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The microbiology of chronic rhinosinusitis with and without nasal polyps

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Abstract

Objective To compare the microbiological features in middle meatus samples from chronic rhinosinusitis (CRS) patients with nasal polyps (CRSwNP) and those without nasal polyps (CRSsNP), and control subjects.

Methods A total of 136 CRSwNP patients, 66 CRSsNP patients, and 49 control subjects who underwent endoscopic surgery in Beijing TongRen Hospital were enrolled between January 2014 and January 2016. Swab samples were obtained from the middle meatus during surgery and processed for the presence of aerobic and non-aerobic bacteria and fungi. Information on the allergic rhinitis, asthma, the percentage of eosinophils in peripheral blood, and the history of smoking and surgery was collected.

Results The overall isolation rate for bacteria was 81.3% for the three groups, with the lowest in the CRSsNP group (77.3%) and the highest in the CRSwNP group (88.4%). There were no significant differences in isolation rates among the three groups ($P=0.349$). The three most common bacterial species were: Coagulase-negative Staphylococcus (24.3%), Corynebacterium (19.9%), and *Staphylococcus epidermidis* (19.1%) in the CRSwNP group; *S. epidermidis* (21.2%), Corynebacterium (21.2%), Coagulase-negative staphylococcus (18.2%), and *Staphylococcus aureus* (13.6%) in the CRSsNP group; *S. epidermidis* (30.6%), Coagulase-negative Staphylococcus (28.6%), and *S. aureus* (14.3%) in the control group. For the bacterial species with high isolation rates, no significant difference in the microbial cultures was observed among the three groups; whereas in the CRSwNP group, a relatively high proportion of Citrobacter (5.9%, a bacterium with low isolation rate) was observed compared with the CRSsNP and control groups (all 0.0%). Furthermore, when samples were categorized into subgroups according to the percentage of eosinophils, some bacterial species showed different rates in the CRSwNP group (e.g., *S. aureus*, 3.3% in the subgroup with normal percentage of eosinophils, 17.2% in the subgroup with increased percentage of eosinophils, $P=0.011$).

Conclusions There were no significant differences in the microbiological features (except Citrobacter) in middle meatus samples from CRSwNP patients, CRSsNP patients, and control subjects. *S. aureus* may promote eosinophilic inflammatory response, while *S. epidermidis* may promote non-eosinophilic inflammatory response.

Keywords Microbiology · Chronic rhinosinusitis · *Staphylococcus epidermidis* · *Staphylococcus aureus*

Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinus mucosa, which is considered as one of severe chronic health problems in Western and Asian countries. CRS can be categorized into several subtypes according to various symptoms of sinus inflammation with the time period of at least 12 weeks [1, 2]. The underlying mechanism of the pathogenesis of CRS is complicated and remains unclear. Local and systemic factors, as well as the microbiological, environmental, genetic and iatrogenic factors play important roles in the pathophysiology of CRS.

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CRS can be considered as a functional abnormality of the host and environment interaction that occurs in contact site of the body and the nasal sinus mucosa [3].

A number of studies have showed that microflora play an important role in promoting the development of early immune function, maintaining immune balance, removing pathogens, and regulating infection and allergic disease sensitivity [4–6]. Recent studies of the airway microflora have demonstrated that the nasal sinuses are not sterile in the healthy state [7–11], indicating that the composition of the microbial community may be a potential regulator of the inflammation progression of CRS [12–17]. Previous studies have reported that patients with chronic inflammatory airway disease have unique microbial characteristics, which suggest the severity of the disease [18, 19]. Another study showed that the sinus microflora of CRS patients exhibit significantly reduced bacterial diversity compared with that of healthy controls [8]. They demonstrated that the multiple, phylogenetically distinct lactic acid bacteria were depleted while the *Corynebacterium tuberculostearicum* was significantly increased in the cohort of CRS patients. Similarly, CRS patients with altered microflora composition and greater abundance of *Staphylococcus aureus* were also reported in the United States [11]. It is worth noting that the bacteria colonizing the nasal airways of Chinese CRS patients and Caucasian CRS patients are not similar. In Western countries, CRS is classified as CRSsNP, a Th1 polarized disorder, or CRSwNP, a Th2 polarized disorder with eosinophilic inflammation as a key feature of White patients with CRSwNP [20]. In contrast, polyps from some Asian CRSwNP patients exhibit Th1, Th17, and KCN cytokine profiles and *S. aureus* appears to be less common with lower isolation rates than that in Caucasian CRSwNP patients [21]. Additionally, the treatment strategy may be inappropriate in Asian patients with neutrophilic interleukin (IL)-17 biased polyps despite the treatment of eosinophils as the first-line therapy for NPs in the Western countries. Furthermore, the sample size of CRS patients reported in the previous studies is relatively small [8].

Therefore, the aim of this study was to compare the microbiological features in middle meatus samples from Chinese patients with CRS with nasal polyps (CRSwNP), without nasal polyps (CRSsNP), and control subjects, and to further analyze the effect of host-related factors on the distribution of microflora.

Materials and methods

Study population

This was a retrospective study. A total of 251 patients who underwent endoscopic surgery in Beijing TongRen Hospital were enrolled between January 2014 and January 2016. According to EPOS2012 diagnostic criteria [3], 136 cases were diagnosed with CRSwNP (male: $n=89$, female: $n=47$; mean age: 45 years), and 66 cases were diagnosed with CRSsNP (male: $n=35$, female: $n=31$; mean age: 42 years). Forty-nine control patients with nasal congestion and snoring (without chronic rhinosinusitis) were selected and they underwent nasal cavity expansion surgery. The mean age of control subjects was 41 years (male: $n=31$, female: $n=18$). All patients underwent sinus CT examination and some received sinus MRI examination before operation. Other information on allergic rhinitis, asthma, the percentage of eosinophils (EOS) in peripheral blood, and history of smoking and surgery was collected. Antibiotics and glucocorticoids were avoided at least 1 month before surgery. Preoperative diagnosis or suspected fungal ball sinusitis, immotile-cilia syndrome and cystic fibrosis, other immune dysfunction, and pregnant and lactating patients were excluded from the study. This study protocol was approved by local ethics committee of Beijing TongRen Hospital. Patient consent was not required because of the retrospective nature of the study.

Sample collection

The specimens were collected by preoperative nasal endoscopy or endoscopic sinus surgery (ESS). Before the collection, the nasal vestibular area was disinfected with Anne iodine. The sterile swabs (Jinzhang, Tianjin, China) were endoscopically guided to the middle meatus, rotated at least three full turns. If the patients experienced significant swelling in their inferior turbinate, ephedrine was used to contract the inferior turbinate, and avoided other parts of the body from contaminating the swabs. Each specimen was labeled with complete patient information including ward, disease number, name, gender, age, diagnosis, and collection site and time. Subsequently, the swabs were placed in a transfer medium immediately to prevent drying (Jinzhang, Tianjin, China). All samples were transferred to the microbial laboratory within 30 min after the collection.

Culture and identification of microflora

All samples were subjected to normal bacterial culture, anaerobic culture and fungal culture.

Normal bacterial culture: the specimens were inoculated in Colombian blood agar medium and Chlamydomonas Glabriate agar medium (BioMérieux, China), incubated for 24 h at 35 °C under 5% CO_2; Specimens were inoculated in the MacConkey Agar Medium (BioMérieux, China) and incubated for 24 h at 35 °C.

Table 1 Characteristics of the three groups: CRSwNP patients, CRSsNP patients, and control subjects

	CRSwNP ($n=136$)	CRSsNP ($n=66$)	Control subjects ($n=49$)	P value
Male, n	88	35	31	
Female, n	47	31	18	
Age, mean ± SD	45.39 ± 13.26	41.88 ± 16.29	41.47 ± 14.38	0.147
Allergic rhinitis, n	61	12	12	0.001
History of asthma, n	37	2	2	< 0.001
Surgery, n	37	17	0	< 0.001
History of smoking, n	23	7	7	0.611

SD standard deviation

Anaerobic culture: the specimens were inoculated in the blister medium (Jinzhang, Tianjing, China) and incubated for 48 h at 35 °C under anaerobic conditions.

Fungal culture: the specimens were inoculated in the Soba dextrose (chloramphenicol) medium (BioMérieux, China), and the fungal growth was monitored at 2, 7 and 14 days after the inoculation. Microbiological isolation and identification were performed according to the methods defined in the Manual of Clinical Microbiology [22].

Statistical analyses

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). A $P<0.05$ (two-tailed) was considered statistically significance. Comparisons for categorical variables were performed by the Pearson Chi-square test or Fisher's exact test. Comparisons for continuous variables were performed using ANOVA or the Kruskal–Wallis test, and multiple comparisons was performed using the Student–Newman–Keuls (SNK) method. Comparisons between two groups for continuous variables were performed by an either t test or Mann–Whitney test.

Results

Table 1 presents the characteristics of the three groups. There were no significant differences in sex, age, and smoking history among the three groups. However, the history of allergic rhinitis and asthma, and the previous history of endoscopic surgery were different in these three groups (Table 1).

In this study, 251 swab samples were collected, and the isolation rate for bacteria was 81.3% for the three groups (204 cases), with the lowest in the CRSsNP group (77.3%) and the highest in the CRSwNP group (88.4%) (Table 2). However, there was no significant difference in the isolation rate of microbial cultures among the three groups ($P=0.349$).

The difference in the number of bacteria in each group was not statistically significant (Table 3). A total of 22 positive strains were detected in 136 cases for the CRSwNP group. Among these cases, 33 (24.3%) were Coagulation-negative staphylococcus, 27 (19.9%) were Corynebacterium and 26 (19.1%) were *Staphylococcus epidermidis*. Twenty positive strains were detected in the CRSsNP group, including *S. epidermidis* (14 cases, 21.2%), Corynebacterium (14 cases, 21.2%), Coagulase-negative staphylococcus (12 cases, 18.2%) and *S. aureus* (9 cases, 13.6%). In the control group, 17 cases (34.7%) were detected with positive strains, among which 15 cases (30.6%) were Staphylococcus epidermal, 14 cases (28.6%) of Coagulase-negative staphylococcus and 7 cases (14.3%) of *S. aureus*. The positive strains were mainly Gram-positive aerobic and facultative anaerobic bacteria in these three groups, including 69.8% in the CRSwNP group, 58 (68.2%) in the CRSsNP group and 46 strains (74.2%) in the control group, followed by Gram-negative aerobic and facultative anaerobic bacteria in the three groups. Moreover, the isolation rates of Gram-negative obligate aerobic

Table 2 The isolation rate of microbial cultures in the three groups: CRSwNP patients, CRSsNP patients, and control subjects

	CRSwNP	CRSsNP	Control subjects	Total
Total number	136	66	49	251
Positive number	115	51	38	204
Positive rate	84.6%	77.3%	77.6%	81.3%

The difference in the isolation rate among the three groups was non-significant ($P=0.349$)

Table 3 Numbers of different bacterial species in specimens from the three groups: CRSwNP patients, CRSsNP patients, and control subjects

Group	No. of bacterial species				
	0	1	2	3	4
CRSwNP ($n=136$)	21	65	38	10	2
CRSsNP ($n=66$)	15	25	20	4	2
Control subjects ($n=49$)	11	16	21	1	0
Total ($n=251$)	47	106	79	15	4

bacteria, anaerobic bacteria and fungi were very low. However, there was no statistically significant difference in the major detectable strains, except Citrobacter (Table 4).

Furthermore, when samples were categorized into subgroups according to the percentage of eosinophils, some bacterial species showed different rates in CRSwNP group (e.g., *S. aureus*, 3.3% in the subgroup with normal percentage of eosinophils, 17.2% in the subgroup with increased percentage of eosinophils, $P = 0.011$). Similarly, for *S. epidermidis*, the corresponding rates were 29.5 and 10.9%, respectively ($P = 0.009$).

Table 4 Bacteria and fungi cultured from middle meatus specimens from the three groups: CRSwNP patients, CRSsNP patients, and control subjects

	CRSwNP ($n = 136$)	CRSsNP ($n = 66$)	Control subjects ($n = 49$)	*P*
Gram-positive aerobic and facultative anaerobic bacteria				
Staphylococcus aureus	15 (11.0%)	9 (13.6%)	7 (14.3%)	0.783
Staphylococcus epidermidis	26 (19.1%)	14 (21.2%)	15 (30.6%)	0.246
Coagulase-negative staphylococcus[a]	33 (24.3%)	12 (18.2%)	14 (28.6%)	0.410
Staphylococcus intermedius	0 (0/0%)	1 (1.5%)	0 (0.0%)	–
Staphylococcus hominis	0 (0.0%)	1 (1.5%)	0 (0.0%)	–
Streptococcus	20 (14.7%)	5 (7.6%)	3 (6.1%)	0.147
Corynebacterium	27 (19.9%)	14 (21.2%)	6 (12.2%)	0.420
Others				
Bacillus	1 (0.7%)	0 (0.0%)	0 (0.0%)	–
Lactobacillus	0 (0.0%)	1 (1.5%)	0 (0.0%)	–
Enterococcus	1 (0.7%)	1 (1.5%)	1 (2.0%)	–
Enterococcus faecium	0 (0.0%)	0 (0.0%)	0 (0.0%)	–
Rhodococcus	2 (1.5%)	0 (0.0%)	0 (0.0%)	–
Gram-negative aerobic and facultative anaerobic bacteria				
Proteus	0 (0.0%)	1 (1.5%)	0 (0.0%)	–
Haemophilus	5 (3.7%)	2 (3.0%)	1 (2.0%)	1.000
Klebsiella	4 (2.9%)	4 (6.1%)	1 (2.0%)	0.531
Citrobacter	8 (5.9%)	0 (0.0%)	0 (0.0%)	0.034
Escherichia coli	1 (0.7%)	1 (1.5%)	0 (0.0%)	–
Enterobacter	5 (3.7%)	3 (4.5%)	4 (8.2%)	0.410
Others				
Denitrifying bacteria	1 (0.7%)	0 (0.0%)	0 (0.0%)	–
Eikenella corrodens	0 (0.0%)	1 (1.5%)	0 (0.0%)	–
Serratia marcescens	0 (0.0%)	0 (0.0%)	1 (2.0%)	–
Serratia liquefaciens	1 (0.7%)	0 (0.0%)	0 (0.0%)	–
E. agglomerans	0 (0.0%)	0 (0.0%)	1 (2.0%)	–
Gram-negative obligate aerobic bacteria				
Neisseria	8 (5.9%)	3 (4.5%)	3 (6.1%)	0.936
Pseudomonas aeruginosa	9 (6.6%)	4 (6.1%)	0 (0.0%)	0.167
Acinetobacter	0 (0.0%)	0 (0.0%)	1 (2.0%)	–
Moraxella nonliquefaciens	2 (1.5%)	0 (0.0%)	0 (0.0%)	–
Stenotrophomonas maltophilia	2 (1.5%)	1 (1.5%)	0 (0.0%)	–
Branhamella catarhalis	2 (1.5%)	0 (0.0%)	1 (2.0%)	–
G-nonfermenters	0 (0.0%)	0 (0.0%)	1 (2.0%)	–
Others				
Delftia acidovorans	0 (0.0%)	1 (1.5%)	0 (0.0%)	–
Onion Burkholderia cepacia	1 (0.7%)	0 (0.0%)	0 (0.0%)	–
Anaerobic bacteria				
Bacteroides fragilis	0 (0.0%)	0 (0.0%)	1 (2.0%)	–
Fungi	5 (3.7%)	6 (9.0%)	1 (2.0%)	0.206

[a]Coagulase-negative staphylococcus may consist of some *S. epidermidis*; however, it cannot be identified in the laboratory. Ditto

Moreover, in the CRSwNP accompanied with or without asthma groups, the isolation rates of Corynebacterium (32.4% vs. 15.7%) and *Pseudomonas aeruginosa* (16.2% vs. 3.4%) were statistically different ($P=0.035$ and $P=0.030$, respectively). Similarly, patients with a history of ESS exhibited a lower isolation rate of Coagulase-negative staphylococcus (13.6%) and higher isolation rate of *P. aeruginosa* (16.2%) compared with patients without surgery (30.1 and 3.2%, respectively), and the differences were significant ($P=0.050$ and $P=0.024$, respectively). However, the other bacteria mentioned above showed no difference in the CRSsNP and control groups (Tables 5, 6, 7). In this study, we did not find the effect of smoking and allergic rhinitis factors on the distribution of microflora in each group.

Table 5 The effect of eosinophils on the distribution of microflora

Macrobacteria	CRSwNP			CRSsNP			Control subjects		
	0 < EOS ≤ 5 ($n=61$)	5 < EOS (n = 64)	*P*	0 < EOS ≤ 5 ($n=44$)	5 < EOS ($n=12$)	*P*	0 < EOS ≤ 5 ($n=38$)	5 < EOS ($n=7$)	*P*
Staphylococcus aureus	2 (3.3%)	11 (17.2%)	0.011	7 (15.9%)	0 (0.0%)	0.325	6 (15.8%)	1 (14.3%)	1.000
Staphylococcus epidermidis	18 (29.5%)	7 (10.9%)	0.009	13 (29.5%)	0 (0.0%)	0.078	11 (28.9%)	4 (57.1%)	0.309
Coagulase-negative staphylococcus	17 (27.9%)	13 (20.3%)	0.323	7 (15.9%)	4 (33.3%)	0.349	10 (26.3%)	2 (28.6%)	1.000
Streptococcus	10 (16.4%)	9 (14.1%)	0.717	4 (9.1%)	0 (0.0%)	1.000	1 (2.6%)	1 (14.3%)	0.290
Corynebacterium	10 (16.4%)	15 (23.4%)	0.325	10 (22.7%)	3 (25.0)	1.000	4 (10.5%)	1 (14.3%)	0.589
Haemophilus	4 (6.6%)	0 (0.0%)	0.116	2 (4.5%)	0 (0.0%)	1.000	1 (2.6%)	0 (0.0%)	1.000
Klebsiella	2 (3.3%)	2 (3.1%)	1.000	3 (6.8%)	0 (0.0%)	1.000	1 (2.6%)	0 (0.0%)	1.000
Citrobacter	2 (3.3%)	5 (7.8%)	0.476	0 (0.0%)	0 (0.0%)	–	0 (0.0%)	0 (0.0%)	–
Enterobacter	2 (3.3%)	3 (4.7%)	1.000	2 (4.5%)	1 (8.3%)	0.522	3 (7.9%)	1 (14.3%)	0.505
Neisseria	4 (6.6%)	4 (6.3%)	1.000	2 (4.5%)	1 (8.3%)	0.522	2 (5.3%)	1 (14.3%)	0.405
Pseudomonas aeruginosa	3 (4.9%)	6 (9.4%)	0.537	2 (4.5%)	1 (8.3%)	0.522	0 (0.0%)	0 (14.3%)	–
Fungi	2 (3.3%)	2 (3.1%)	1.000	3 (6.8%)	1 (8.3%)	0.630	1 (2.6%)	0 (0.0%)	–

Table 6 The effect of asthma on the distribution of microflora

Macrobacteria	CSRwNP			CRSsNP			Control subjects		
	Yes ($n=89$)	No ($n=37$)	*P*	Yes ($n=52$)	No ($n=2$)	*P*	No ($n=35$)	Yes ($n=2$)	*P*
Staphylococcus aureus	9 (10.1%)	5 (13.5%)	0.809	5 (9.6%)	0 (0.0%)	1.000	6 (17.1%)	0 (0.0%)	–
Staphylococcus epidermidis	20 (22.5%)	6 (16.2%)	0.429	13 (25.0%)	0 (0.0%)	1.000	14 (40.0%)	1 (50.0%)	–
Coagulase-negative staphylococcus	22 (24.5%)	7 (18.9%)	0.481	10 (19.2%)	0 (0.0%)	1.000	12 (34.3%)	0 (0.0%)	–
Streptococcus	14 (15.7%)	5 (13.5%)	0.751	4 (7.7%)	0 (0.0%)	1.000	2 (5.7%)	0 (0.0%)	–
Corynebacterium	14 (15.7%)	12 (32.4%)	**0.035**	13 (25.0%)	0 (0.0%)	1.000	5 (14.3%)	0 (0.0%)	–
Haemophilus	5 (5.6%)	0 (0.0%)	0.332	2 (3.8%)	0 (0.0%)	1.000	1 (2.9%)	0 (0.0%)	1.000
Klebsiella	2 (2.2%)	2 (5.4%)	0.717	3 (5.8%)	0 (0.0%)	1.000	1 (2.9%)	0 (0.0%)	1.000
Citrobacter	5 (5.6%)	3 (8.1%)	0.904	0 (0.0%)	0 (0.0%)	–	0 (0.0%)	0 (0.0%)	–
Enterobacter	3 (3.4%)	2 (5.4%)	0.975	2 (3.8%)	0 (0.0%)	–	3 (8.6%)	0 (0.0%)	–
Neisseria	5 (5.6%)	3 (8.1%)	0.904	2 (3.8%)	1 (50.0%)	–	2 (5.7%)	1 (50.0%)	–
Pseudomonas aeruginosa	3 (3.4%)	6 (16.2%)	**0.030**	2 (3.8%)	1 (50.0%)	–	0 (0.0%)	0 (0.0%)	–
Fungi	3 (3.4%)	1 (2.7%)	1.000	4 (7.7%)	0 (0.0%)	–	1 (2.9%)	0 (0.0%)	–

Bold values indicate statistically significant difference

Table 7 The effect of history of nasal surgery on the distribution of microflora

Macrobacteria	CSRwNP			CRSsNP			Control subjects		
	Yes ($n=37$)	No ($n=93$)	P	Yes ($n=17$)	No ($n=42$)	P	Yes ($n=0$)	No ($n=49$)	P
Staphylococcus aureus	5	8	0.604	2	5	1.000	0	7	–
Staphylococcus epidermidis	9	17	0.437	4	9	1.000	0	15	–
Coagulase-negative staphylococcus	5	28	**0.050**	3	8	1.000	0	14	–
Streptococcus	6	14	0.868	1	3	1.000	0	3	–
Corynebacterium	7	20	0.743	7	7	0.096	0	6	–
Haemophilus	3	2	0.276	2	0	0.079	0	1	–
Klebsiella	1	3	1.000	1	2	0.647	0	1	–
Citrobacter	0	7	0.199	0	0	–	0	0	–
Enterobacter	2	3	0.938	1	1	–	0	4	–
Neisseria	1	7	0.530	2	1	–	0	3	–
Pseudomonas aeruginosa	6	3	**0.024**	2	1	–	0	2	–
Fungi	2	2	0.684	2	2	–	0	1	–

Bold values indicate statistically significant difference

Discussion

In this study, we found that the isolation rates for bacteria were not significantly different among the three study groups, and aerobic and facultative anaerobic bacteria were the main species. Specifically, Coagulase-negative staphylococcus and *S. epidermidis* were the most common species among the three groups. No significant differences in the isolation rates were observed among the three groups for the bacterial species with high isolation rates, whereas a high rate of Citrobacter with low isolation rates was observed in the CRSwNP group. Furthermore, when study samples were categorized into subgroups according to the percentage of eosinophils, some bacterial species showed different rates in the CRSwNP group. We did not find the effect of smoking and allergic rhinitis factors on the distribution of microflora in each group. These findings provide important insights into the mechanism underlying CRS and suggest the immunomodulatory effects of microflora in CRS.

The isolation rates for bacteria were not significantly different among the three study groups. The rates are generally consistent with that reported in the literature [23–25]. In this study, aerobic and facultative anaerobic bacteria were the main species, followed by Gram-negative aerobic or facultative anaerobic bacteria, strictly Gram-negative bacteria, and the isolation rates of anaerobic bacteria and fungi were very low. To date, different order and types of bacterial species have been reported. For example, Liu et al. [26] found that the aerobic bacteria were mainly Coagulase-negative staphylococcus, α-hemolytic streptococcus, whereas anaerobic bacteria were mainly Streptococcus and Streptomyces in 42 cases of adult maxillary sinusitis. Rombaux et al. [24] reported an order: Coagulase-negative staphylococcus, *S. aureus*, Streptococcus, other Gram-positive cocci, *Haemophilus influenzae*, non-fermented Gram-negative bacilli, Enterobacter, and anaerobic bacteria. In a study of 31 patients with or without nasal polyps, Niederfuhr et al. [25] found that the order of bacteria was Coagulase-negative staphylococcus, Bacteroides, *S. aureus*, α-hemolytic streptococcus. Liu et al. [27] reported that the common positive strains were Coagulase-negative staphylococcus, Corynebacterium, *S. aureus*, and *H. influenzae*. In our study, the most common bacterial species were Coagulase-negative staphylococcus, Corynebacterium, and *S. epidermidis* in the CRSwNP group; *S. epidermidis*, Corynebacterium, Coagulase-negative staphylococcus, and *S. aureus* in the CRSsNP group; *S. epidermidis*, Coagulase-negative staphylococcus, and *S. aureus* in the control group.

We found no significant differences in the rates for bacterial species with high isolation rates in middle meatus samples from CRSwNP patients, CRSsNP patients, and control subjects. This is consistent with results from prior studies [25, 27, 28]. Note that a high rate of Citrobacter (a bacterium with low isolation rate) was observed in the CRSwNP group. Citrobacter is a Gram-negative facultative anaerobic bacterium and is a common intestinal colonization bacterium. There are few studies on the colonization state of Citrobacter in nasal sinus and its role in the immune function of the host in CRSwNP patients, and more studies are needed in the future.

Recent studies have shown that there may be interactions between local microflora and the immune system. Smeekens et al. [29] have found that the number of normal bacteria (e.g., Corynebacterium) in patients with high IgE syndrome (HIES) was reduced, and the number of Gram-negative bacilli was increased (e.g., Pseudomonas). In peripheral blood mononuclear cell stimulation experiments, Pseudomonas can inhibit the cytotoxin against *S. aureus*, but the

normal Corynebacterium does not have this inhibitory effect. Ba et al. [21] have found that the colonization of Gram-positive bacteria was more common in Chinese CRS patients with IL-5 positive nasal polyps, whereas the colonization of Gram-negative bacteria was more common in Chinese CRS patients with KCN (Key Cytokine-negative Nasal Polyps). Therefore, they suggested that the colonization of bacteria might be related to local IL-5 levels [21]. Ramakrishnan et al. [30] have found that the composition of microflora in CRS patients with complicated asthma and local purulent secretions showed some differences compared with patients with other phenotypes, suggesting that changes in microflora could disrupt the immune balance, and thus cause persistent inflammation status. Aurora et al. [28] have found that the blood leukocytes in CRS patients produced excessive IL-5 when they were exposed to symbiotic bacteria. Other studies also have observed that the composition of airway microflora may be associated with bronchial hyper responsiveness, increased eosinophil counts and total IgE levels [31, 32]. These findings suggest that changes in airway microflora may be associated with specific inflammatory processes.

In this study, we found that the isolation rate of *S. aureus* in the CRSwNP group was 11.0%, which was significantly lower than that reported in Europe [33–35], but was consistent with other studies in China [21, 36]. Furthermore, we found that the distributions of *S. aureus* and *S. epidermidis* showed opposite trend according to different EOS phenotypes in the peripheral blood. Compared with the subgroup with normal percentage of eosinophils, the isolation rate of *S. aureus* (3.3%) was lower than that in the subgroup with increased percentage of eosinophils (17.2%). However, the corresponding isolation rate of *S. epidermidis* decreased (29.5 and 10.9%, respectively). As there is a correlation between the phenotype of EOS in the peripheral blood and the local EOS phenotype of the nasal mucosa [37, 38], we postulate that similar results may be present according to the different local EOS phenotypes of the nasal mucosa. In the study on children with atopic dermatitis (Atopic dermatitis, AD), Laborel-Préneron et al. [39] found that *S. aureus* may promote inflammatory response through concomitant Th2 cell activation and Treg cell inhibition, and thereby promote the inflammatory response process of Th2 type, whereas normal flora such as *S. epidermidis* may counteract this effect by inducing skin DC cells to produce IL-10. It is unknown whether such a similar immune regulation mechanism exists in the mucosa of CRSwNP patients. That is, through the above mechanism, *S. aureus* induces eosinophilic inflammation, and promotes the inflammatory process of Th2 type, whereas *S. epidermidis* acts against this process, and promotes non-eosinophilic granulocyte inflammation. This hypothesis needs to be further verified.

In this study, we found that the isolation rate of *P. aeruginosa* and Corynebacterium was higher in CRSwNP patients with asthma than that in those without asthma. But there was no effect of asthma on the isolation rate of *S. aureus* in this group. Similarly, other researchers did not find any effect of asthma on the isolation rate of *S. aureus* based on Chinese populations [27, 29]. However, studies in Caucasian populations found that the isolation rate of *S. aureus* was the highest in CRSwNP patients with asthma [33–35]. We postulate that this disparity may be due to the differences in gene expression profiles and living conditions. Regarding the impact of asthma on the isolation rate of *P. aeruginosa* and Corynebacterium in CRSwNP patients, it is suggested that: (1) studies with large sample size are needed in the future; (2) consider the perspective of the interaction with microflora immune function.

This study found that the history of nasal surgery may have an impact on the isolation rate of nasal microbacteria. Prince et al. [40] reported that the isolation rate of nasal biofilm was 30.7% in patients with a prior history of functional endoscopic sinus surgery (FESS), which was higher than that in those without FESS (15.0%). In CRSwNP patients with surgical history, the isolation rate of *P. aeruginosa* increased, and the isolation rate of Coagulase-negative staphylococcus decreased. This suggests that surgical procedures may reduce the colonization of Coagulase-negative staphylococcus (normal flora) colonization, and increase the colonization of Pseudomonas, and thus affect the local microbial community status, and plays a role in local epithelial immune regulation.

Our study has some implications in clinical setting. For example, the isolation of microbacteria from the middle meatus during ESS becomes useful in the daily clinical practice because the corresponding results can help determine if the antibiotic therapy is needed. Furthermore, since the isolation rate of *P. aeruginosa* and Corynebacterium was higher in CRSwNP patients with asthma, and those patients also had high risk for recurrences of nasal polyps, the microbacterial profile might be useful in the future for early predicting more aggressive rhinosinusitis.

Conclusions

This study found no significant differences in the microbiological features in middle meatus samples from CRSwNP patients, CRSsNP patients, and control subjects, except for Citrobacter. The clinical significance needs further study. Furthermore, the distributions of *S. aureus* and *S. epidermidis* showed opposite trend according to different EOS phenotype in peripheral blood. The colonization rate of *S. aureus* was increased in subgroup with increased percentage of eosinophils, whereas the colonization rate of *S. epidermidis* was increased in subgroup with normal percentage of eosinophils. This finding suggests that *S. aureus*

may promote eosinophilic inflammatory response, while *S. epidermidis* may promote non-eosinophilic inflammatory response. Our results suggest the immunomodulatory effects of microflora, and the non-infective factor may play a role in the pathogenesis of CRS.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kato A (2015) Immunopathology of chronic rhinosinusitis. Allergol Int 64(2):121–130
2. Akdis CA, Bachert C, Cingi C et al (2013) Endotypes and phenotypes of chronic rhinosinusitis: a PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol 131(6):1479–1490
3. Fokkens WJ, Lund VJ, Mullol J et al (2012) EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. Rhinology 50(1):1–12
4. McLoughlin RM, Mills KH (2011) Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. J Allergy Clin Immunol 127(5):1097–1107
5. Frank DN, Pace NR (2008) Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol 24(1):4–10
6. Tabas I, Glass CK (2013) Anti-inflammatory therapy in chronic disease: challenges and opportunities. Science 339(6116):166–172
7. Ramakrishnan VR, Feazel LM, Gitomer SA, Ir D, Robertson CE, Frank DN (2013) The microbiome of the middle meatus in healthy adults. PLoS ONE 8(12):e85507
8. Abreu NA, Nagalingam NA, Song Y et al (2012) Sinus microbiome diversity depletion and *Corynebacterium tuberculostearicum* enrichment mediates rhinosinusitis. Sci Transl Med 4(151):151ra124–151ra124
9. Yan M, Pamp SJ, Fukuyama J et al (2013) Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and *S. aureus* carriage. Cell Host Microbe 14(6):631–640
10. Boase S, Foreman A, Cleland E et al (2013) The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection. BMC Infect Dis 13(1):210
11. Feazel LM, Robertson CE, Ramakrishnan VR, Frank DN (2012) Microbiome complexity and *Staphylococcus aureus* in chronic rhinosinusitis. Laryngoscope 122(2):467–472
12. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett C, Knight R, Gordon JI (2007) The human microbiome project: exploring the microbial part of ourselves in a changing world. Nature 449(7164):804
13. Ivanov II, Atarashi K, Manel N et al (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 139(3):485–498
14. Ivanov II, de Llanos Frutos R, Manel N et al (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe 4(4):337–349
15. Sudo N, Sawamura S-A, Tanaka K, Aiba Y, Kubo C, Koga Y (1997) The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 159(4):1739–1745
16. Worbs T, Bode U, Yan S et al (2006) Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. J Exp Med 203(3):519–527
17. Atarashi K, Tanoue T, Shima T et al (2011) Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331(6015):337–341
18. Sze MA, Dimitriu PA, Hayashi S et al (2012) The lung tissue microbiome in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 185(10):1073–1080
19. Marri PR, Stern DA, Wright AL, Billheimer D, Martinez FD (2013) Asthma-associated differences in microbial composition of induced sputum. J Allergy Clin Immunol 131(2):346–352. e343
20. Tomassen P, Zele TV, Zhang N et al (2011) Pathophysiology of chronic rhinosinusitis. Proc Am Thorac Soc 8(1):115–120
21. Ba L, Zhang N, Meng J et al (2011) The association between bacterial colonization and inflammatory pattern in Chinese chronic rhinosinusitis patients with nasal polyps. Allergy 66(10):1296–1303
22. Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW (2011) Manual of clinical microbiology, 10th edn. American Society of Microbiology, Washington, DC
23. Brook I (1989) Bacteriology of chronic maxillary sinusitis in adults. Ann Otol Rhinol Laryngol 98(6):426–428
24. Rombaux P, Gigi J, Hamoir M, Eloy P, Bertrand B (2002) Bacteriology of chronic sinusitis: the bulla ethmoidalis content. Rhinology 40(1):18–23
25. Niederfuhr A, Kirsche H, Riechelmann H, Wellinghausen N (2009) The bacteriology of chronic rhinosinusitis with and without nasal polyps. Arch Otolaryngol Head Neck Surg 135(2):131–136
26. Liu Z, Gao QX, Cui YH, Tao YL (1998) Bacteriological study of chronic maxillary sinusitis in adults and observation of susceptibility to antibiotics. J Clin Otorhinolaryngol 12(12):545–548
27. Liu Q, Lu X, Bo M, Qing H, Wang X, Zhang L (2014) The microbiology of chronic rhinosinusitis with and without nasal polyps. Acta Otolaryngol 134(12):1251–1258
28. Aurora R, Chatterjee D, Hentzleman J, Prasad G, Sindwani R, Sanford T (2013) Contrasting the microbiomes from healthy volunteers and patients with chronic rhinosinusitis. JAMA Otolaryngol Head Neck Surg 139(12):1328–1338
29. Smeekens SP, Huttenhower C, Riza A et al (2014) Skin microbiome imbalance in patients with STAT1/STAT3 defects impairs innate host defense responses. J Innate Immun 6(3):253–262
30. Ramakrishnan VR, Hauser LJ, Feazel LM, Ir D, Robertson CE, Frank DN (2015) Sinus microbiota varies among chronic rhinosinusitis phenotypes and predicts surgical outcome. J Allergy Clin Immunol 136(2):334–342.e331
31. Bisgaard H, Hermansen MN, Buchvald F et al (2007) Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med 357(15):1487–1495
32. Huang YJ, Nelson CE, Brodie EL et al (2011) Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. J Allergy Clin Immunol 127(2):372–381. e371–e373
33. Van Zele T, Gevaert P, Watelet JB et al (2004) *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy Clin Immunol 114(4):981–983
34. Corriveau MN, Zhang N, Holtappels G, Van Roy N, Bachert C (2009) Detection of *Staphylococcus aureus* in nasal tissue with peptide nucleic acid-fluorescence in situ hybridization. Am J Rhinol Allergy 23(5):461–465
35. Sachse F, Becker K, von Eiff C, Metze D, Rudack C (2010) *Staphylococcus aureus* invades the epithelium in nasal polyposis and induces IL-6 in nasal epithelial cells in vitro. Allergy 65(11):1430–1437

36. Jiang RS, Hsu CY, Jang JW (1998) Bacteriology of the maxillary and ethmoid sinuses in chronic sinusitis. J Laryngol Otol 112(9):845–848
37. Hu Y, Cao PP, Liang GT, Cui YH, Liu Z (2012) Diagnostic significance of blood eosinophil count in eosinophilic chronic rhinosinusitis with nasal polyps in Chinese adults. Laryngoscope 122(3):498–503
38. Wang MJ, Zhou B, Li YC, Huang Q (2013) The role of peripheral blood eosinophil percentage in classification of chronic rhinosinusitis with nasal polyps. Chin J Otorhinolaryngol Head Neck Surg 48(8):650–653
39. Laborel-Preneron E, Bianchi P, Boralevi F et al (2015) Effects of the *Staphylococcus aureus* and *Staphylococcus epidermidis* secretomes isolated from the skin microbiota of atopic children on CD4 + T cell activation. PLoS ONE 10(10):e0141067
40. Prince AA, Steiger JD, Khalid AN et al (2008) Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. Am J Rhinol 22(3):239–245