



Published in final edited form as:

*Int Forum Allergy Rhinol.* 2015 March ; 5(3): 185–190. doi:10.1002/alr.21467.

## Impact of saline irrigation and topical corticosteroids on the post-surgical sinonasal microbiota

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### Abstract

**Introduction**—Topical treatments with nasal saline irrigation, topical steroid sprays, or corticosteroid rinses can improve sinonasal symptoms in chronic rhinosinusitis (CRS). However, the impact of these therapies on commensals (*Corynebacterium*) and on biofilm pathogens associated with CRS (*Staphylococcus aureus* and *Pseudomonas*) is not well characterized.

**Methods**—Paired nasal and sinus swabs were collected endoscopically from 28 controls and 14 CRS patients with nasal polyposis (CRSwNP) who had not received systemic antibiotics or corticosteroids in the previous eight weeks. Total DNA from swab eluents were extracted and analyzed by 16S rRNA gene-based pyrosequencing. A total 359,077 reads were obtained and classified taxonomically. The association of use of topical therapies with sinonasal microbiota composition was assessed by factor and vector-fitting. The proportional abundances of sinonasal bacteria between topical therapy users and non-users were further compared by two-tailed Kolmogorov-Smirnov test among controls and among CRSwNP participants.

**Result**—Nasal saline irrigation, with or without added budesonide, was not associated with significantly distinct sinonasal microbiota composition or significantly decreased *Pseudomonas* or *S. aureus* abundances among either controls or CRSwNP participants. *Corynebacterium* was slightly lower in controls that reported using saline irrigation than those who did not. No significant association was found between nasal saline irrigation and the proportional abundances of *Pseudomonas*, *S. aureus*, and *Corynebacterium* in CRSwNP participants. However, male CRSwNP patients were noted to have significantly higher *Corynebacterium* proportional

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**CONFLICT OF INTEREST:** The authors have no other financial disclosures or conflict of interest.

abundances than their female counterparts. The use of topical steroid sprays was associated with a distinct microbiota in control subjects, characterized by higher proportional abundances of *Dolosigranulum* and *Simonsiella* and a lower proportional abundance of *Campylobacter*.

**Conclusion**—Nasal saline irrigation is not associated with a distinct alteration in the proportional abundance of commensal bacteria or biofilm-forming pathogens in CRSwNP patients. However, use of topical intranasal corticosteroid sprays in control subjects is associated with a distinct sinonasal microbiota.

### Keywords

Sinus microbiota; 16S rRNA gene; 16S rRNA gene-based pyrosequencing; Nasal rinse; Irrigation; Nasal saline irrigation; Intranasal steroid spray; Intranasal steroid therapy; Topical Therapy; Chronic Rhinosinusitis; Chronic Sinusitis; Bacteriology; Medical Therapy of Chronic Rhinosinusitis; Steroid therapy

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## INTRODUCTION

Nasal saline irrigation is common adjuvant therapy for chronic rhinosinusitis patients (CRS), including as a postoperative adjuvant. Clinical trials and meta-analyses have shown irrigation to be effective in reducing CRS symptoms and improving quality of life in both pre-surgical and post-operative periods<sup>1–6</sup>. This, together with its low-cost and favorable safety profile, makes nasal saline irrigation a robust choice for the management of sinonasal symptoms.

Nasal saline irrigation is thought to address sinonasal symptoms through several potential mechanisms, including improving mucociliary clearance<sup>7</sup>, reducing mucosal inflammation<sup>4</sup>, maintaining moisture and decreasing postoperative crusting. In addition, nasal saline irrigation is thought to improve symptoms by reducing biofilms and bacterial antigens<sup>1</sup>, either by direct removal or secondarily by increasing their clearance by the mucociliary mechanism. However, there are limited data regarding the microbiological effects of nasal saline irrigation. This study seeks to determine the association between the sinonasal microbiota and nasal saline irrigation. Specifically, the proportional abundances are examined of *Corynebacterium*, a common sinonasal bacteria<sup>8,9</sup>, and two biofilm-associated pathogens in CRS: *Staphylococcus aureus*, which has also been implicated as a potential contaminant of nasal rinse, and *Pseudomonas*, a common waterborne bacteria<sup>10–14</sup>. If a distinct sinonasal microbiota profile is associated with nasal saline irrigation, this would support the concept that nasal saline irrigation reduces sinonasal symptoms, at least in part, by removal of colonizing or infecting pathogens. Alternatively, if nasal saline irrigation is associated with increased *S. aureus* or *Pseudomonas*, this might suggest that nasal lavage results in contamination. To evaluate these possibilities, the sinonasal microbiota was assessed in 28 control subjects and 14 patients with active exacerbation of CRS with nasal polyps (CRSwNP).

## MATERIALS AND METHODS

### Study design and subjects

Adult patients of both sexes were recruited from the Johns Hopkins Outpatient Rhinology Clinic in 2011. All subjects enrolled had patent maxillary anastomies following endoscopic sinus surgery greater than one year prior to enrollment. Subjects were excluded if they had used oral antibiotics in the previous eight weeks, or if they had a known history of cystic fibrosis, immunodeficiency, or autoimmune diseases. All participants signed written consent at the time of enrollment. Patients with chronic rhinosinusitis with nasal polyps (CRSwNP) were defined by historical and endoscopic criteria, meeting the definition of the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force. Patients had continuous symptoms of rhinosinusitis for greater than 12 consecutive weeks, which was associated with bilateral mucosal disease of the sinuses. The control participants demonstrated normal mucosa on endoscopic exam and had no symptoms or signs of active rhinosinusitis. CRSwNP subjects had endoscopic evidence of polypoid mucosal inflammation.

Each participant's nasal cavity and maxillary sinus mucosa were sampled separately by calcium alginate swab (MPC, Amarillo, CA) under endoscopic guidance. Each swab was immediately flash frozen on dry ice, then stored at  $-80^{\circ}\text{C}$  until analysis. At the time that the samples were taken, information was collected as to the patients' current self-reported use of topical sinonasal therapies, including saline irrigations and corticosteroids. The study was approved by three institutional review boards: the Northern Arizona University Institutional Review Board (Flagstaff, AZ), the Johns Hopkins Medicine Institutional Review Board (Baltimore, MD), and the Western Institutional Review Board (Olympia, WA), the IRB of record for the Translational Genomics Research Institute.

### Sample processing and pyrosequencing analysis

Sample processing and pyrosequencing analysis was performed as previously described<sup>15</sup>. Briefly, cell lysis was performed using a combination of enzyme-free chemical and mechanical lysis and the nucleic acid purified using the Qiagen AllPrep Mini Kit (Qiagen, Valencia, CA, USA). Barcoded amplicons of the V3V6 hypervariable region were generated using broad-coverage fusion PCR primers and pooled for sequencing on the Genome Sequencer FLX (Roche Applied Sciences, Branford, USA). The resultant pyrosequences were chimera-checked, demultiplexed, and quality-checked. A total of 407,863 16S rRNA gene sequences underwent taxonomic classification using a custom classifier adapted from the Ribosomal Database Project Naïve Bayesian Classifier<sup>16</sup>, which was trained with a large curated set of *Staphylococcus aureus* and *Staphylococcus epidermidis* sequences to ensure accurate species-level classification of the two species.

### Sinonasal microbiota analyses

All sinonasal microbiota comparisons were performed in R version 2.13.1<sup>17</sup>. We calculated proportional abundances as:  $(\text{Total number of 16S rRNA gene sequences classified as genus } A \text{ from the sample}) / (\text{Total number of 16S rRNA gene sequences classified as bacteria from$

the sample). All statistical analyses were performed separately in controls and in CRSwNP participants.

We began by evaluating the role of host factors, including nasal saline irrigation on the overall sinonasal microbiota. Using proportional abundance data in Bray-Curtis distance, we visualized the sinonasal microbiota composition by non-metric multidimensional scaling (nMDS) using the R package *vegan*<sup>18</sup>. Next, we assessed the association between microbiota composition with host factors (sex, sampling site, and self-reported use of intranasal steroid spray and nasal irrigation) by fitting them as factors or vectors on the nMDS plots<sup>18</sup> and plotted 95% confidence intervals. We used indicator analysis to identify the sinonasal bacteria (i.e., the indicator bacteria) uniquely linked to each host factor statistically significantly associated with overall sinonasal microbiota from the factor/vector-fitting analysis. We then examined these indicator bacteria's proportional abundances using scatterplots.

Net, we assessed the association of nasal saline irrigation with *Corynebacterium*, *Staphylococcus aureus*, and *Pseudomonas*. We compared the proportional abundances of these bacteria in irrigators versus non-irrigators using scatterplots and two-tailed Kolmogorov-Smirnov test with  $\alpha = 0.05$ . The prevalence and proportional abundance of all sinonasal bacteria detected were summarized in Tables S1–2.

## RESULTS

### Participant demographics and clinical history

We enrolled 42 participants with patent maxillary anrostomies, who had not received systemic antibiotics or corticosteroids in the previous eight weeks. Among the 42 participants, 28 were non-diseased controls with normal sinonasal mucosa on endoscopy and 14 were participants with chronic rhinosinusitis with nasal polyposis (CRSwNP) who had endoscopic evidence of polypoid mucosa with inflammation (Table 1).

The controls comprised of 16 women and 12 men, with a median age of 53.5 years (SD = 12.2, range = 25–77), with 10 who reported using any nasal saline irrigation and 10 who reported using any intranasal steroid spray. Among those who used saline irrigation, 5 concurrently used intranasal steroid spray and one individual used steroid irrigation (Table 1). The CRSwNP group comprised of 11 women and 3 men, with a median age of 52.0 years (SD = 12.4, range = 27–65), with 8 who reported using nasal saline irrigation of any type and 9 who reported using intranasal steroid spray. All CRSwNP participants who used saline irrigation also used some form of topical steroid (i.e., spray or irrigation). We collected and analyzed paired ipsilateral nasal and sinus swabs from each participant.

### Intranasal steroid spray, but not nasal saline irrigation, was associated with distinct sinonasal microbiota in controls

We found no evidence that nasal saline irrigation—with or without intranasal steroid spray—was associated with distinct sinonasal microbiota in either controls or CRSwNP participants ( $r^2 = 0.02$ ,  $p = 0.4$  in controls;  $r^2 = 0.01$ ,  $p = 0.7$  in CRSwNP); however, we found that any steroid spray use was associated with distinct microbiota in controls ( $r^2 =$

0.11,  $p = 0.002$ ). Further analysis revealed that the difference in sinonasal microbiota were driven by steroid spray users having higher proportional abundances of *Dolosigranulum* ( $p < 0.001$ ) and a genetic near neighbor of *Simonsiella* ( $p = 0.01$ ) and a lower proportional abundance in *Campylobacter* ( $p = 0.05$ ) (Figure S1A–C).

Among CRSwNP participants, we found evidence of sex-specific differences in sinonasal microbiota composition ( $r^2 = 0.09$ ,  $p = 0.08$ ) (Figure 1). Further analysis showed that the differences in CRSwNP sinonasal microbiota between men and women were driven mostly by the significantly higher proportional abundances of *Corynebacterium* ( $p = 0.002$ ) and two other sinonasal bacteria: *Serratia* ( $p = 0.003$ ), *Finnegoldia* ( $p = 0.03$ ) in men (Figure S1D–G).

### **Nasal irrigation was not associated with differences in *S. aureus*, *Pseudomonas*, or *Corynebacterium* abundances in controls or CRSwNP participants**

We found that nasal saline irrigation was not associated with lower *Pseudomonas* or *S. aureus* abundances in sinonasal microbiota in controls. In addition, the abundance of *Corynebacterium* was lower in the sinus of controls that reported using any nasal saline irrigation ( $n = 10$ ) ( $Mean = 0.09$ ,  $Range = 0.00 - 0.43$ ) than those who did not ( $n = 18$ ) ( $Mean = 0.18$ ,  $Range = 0.00 - 0.29$ ), but the difference was not statistically significant ( $p = 0.49$ ) (Figure 2A–C) (Tables S1–2).

Likewise, even though nasal saline irrigation was also associated with lower *Corynebacterium* abundance in CRSwNP participants, the difference was not statistically significant between CRSwNP irrigators ( $n = 7$ ) ( $Mean = 0.02$ ,  $Range = 0.00 - 0.24$ ) and non-irrigators ( $n = 7$ ) ( $Mean = 0.09$ ,  $Range = 0.00 - 0.56$ ) ( $p = 0.18$ ). We also found no significant in *Pseudomonas* abundance between CRSwNP irrigators ( $Mean = 0.02$ ,  $Range = 0.00 - 0.07$ ) and non-irrigators ( $Mean = 0.11$ ,  $Range = 0.00 - 0.80$ ) ( $p = 0.9$ ), or in *S. aureus* abundance between CRSwNP irrigators ( $Mean = 0.15$ ,  $Range = 0.00 - 0.85$ ) and non-irrigators ( $Mean = 0.14$ ,  $Range = 0.00 - 0.88$ ) ( $p = 0.9$ ) (Figure 2D–F). The prevalence and proportional abundance of all detected sinonasal bacteria are reported in detail in Tables S1–2.

## **DISCUSSION**

Nasal saline irrigation is thought to reduce sinonasal symptoms partly by removing colonizing or infecting pathogens. In this study, no significant association between nasal saline irrigation was found with overall sinonasal microbiota composition or the nasal bacteria's proportional abundances. This suggests that the clinical benefits of nasal saline irrigation likely occur through non-microbiological means. An additional implication, however, is that even though high rates of nasal irrigation bottle contamination by *S. aureus* have been reported<sup>11</sup>, nasal irrigation was not associated with higher *S. aureus* abundances in our participants.

Interestingly, the present study reveals that intranasal corticosteroid spray, a common topical therapy for most sinonasal inflammatory diseases, was associated with a unique microbiota profile in subjects with normal sinonasal mucosa. This may have clinical relevance, since intranasal steroid spray has shown some positive efficacy in adult and pediatric acute

bacterial sinusitis<sup>19,20,21,22</sup>. Thus, the data suggest that, in addition to its well-known anti-inflammatory effects, intranasal steroid spray might have microbiological effects. The clinical implication of the increases in *Dolosigranulum* and *Simonsiella* with intranasal steroid spray is still unknown, but *Dolosigranulum* has been associated with decreased risk for acute otitis media in pediatric patients<sup>23</sup> and characterized primarily in children using culture-independent methods<sup>23,24</sup>, whereas *Simonsiella* has been described as commensal oral bacteria<sup>25</sup>.

Gender-specific differences in the CRSwNP sinonasal microbiota were also demonstrated in this study. Specifically, men had significantly higher *Corynebacterium* proportional abundances than did women. Even though *Corynebacterium tuberculoostearicum* has been linked to the development of chronic rhinosinusitis<sup>8</sup>, most sinonasal studies have shown *Corynebacterium* to correlate negatively with colonization by CRS pathogens, such as *S. aureus*<sup>9</sup>. Consistent with this relationship, women in the present study were more likely than men to demonstrate *S. aureus*.

There are several limitations to this study that are primarily attributable to the cross-sectional design and small sample size. Although all subjects were prescribed high-volume high-pressure isotonic saline irrigations, patient compliance was not recorded or accounted for, including whether some patients varied in technique or saline tonicity. Given the small CRSwNP sample, it was not possible to stratify based on disease characteristics such as aspirin sensitivity or asthma. In addition, our cross-sectional design did not allow us to measure the microbiota change associated with nasal saline irrigation by comparing pre- and post-irrigation, which will be important to address in future studies.

## CONCLUSION

Saline irrigation of the sinonasal cavities in post-surgical patients is not associated with a distinct sinonasal microbiota. Although nasal lavage seems logically more capable of modifying the microbiome, the present study shows that intranasal topical corticosteroids are associated with distinct alterations in sinonasal bacterial proportional abundances. These findings support the concept that non-microbiological mechanisms, such as improved mucociliary clearance and removal of inflammatory mediators, contribute to the therapeutic benefits of saline. The sinonasal microbial changes associated with topical corticosteroids warrant further exploration, and may suggest unrecognized microbial mechanisms in addition to the known anti-inflammatory effects of these medications.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

**FUNDING SOURCES:** Financial support for this work was provided by the National Institutes of Health (1R15DE021194-01). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

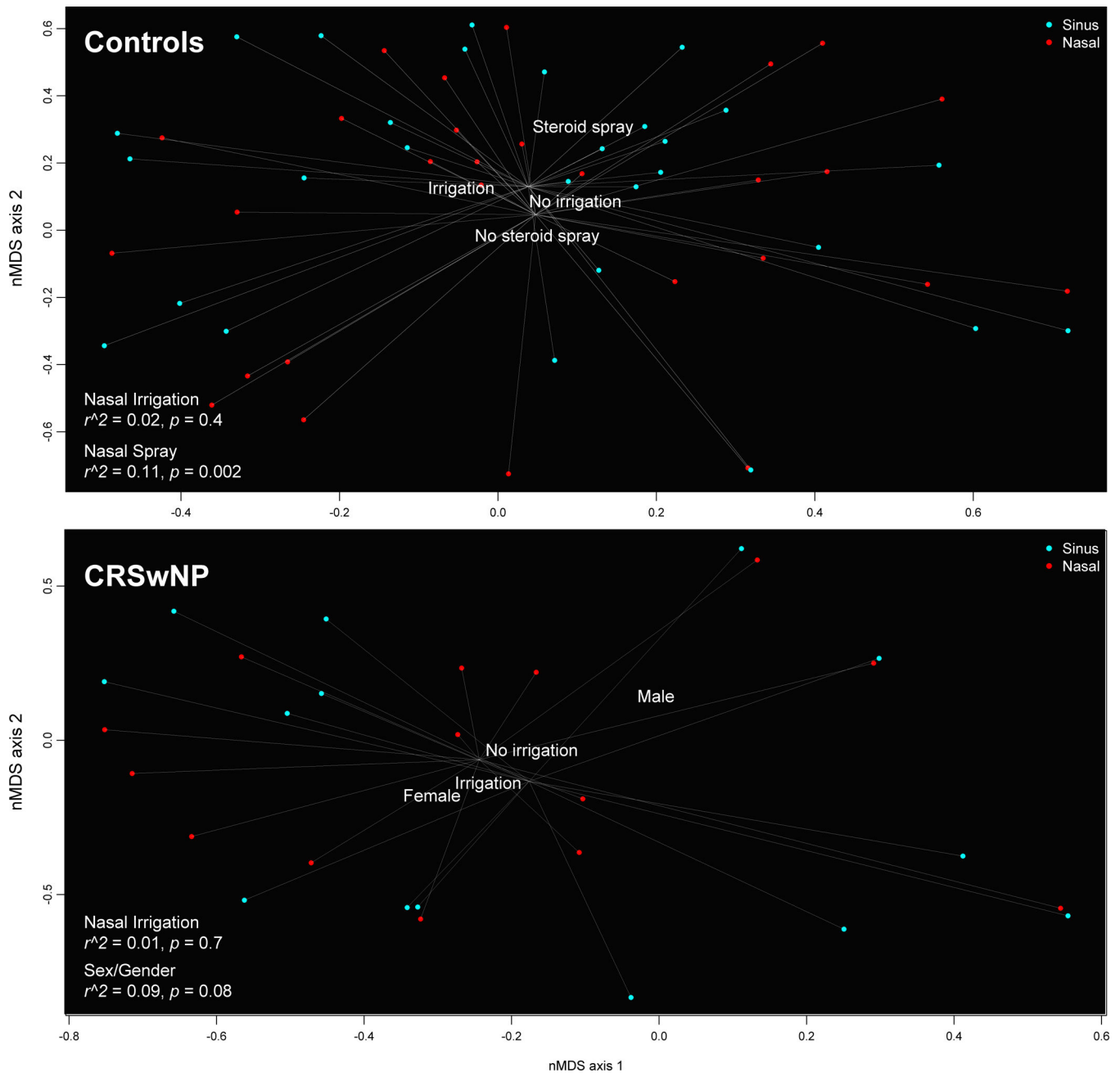


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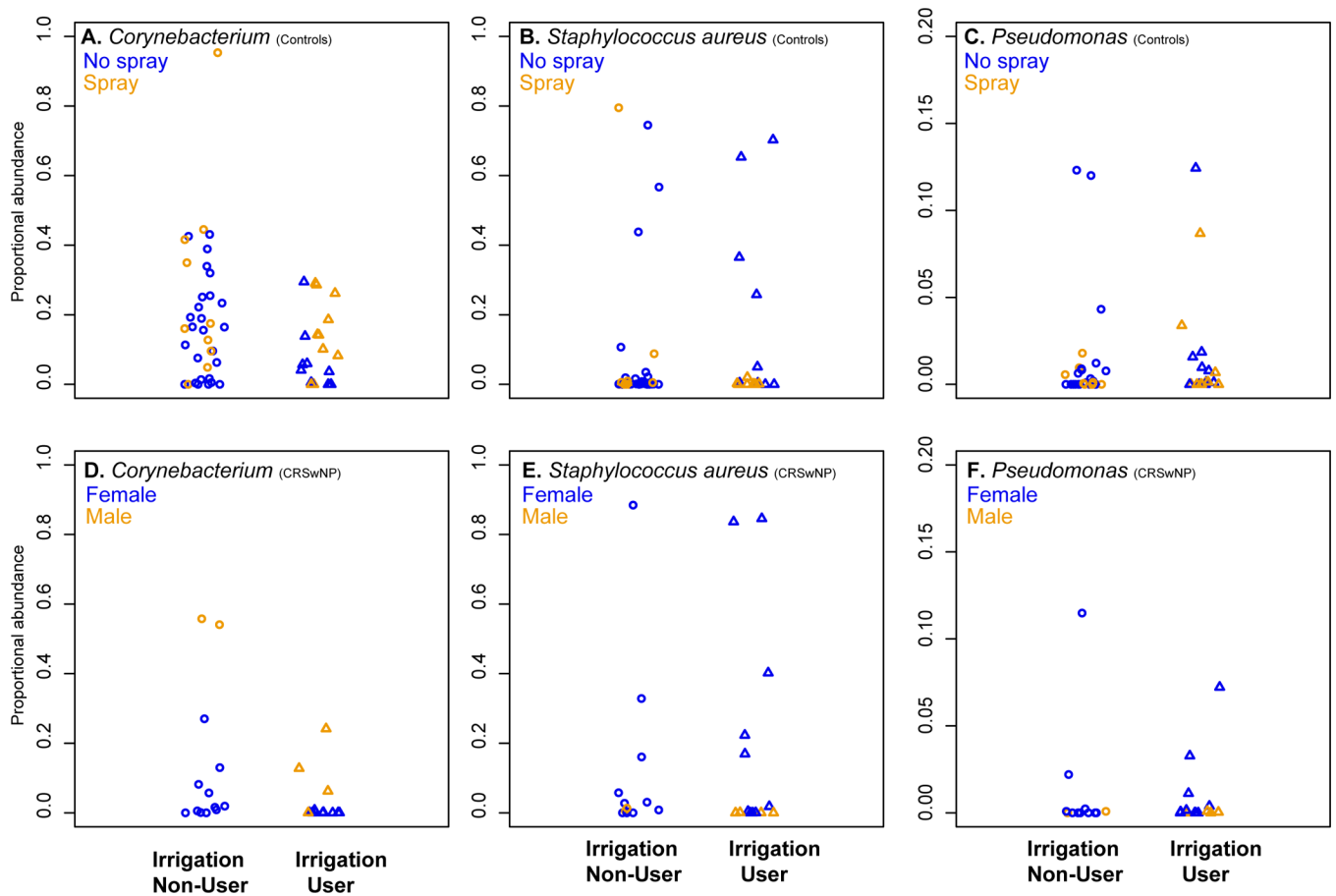




**Figure 1.**

**A–B. Visualization of association between nasal saline irrigation and sinonasal microbiota composition by non-metric multidimensional scaling in controls and CRSwNP participants.** In these two non-metric multidimensional scaling plots, each data point represents the collective nasal/sinus microbiota in a single specimen from a given individual. Data points that are located more closely together represent more similar microbiota compositions than points that are located farther apart. Each data point is also linked to the group's nasal (*red*) and sinus (*blue*) centroid, respectively. Nasal saline irrigation was not associated with a distinct sinonasal microbiota profile, as shown in the

controls (**Figure 1A**) ( $p = 0.4$ ) and CRSwNP participants (**Figure 1B**) ( $p = 0.7$ ). However, intranasal steroid spray users had distinct microbiota than non-users in the controls (**Figure 1A**) ( $p = 0.002$ ) and sex-specific differences were seen in CRSwNP participants (**Figure 1B**) ( $p = 0.08$ )



**Figure 2.**

**A–F. Scatterplots of sinonasal *Corynebacterium*, *Staphylococcus aureus*, and *Pseudomonas* proportional abundances in controls and CRSwNP participants.** This panel of scatterplots depicts the proportional abundances of three sinonasal bacteria (*Corynebacterium*, *S. aureus*, and *Pseudomonas*) in non-irrigators (circles) and irrigators (triangles) among controls (**Figure 2A–C**) and CRSwNP participants (**Figure 2D–F**). The host factors shown to be associated significantly with microbiota composition was further denoted in controls (intranasal steroid spray use) and in CRSwNP (male versus female) participants.

Table 1

Demographics and clinical history of study participants

	Controls (n =28)		CRSwNP (n =14)	
	No irrigation (n = 18)	Any irrigation (n = 10)	No irrigation (n = 7)	Any irrigation (n = 7)
	n (%)			
<b>Age</b>				
21–29	0 (0.0)	1 (10.0)	1 (14.3)	0 (0.0)
30–39	2 (11.1)	0 (0.0)	1 (14.3)	2 (28.6)
40–49	5 (27.8)	3 (30.0)	2 (28.6)	0 (0.0)
50–59	5 (27.8)	3 (30.0)	1 (14.3)	5 (71.4)
60	6 (33.3)	3 (30.0)	2 (28.6)	0 (0.0)
<b>Previous indication for FESS</b>				
CRS	10 (55.6)	8 (80.0)	7 (100.0)	7 (100.0)
non-CRS	8 (44.4)	2 (20.0)	0 (0.0)	0 (0.0)
<b>Side sampled</b>				
Right	9 (50.0)	5 (50.0)	4 (57.1)	2 (28.6)
Left	9 (50.0)	4 (40.0)	3 (42.9)	5 (71.4)
Unknown	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)
<b>Endoscopic findings</b>				
Normal mucosa	18 (100.0)	10 (100.0)	0 (0.0)	0 (0.0)
Polyps				
w/ inflammation only	0 (0.0)	0 (0.0)	3 (42.9)	0 (0)
w/ mucopurulence	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)
w/ mucin	0 (0.0)	0 (0.0)	4 (57.1)	6 (85.7)
<b>Nasal steroid spray use</b>				
Yes	5 (27.8)	5 (50.0)	3 (42.9)	6 (85.7)
No	13 (72.2)	5 (50.0)	4 (57.1)	1 (14.3)