative Recurrence

Based on preoperative sequencing data, the MRA values of bacteria at the phylum and genus levels in CRS patients with (n=11) and without postoperative recurrence were compared (Table 4, Fig. 2). The proportions of Bacteroides, Pseudomonas and Streptococcus in Proteobacteria, Firmicutes and Bacteroidetes were significantly different between patients with and without postoperative recurrence, being especially higher in those with recurrence.

Correlation Analysis of Dominant Bacterial Species and Recurrence in CRS Patients with Postoperative Recurrence

Correlation analysis was carried out between preoperative MRA values at the genus level and postoperative recurrence using VAS (Fig. 3) and

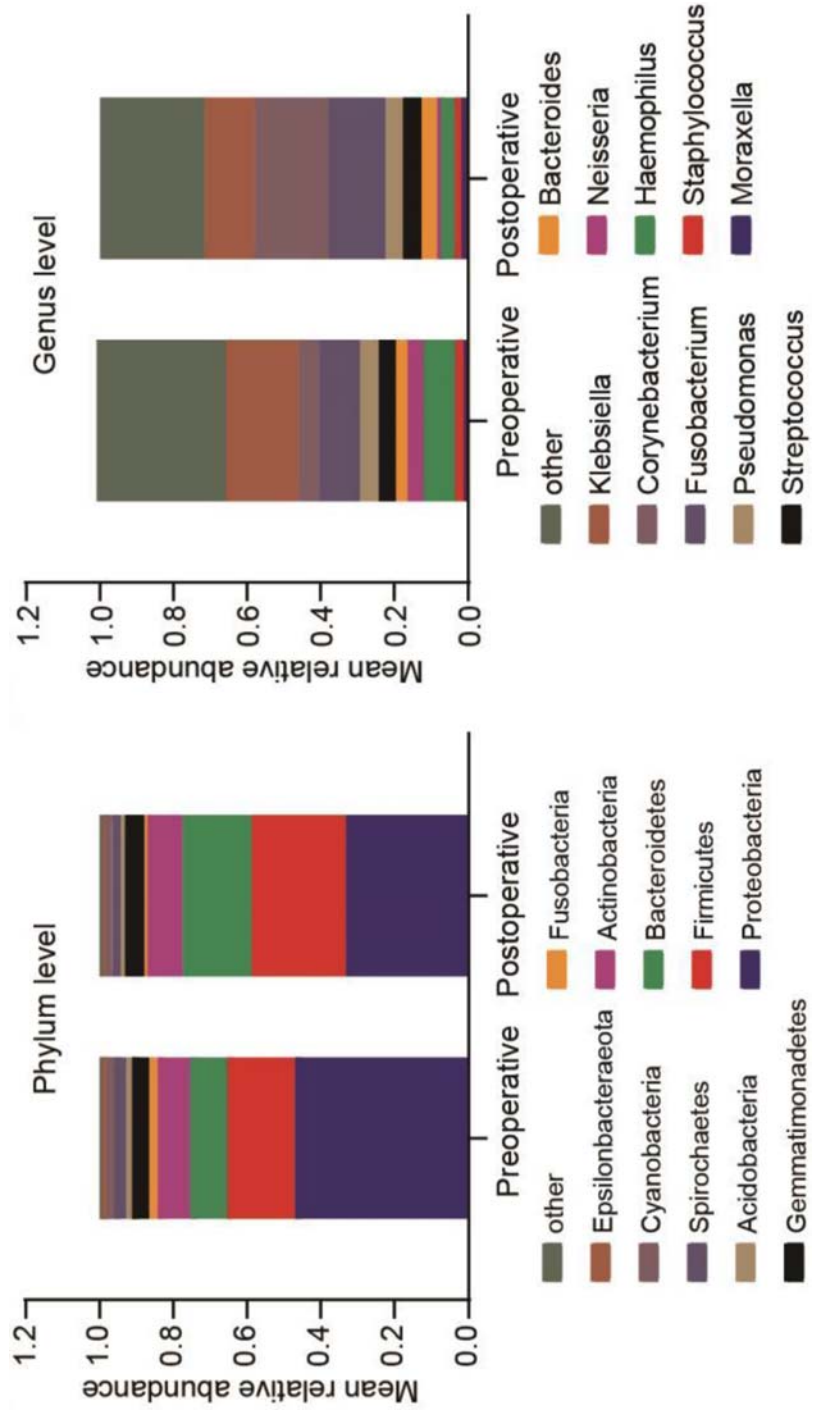

Fig. 1. Composition of dominant bacteria at the phylum/genus level before and after operation

Table 3: Postoperative recurrence in CRS patients

Group	*Gender*		*Age*	*VAS score*	*Lund-Mackay score*
	Male	*Female*			
Recurrence group (n=11)	6	5	46.89±3.04	6.56±3.04	8.14±2.66
Non-recurrence group (n=38)	19	19	44.35±2.30	3.43±1.41	4.64±1.34
χ^2/t	0.9	86	0.786	6.898	10.679
P	>0	.05	>0.05	<0.05	<0.05

CRS: Chronic rhinosinusitis.

Table 4: Difference of dominant bacteria at the phylum/genus level before operation in patients with recurrence

Category	*Name*	*MRA in non-recurrence group*	*MRA in recurrence group*	*P*
Phylum Level	Proteobacteria	0.25	0.464	0.043
	Firmicutes	0.28	0.078	0.015
	Bacteroidetes	0.05	0.12	0.02
Genus Level	Moraxella	0.14	0.09	0.055
	Staphylococcus	0.135	0.11	0.12
	Neisseria	0.05	0.008	0.498
	Bacteroides	0.032	0.24	0.001
	Streptococcus	0.13	0.23	0.043
	Pseudomonas	0.13	0.24	0.02
	Fusobacterium	0.014	0.001	0.052
	Corynebacterium	0.015	0.012	0.671
	Klebsiella	0.019	0.005	0.051
	Others	0.335	0.064	0.002

MRA: Mean relative abundance

Lund-Mackay scores (Fig. 4) after operation. Among the dominant bacteria, Bacteroides (r=0.890, P=0.001), Pseudomonas (r=0.730, P=0.023) and Streptococcus (r=0.840, P=0.002) were significantly different between the two groups and positively correlated with postoperative recurrence (Table 5).

Table 5: Correlations between composition of dominant bacteria at the genus level before operation and postoperative recurrence (VAS score)

Bacterium	*Postoperative recurrence*	
	r	*P*
Bacteroides	0.89	0.001
Streptococcus	0.776	0.023
Pseudomonas	0.894	0.002
Moraxella	0.034	0.089
Staphylococcus	0.192	0.051
Neisseria	-0.345	0.173
Fusobacterium	-0.124	0.124
Corynebacterium	-0.076	0.67
Klebsiella	0.783	0.052

VAS: Visual analogue scale

DISCUSSION

The failure rate after surgical treatment for CRS is 3-20 percent, and patients often suffer from intermittent recurrent or persistent mucopurulent nasal discharge, some of whom need long-term administration of antibiotics (Jiang et al. 2022). The nasal cavity is colonized by microbes, and these commensal microbial floras may be indispensable components to stabilize the internal environment of the nasal cavity (Man et al. 2017; Bomar et al. 2018). The nasal microbial floras alter when nasal infection occurs, thus affecting clinical symptoms possibly through modulating the host innate immune response (Lehtinen et al. 2018; Korten et al. 2019). Hence, it is crucial to compare preoperative and postoperative bacteriological properties in patients with CRS, to explore the predisposing factors of postoperative recurrent infection, and to investigate the role of bacteria in the disease course of CRS. Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, coagulase-negative staphy-

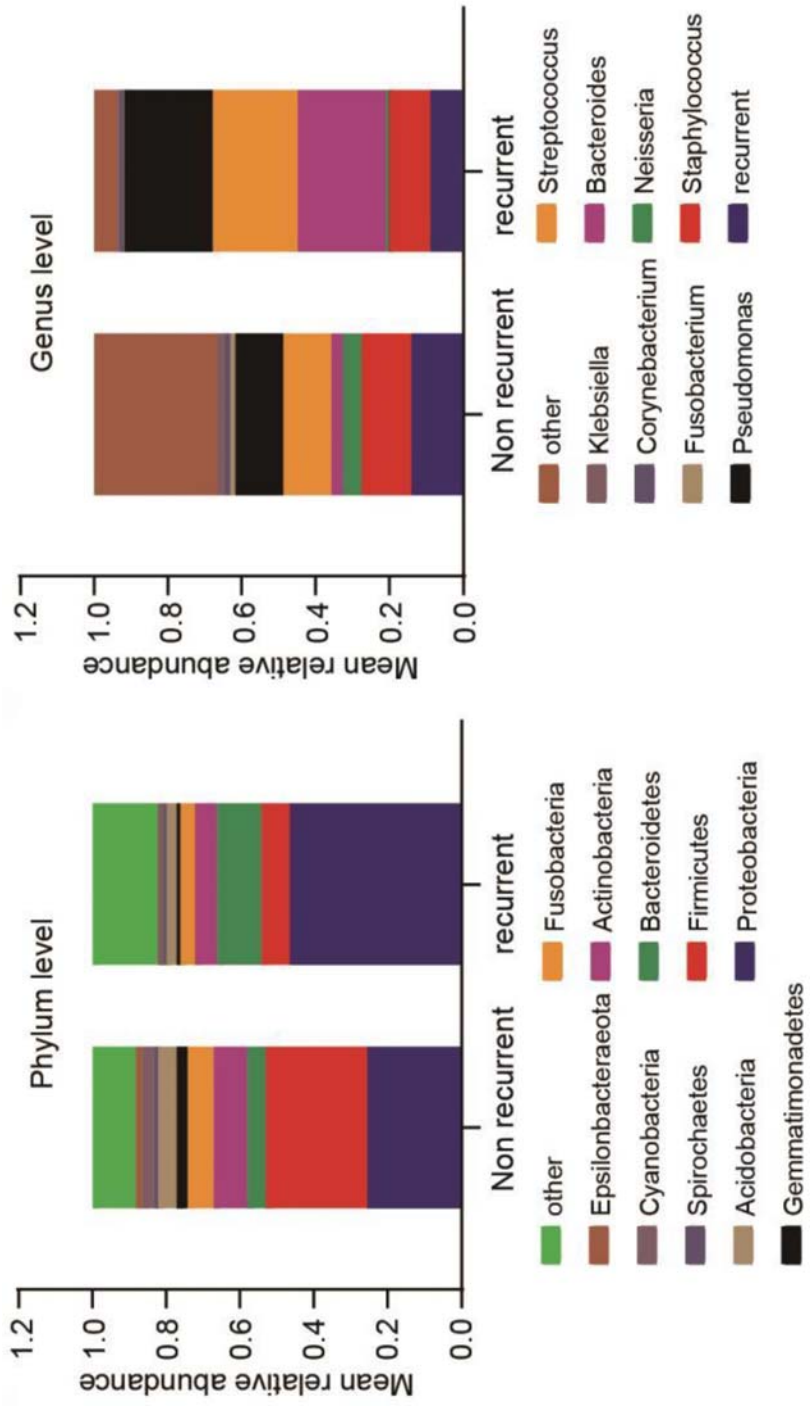

Fig. 2. Composition of dominant bacteria at the phylum/genus level before operation in patients with recurrence

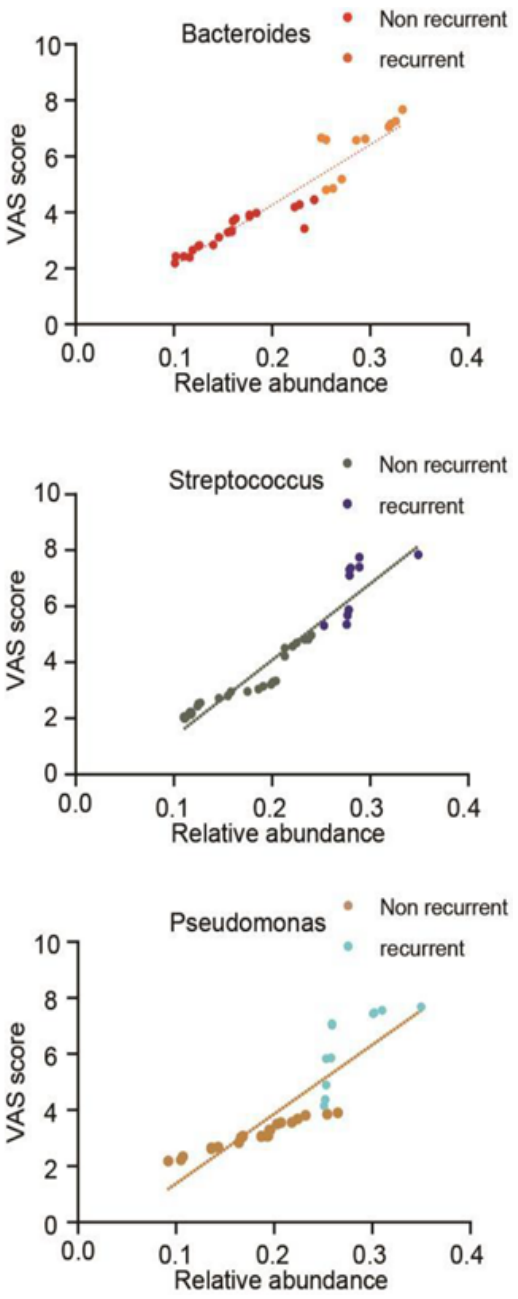

Fig. 3. Correlations between composition of dominant bacteria at the genus level evaluated for postoperative recurrence based on VAS score. VAS: Visual analogue scale

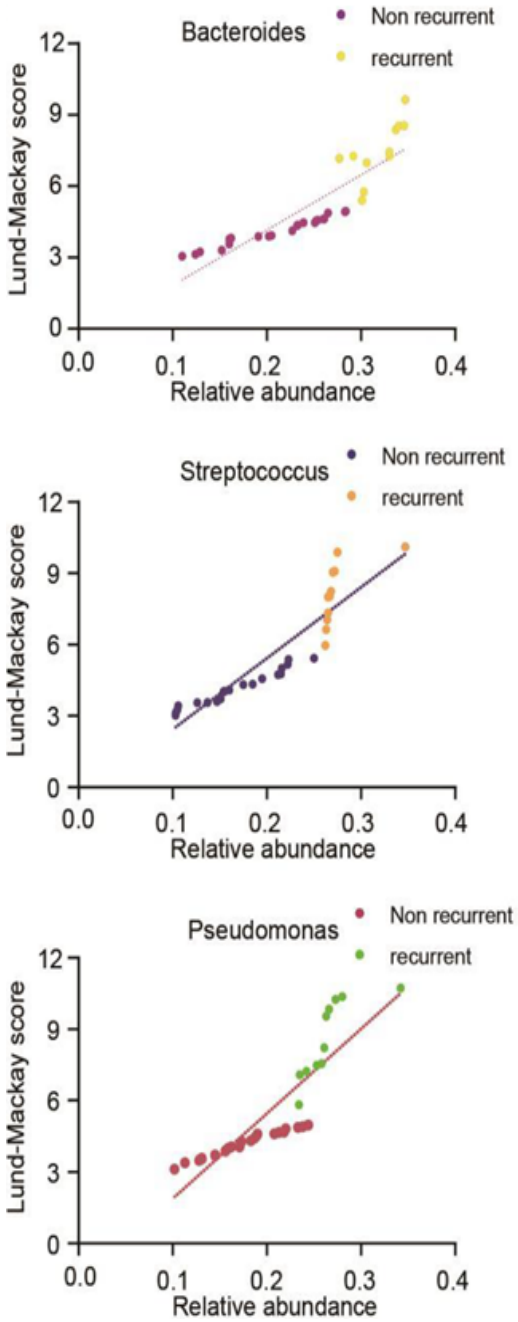

Fig. 4. Correlations between composition of dominant bacteria at the genus level evaluated for postoperative recurrence based on Lund-Mackay score

lococci and anaerobes are common dominant bacteria in CRS patients (Farajzadeh et al. 2016). Similar to the findings of Cleland et al. (2016), the researchers herein found that the bacterial diversity was not significantly different before and after operation.

At present, microbial classification and identification are mainly performed by 16S rDNA sequence analysis (Zhang et al. 2022). It has been extensively applied in the fields such as bacterial identification, comparative analysis of microbial communities, as well as evaluation of phylogeny in community and microbial species diversity (Peleg et al. 2019). By using this technology, the researchers herein demonstrated that there was no significant difference in the bacterial composition at the phylum level before and after operation. At the genus level, dominant bacteria including Moraxella and Neisseria had different proportions, suggesting that such bacteria may be involved in the onset and progression of CRS.

The postoperative recurrence rate of CRS patients in this study was 22.45 percent, which is similar to that reported before (Bachert et al. 2021). Different colonized bacteria in the nasal cavity of CRS patients have been closely associated with clinical severity, host immune response and disease recurrence (Kim et al. 2020; Cho et al. 2022). In this study, Bacteroides, Pseudomonas and Streptococcus in Proteobacteria, Firmicutes and Bacteroidetes were identified as the dominant bacteria. Besides, the proportion of dominant bacteria may be utilized to predict the prognosis after operation. In a recent study, the dominant bacteria significantly differed between patients with and without recurrence (Zhao et al. 2022). Purulent phenotypes are associated with significant amplifications of Bacteroides and Fusobacteria, and may be obligate anaerobes of opportunistic pathogens (Ramakrishnan et al. 2015). The content of Pseudomonas is higher in patients with CRS accompanied by asthma and severe mucosal inflammation, and Pseudomonas aeruginosa, frequently found in the nasal cavity and low respiratory tract of patients with cystic fibrosis, is associated with the generation of tumor necrosis factors and may cause damage to the integrity of respiratory mucosal epithelium (Hardyman et al. 2013), thereby aggravating the disease. Furthermore, Peptostreptococcus has been proven to increase adverse events in smokers and patients with pneumonia (Millares et al. 2019). In this study, they may be closely correlated with postoperative recurrence. Staphylococcus aureus accounts for a higher proportion in terms of postoperative infection (Shah et al. 2022), which is attributed to the use of systemic hormones. However, the difference in the proportion of Staphylococcus aureus was not found in this study, which may be associated with the exclusion criteria.

CONCLUSION

In conclusion, Bacteroides, Pseudomonas and Streptococcus may be implicated in the poor prognosis and postoperative recurrence, as the predictors of CRS.

RECOMMENDATIONS

In the future, establishing and validating multivariate prediction models which combine bacterial composition with factors such as asthma, smoking, surgery or medication history are needed. Considering that the sample size was small in this study, additional samples should be collected to identify the correlations between bacterial composition and postoperative recurrence.

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ABBREVIATIONS

CRS: Chronic Rhinosinusitis;
CT: Computed Tomography;
MRA: Mean Relative Abundance;
OTU: Operational Taxonomic Unit;
RA: Relative Abundance;
rDNA: ribosomal deoxyribonucleic acid;
VAS: Visual Analogue Scale.

REFERENCES

Aldajani A, Alroqi A, Alromaih S et al. 2022. Adverse events of biological therapy in chronic rhinosinusitis with nasal polyps: A systematic review. *Am J Otolaryngol*, 43: 103615.

Arancibia C, Langdon C, Mullol J et al. 2022. Twelve-year long-term postoperative outcomes in patients with chronic rhinosinusitis with nasal polyps. *Rhinology*, 60: 109-117.

Bachert C, Han JK, Wagenmann M et al. 2021. EUFOREA expert board meeting on uncontrolled severe chronic rhi-

nosinusitis with nasal polyps (CRSwNP) and biologics: Definitions and management. *J Allergy Clin Immunol*, 147: 29-36.
Bachert C, Zhang N, Cavaliere C et al. 2020. Biologics for chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol*, 145: 725-739.
Bankova LG, Barrett NA 2020. Epithelial cell function and remodeling in nasal polyposis. *Ann Allergy Asthma Immunol*, 124: 333-341.
Bomar L, Brugger SD, Lemon KP 2018. Bacterial microbiota of the nasal passages across the span of human life. *Curr Opin Microbiol*, 41: 8-14.
Cho SW, Kim DY, Choi S et al. 2021. Microbiome profiling of uncinate tissue and nasal polyps in patients with chronic rhinosinusitis using swab and tissue biopsy. *PLoS One*, 16: e0249688.
Cleland EJ, Bassiouni A, Vreugde S et al. 2016. The bacterial microbiome in chronic rhinosinusitis: Richness, diversity, postoperative changes, and patient outcomes. *Am J Rhinol Allergy*, 30: 37-43.
Copeland E, Leonard K, Carney R et al. 2018. Chronic rhinosinusitis: Potential role of microbial dysbiosis and recommendations for sampling sites. *Front Cell Infect Microbiol*, 8: 57.
Farajzadeh Sheikh A, Ahmadi K, Nikakhlagh S 2016. Detection of Streptococcus pneumoniae and Moraxella catarrhalis in patients with paranasal chronic sinusitis by polymerase chain reaction method. *J Chin Med Assoc*, 79: 440-444.
Feazel LM, Robertson CE, Ramakrishnan VR et al. 2012. Microbiome complexity and Staphylococcus aureus in chronic rhinosinusitis. *Laryngoscope*, 122: 467-472.
Gorgannezhad L, Stratton H, Nguyen NT 2019. Microfluidic-based nucleic acid amplification systems in microbiology. *Micromachines*, 10: 408.
Hardyman MA, Wilkinson E, Martin E et al. 2013. TNF-á-mediated bronchial barrier disruption and regulation by src-family kinase activation. *J Allergy Clin Immunol*, 132: 665-675.e8.
Hopkins C, Lee SE, Klimek L et al. 2022. Clinical assessment of chronic rhinosinusitis. *J Allergy Clin Immunol Pract*, 10: 1406-1416.
Jiang RS, Liang KL 2022. Effect of hypochlorous acid nasal spray as an adjuvant therapy after functional endoscopic sinus surgery. *Am J Otolaryngol*, 43: 103264.
Kim JH, Kim SH, Lim JY et al. 2020. Association between the sinus microbiota with eosinophilic inflammation and prognosis in chronic rhinosinusitis with nasal polyps. *Exp Mol Med*, 52: 978-987.
Korten I, Ramsey K, Mika M et al. 2019. Nasal microbiota and respiratory tract infections: The role of viral detection. *Am J Respir Crit Care Med*, 199: 919-922.
Lehtinen MJ, Hibberd AA, Männikkö S et al. 2018. Nasal microbiota clusters associate with inflammatory response, viral load, and symptom severity in experimental rhinovirus challenge. *Sci Rep*, 8: 11411.
Man WH, de Steenhuijsen Piters WA, Bogaert D 2017. The microbiota of the respiratory tract: Gatekeeper to respiratory health. *Nat Rev Microbiol*, 15: 259-270.
Millares L, Pascual S, Montón C et al. 2019. Relationship between the respiratory microbiome and the severity of airflow limitation, history of exacerbations and circulating eosinophils in COPD patients. *BMC Pulm Med*, 19: 112.
Peleg O, Blinder D, Yudovich K et al. 2019. Microflora of normal maxillary sinuses: Does it justify perioperative antibiotic treatment in sinus augmentation procedures. *Clin Oral Investig*, 23: 2173-2177.
Ramakrishnan VR, Hauser LJ, Feazel LM et al. 2015. Sinus microbiota varies among chronic rhinosinusitis phenotypes and predicts surgical outcome. *J Allergy Clin Immunol*, 136: 334-342.e1.
Shah SJ, Hawn VS, Zhu N et al. 2022. Postoperative infection rate and associated factors following endoscopic sinus surgery. *Ann Otol Rhinol Laryngol*, 131: 5-11.
Shi JB, Fu QL, Zhang H et al. 2015. Epidemiology of chronic rhinosinusitis: Results from a cross-sectional survey in seven Chinese cities. *Allergy*, 70: 533-539.
Stevens WW, Schleimer RP, Kern RC 2016. Chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol Pract*, 4: 565-572.
Ursenbach A, Schramm F, Séverac F et al. 2021. Impact of 16S rDNA sequencing on clinical treatment decisions: A single center retrospective study. *BMC Infect Dis*, 21: 190.
Zhang K, Zhang X, Yang Q 2022. Study on the classification and identification of microorganisms in municipal sludge. *Appl Nanosci*. doi: 10.1007/s13204-021-02078-x.
Zhao Y, Chen J, Hao Y et al. 2022. Predicting the recurrence of chronic rhinosinusitis with nasal polyps using nasal microbiota. *Allergy*, 77: 540-549.

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