The effect of corticosteroids on sinus microbiota in chronic rhinosinusitis patients with nasal polyposis

by
Yousif AlAmmar, MBBS

Department of Otolaryngology- Head & Neck Surgery
McGill University, Montreal
August 2019

A thesis submitted to McGill University
in partial fulfillment of the requirements of the degree of
Master of Science

Dedicated

With love to my parents, wife and siblings,

Thank you for being a true believer, supporters and for being you.

Abstract

Background:

Chronic rhinosinusitis with nasal polyposis (CRSwNP) is a multifactorial disease with no known single cause, but it is thought that bacteria play a role in the disease process. The short-term response of bacterial communities to corticosteroid therapy has been found to be unpredictable. As a result, this pilot study aims to assess the long-term effect of corticosteroid therapy on sinus microbiota in chronic rhinosinusitis patients with nasal polyposis (CRSwNP).

Methods:

A longitudinal prospective case-control study was done on patients with CRSwNP and on healthy subjects. Patients with CRSwNP were randomly allocated to a maximal medical therapy (corticosteroids and antibiotics) treatment group or a corticosteroid-only treatment group. Data was collected at 3 time points (before treatment, 1 and 3 months after treatment): A guarded sinus swab was collected from the middle meatus; the SNOT-22 questionnaire was used to assess clinical symptoms. Specimens were cultured and Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) was used as a bacterial detection method. The raw data produced was analyzed to characterize the sample and to assess the response to each medical treatment.

Results:

Data from 29 patients with CRSwNP (16 maximal medical therapy; 13 corticosteroids only) was compared to 15 healthy subjects. Patients reported significant symptoms improvement initially (1-month), but not on the long-term (3-months). This result was found as a response to both treatment

groups, whether or not antibiotics was used. There was no significant difference in the sinus microbiota prevalence between CRSwNP patients and normal subjects. After three months from treatment, *Corynebacterium* genera tended to increase in the maximal medical therapy group, while *Staphylococcus* and gram-negative genera (*Pseudomonas)* tended to increase after corticosteroid treatment. Smoking, aspirin sensitivity and previous endoscopic sinus surgery were found to be co-factors significantly associated with the response to systemic corticosteroid therapy.

Conclusion:

In this pilot study both treatment options were effective on the short-term, but not on the long-term with no clear sinus microbiota response linked. As a result, this study agrees with previous reports that discourage the use of systemic antibiotics without evidence of active infection.

Résumé

Contexte :

La rhinosinusite chronique avec polypes nasaux (CRSwNP) est une maladie multifactorielle sans cause unique connue, mais on pense que les bactéries jouent un rôle dans le processus de la maladie. La réponse à court terme des communautés bactériennes au traitement par corticostéroïdes s'est avérée imprévisible. En conséquence, cette étude pilote vise à évaluer l'effet à long terme de la corticothérapie sur le microbiote des sinus chez les patients atteints de rhinosinusite chronique et de polypes nasaux (CRSwNP).

Les méthodes:

Une étude longitudinale prospective cas-témoins a été menée chez des patients atteints de CRSwNP et des sujets sains. Les patients atteints de CRSwNP ont été assignés au hasard à un groupe de traitement médical maximal (corticostéroïdes et antibiotiques) ou à un groupe de traitement composé uniquement de corticostéroïdes. Les données ont été collectées à 3 moments différents (avant le traitement, 1 et 3 mois après le traitement): un écouvillon de sinus gardé a été collecté à partir du méat moyen; le questionnaire SNOT-22 a été utilisé pour évaluer les symptômes cliniques. Les échantillons ont été cultivés et la spectrométrie de masse (MS) MAL-TOF (ionisation par désorption laser à temps de vol assistée par matrice) a été utilisée comme méthode de détection bactérienne. Les données brutes produites ont été analysées pour caractériser l’échantillon et évaluer la réponse à chaque traitement médical.

Résultats:

Les données de 29 patients atteints de CRSwNP (16 traitements médicaux maximal; 13 corticostéroïdes uniquement) ont été comparées à 15 sujets en bonne santé. Les patients ont signalé une amélioration significative des symptômes au départ (1 mois), mais pas à long terme (3 mois). Ce résultat a été trouvé en réponse aux deux groupes de traitement, que des antibiotiques aient été utilisés ou non. Il n'y avait pas de différence significative dans la prévalence du microbiote sinusal entre les patients atteints de CRSwNP et les sujets normaux. Après trois mois de traitement, les genres de *Corynebacterium* avaient tendance à augmenter dans le groupe de traitement médical maximal, alors que les genres *Staphylococcus* et Gram négatif (*Pseudomonas*) avaient tendance à augmenter après le traitement par les corticostéroïdes. Le tabagisme, la sensibilité à l'aspirine et les chirurgies endoscopiques antérieures des sinus se sont avérés des facteurs concomitants ayant une incidence sur la réponse au traitement systématique aux corticostéroïdes.

Conclusion:

Dans cette étude pilote, les deux options de traitement étaient efficaces à court terme, mais pas à long terme, sans réponse claire du microbiote des sinus liée. En conséquence, cette étude est en accord avec les rapports précédents qui découragent l'utilisation d'antibiotiques systémiques sans preuve d'infection active.

Preface

Contributions of authors

Dr. Yousif AlAmmar did the literature review in its entirety, the data collection, and the analysis of results. Dr. Marc Tewfik provided supervision and guidance regarding clinical relevance, concept, design and review of this thesis. Dr. Simon Rousseau provided the research design and implementation framework, supervision, and guidance on basic science relevance along with the revision of this thesis.

Claim of originality

This is the first pilot study to assess the long-term effect of corticosteroids on sinus microbiota in chronic rhinosinusitis patients with nasal polyposis. It added clarification and supported previous reports that discourage the use of systemic antibiotics without justification. In addition, it highlights a potential role of *Staphylococcus* and *Pseudomonas* in the disease response to medical treatment.

Acknowledgment

I would like to express my deepest appreciation to Dr. Marc Tewfik M.D. M.Sc. FRCSC who provided an outstanding support, mentoring, knowledge and expertise in sinusitis and networking during this entire study. I am also very much thankful to Dr. Simon Rousseau Ph.D. for his much-needed support and guidance in the basic science part of this thesis and I am extremely grateful for his support and mentorship. I am also grateful to Dr. Rousseau's laboratory members for their technical assistance. Special thanks to Dr. Bernard Segal Ph.D. for his excellent critical review of this work.

Finally, I would like to thank my home institution King Saud university and the scholarship program administration for believing in me and providing the opportunity to pursue this degree at McGill university.

Table of Contents

Abstract 3

Résumé 5

Preface 7

Contributions of authors 7

Claim of originality 7

Acknowledgements 8

List of Tables 11

List of Figures 12

List of Abbreviations 13

1 Chapter One: Introduction 14

1.1 Rationale 14

1.2 Objective 15

1.3 Hypothesis 16

2 Chapter Two: Literature Review 17

2.1 Current Knowledge of Chronic Rhinosinusitis with nasal polyposis 17

2.2 Bacterial detection methods in CRS 18

2.3 Microbiota in CRSwNP vs healthy 19

2.4 Systemic antibiotics and corticosteroids in recent CRSwNP management guidelines 20

2.5 The effect of medical management on microbiota 21

3 Chapter Three: Methods 24

3.1 Study design and Population…….…..24

3.2 Sample size……...…….25

3.3 Intervention……..……25

3.4 Sample collection…….…….……25

3.5 Sample processing……..……..26

3.6 Microbial extraction…….……...……27

3.7 Microbial Identification……..…….28

3.8 Statistical Analysis……...…..……28

4 Chapter Four: Results…….....…30

4.1 Patient demographics and clinical characteristics…….30

4.2 Control……..……31

4.3 Control vs CRSwNP…….32

4.4 CRSwNP Maximal medical therapy cohort (CRSwNPM)……..33

4.5 CRSwNP Corticosteroid medical therapy cohort (CRSwNPS)……..…36

4.6 Comparing CRSwNP maximal medical therapy cohort to corticosteroid medical therapy cohort……..39

5 Chapter Five: Discussion…….…….41

6 Chapter Six: Summary and Conclusion……..45

7 Chapter Seven: List of References……47

List of Tables

Table 2-1 Summary of studies discussing the effect of medical treatment on sinus microbiota ….23

Table 4 -1: Patient demographics and clinical characteristics…….…….30

Table 4-2: Comparing CRSwNP Maximal medical therapy cohort characteristics over the three different time points……..…….33

Table 4-3: Comparing CRSwNP Corticosteroid therapy cohort characteristics over the three different time points……..…….36

List of Figures

Figure 4 -1. The prevalence of microbiota in the control cohort on a genus level……..……31

Figure 4 -2. Comparison of the most prevalent microbiota between the control cohort and the CRSwNP cohort (case group)……..……..32

Figure 4 -3. Comparing SNOT-22 scoring between the three different time points in the chronic rhinosinusitis with nasal polyposis group who received maximal medical therapy (CRSwNPM)……..34

Figure 4 -4. Comparing the most prevalent genera between the three different time points in the chronic rhinosinusitis with nasal polyposis group who received maximal medical therapy (CRSwNPM)……..35

Figure 4 -5. Comparing SNOT-22 scoring between the three different time points in the chronic rhinosinusitis with nasal polyposis group who received Corticosteroid therapy (CRSwNPS)…….……..37

Figure 4 -6. Comparing the most prevalent genera between the three different time points in the chronic rhinosinusitis with nasal polyposis group who received corticosteroid therapy (CRSwNPS)……...38

Figure 4-7. Comparing the most prevalent genera between the two different treatment groups…….40

List of Abbreviations

CRS Chronic Rhinosinusitis

ARS Acute Rhinosinusitis

CRSsNP Chronic Rhinosinusitis without nasal polyposis

CRSwNP Chronic Rhinosinusitis with nasal polyposis

AERD Aspirin Exacerbated Respiratory Disease

Th T-helper

IL Interleukin

MALDI-TOF Matrix-assisted laser desorption ionization–time of flight

MS Mass spectrometry

INCS Intranasal corticosteroids

CRSwNPM Chronic Rhinosinusitis with nasal polyposis maximal medical therapy group

CRSwNPS Chronic Rhinosinusitis with nasal polyposis corticosteroid therapy group

ESS Endoscopic sinus surgery

Chapter One: Introduction

1.1 Rational:

Chronic Rhinosinusitis (CRS) is a common disabling inflammatory disease affecting the nasal mucosa and the paranasal sinuses [1]. The estimated prevalence in the American population is up to 16 % and in the European population is around 10.9 % [2,3]. A survey conducted on Canadian households reported the prevalence to be around 5%, with 2.89 million prescriptions dispensed in 2006 for acute rhinosinusitis (ARS) or CRS [4,5].

The American Academy of Otolaryngology & Head and Neck Surgery (AAO-HNS) defined CRS as presence of the two or more of the following symptoms: mucopurulent drainage, nasal obstruction, facial pain/pressure/fullness, or decreased sense of smell, in addition to objective documentation for the inflammation by either nasal endoscopy or radiographic imaging [6]. CRS can be subdivided further phenotypically into Chronic rhinosinusitis without nasal polyposis (CRSsNP), or Chronic rhinosinusitis with nasal polyposis (CRSwNP), with CRSwNP being found in 20% to 30 % of CRS patients [7,8].

CRSwNP is a multifactorial disease with no known single cause. Many theories have been proposed to better understand the disease pathogenesis, which can be divided into either (a) host related factors such as the immunity barrier hypothesis, or (b) environmental factors such as the microbiome hypothesis, which has been gaining interest over the last few years [9]. However, the causative factors continue to be an area of ongoing research. Often, the most prevalent bacteria in CRS was *Staphylococcus aureus* [10]. However with increased use of culture-independent

bacterial identification methods, recent studies concluded that *Corynebacterium* and *Staphylococcus* were more often found in healthy sinuses, and there were changes in the abundance and richness seen in the CRSwNP patients. *Pseudomonas*, *Moraxella* were more frequently identified in CRSwNP patients as well [11,12,13].

There have been different approaches for managing the disease medically. The first approach uses maximal or appropriate medical therapy, which includes oral and topical corticosteroids. A second adds the option of additional antibiotics [14]. Few studies have assessed the effect of these treatment options on the sinus microbiota, focusing on the immediate changes within the first month of receiving the treatment [12]. However, studying the effect of these treatments on the sinus microbiota might explain a non-respondent patient's pathogenesis, which might lead to alternative effective treatment options, and to a decreased need for surgical intervention.

1.2 Objective:

The aim of this thesis is to use a pilot study to identify the long-term impact of corticosteroid therapy on the sinus microbiota in Chronic Rhinosinusitis patients with nasal polyposis (CRSwNP).

1.3 Hypothesis:

The working hypothesis of this thesis is that the sinus microbiota in patients with CRSwNP is different from subjects without CRS. Using corticosteroid therapy will alter the sinus flora leading to clinical improvement.

Chapter Two: Literature review

2.1 Current Knowledge of Chronic Rhinosinusitis with nasal polyposis:

One of the common subtypes of CRS is Chronic rhinosinusitis with nasal polyposis (CRSwNP) characterized by the presence of bilateral nasal polyposis in the nasal cavity and paranasal sinuses [8]. Nasal polyposis are benign, edematous growths, containing inflammatory material and exudates [15]. Four percent of the general population are affected with nasal polyposis [3, 16], with 2:1 male to female ratio [17]. A well-known association has been noted between CRSwNP and asthma (in about 34-50% of patients [18]) and with aspirin sensitivity. This triad of CRSwNP, adult onset of asthma and aspirin sensitivity is known as "aspirin-exacerbated respiratory disease (AERD)" or "Samter's triad "[19].

The etiology of CRS is still a subject of an ongoing research. Environmental factors such as allergens [20] and toxins [3] were hypothesized to be causative factors, in addition to other host related factors as an example genetic predisposition [21] or a defect in the immune system [9]. A recent study by our group investigating the immune system defect in CRSwNP patients observed a persistent elevation of key Th1 inflammatory cytokines (i.e. TNF-α. and IL-1.) despite the known theory suggesting Th2 response as the key inflammatory pathway in CRSwNP. Study findings also suggested that persistent elevation of TNF-α may contribute to corticosteroids resistance and to CRSwNP recalcitrance [22].

Furthermore, a growing interest over the last few years has been noted in superantigens [23], fungi [24], biofilms [25], bacterial intramucosal residence [26] and microbes [24,26] as a potential causative factor for CRS. Recent literature concluded that an alteration in the sinonasal microbial communities was seen in CRS patients, and this could be indirectly influenced by environmental factors [27], host related factors such as asthma [10] and treatment factors (for example: antibiotics) [28], which make establishing disease causation challenging and higher quality longitudinal studies are required [26].

2.2 Bacterial detection methods in CRS:

In the past, axenic cultivation was the main pathogenetic detection method used in CRS, which requires subcultivation and biochemical assays to be able speciate pathogens in a slow process that varies between 2 to 30 days. Microscopy and nuclear acid testing are two different culture-independent identification methods used in the field. Nuclear acid testing methods clarified the polymicrobial behaviour of CRS and has been the favoured method in the field since it is a faster and accurate detection method with the advantage of testing for multipathogens. However, the cost and the need for experienced laboratory personnel are the downsides for this detection method. [26, 29]

Over the last few years, Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) has been used as a bacterial detection method with significant potential. It detects pathogens by measuring a unique proteomic fingerprint. A spectrum is generated after

measuring the highly abundant protein of an organism and then matched with a reference database for identification. This identification method is fast, accurate, less expansive in comparison to nuclear acid testing methods and no extensive training is required. Although, the initial cost of the equipment is a clear disadvantage. [30]

A limited number of studies have used MALDI-TOF-MS as a detection method in the CRS research literature with promising outcomes [31,32,33]. Interestingly, Kasper et al, in their comparison between the cultivable and the molecular fingerprints were able to correlate only 6.2% of the phylotypes, with a higher level of species identification in the culturemoic approach [32].

2.3 Microbiota in CRSwNP vs healthy:

Historically, it was thought that the sinus mucosa is sterile in healthy subjects [34]. However, in 2009, a landmark study was published by Abou-Hamad defeated this theory [35]. The microbiota of healthy and diseased sinus has been under extensive research over the last few years. Total amount of bacteria was found to be similar in several studies between both healthy and diseased sinuses [11,13,36]. Several studies identified *Staphylococcus*, *Corynebacterium* and *Propionibacterium* as the most common genera in the healthy sinuses [13, 37].

To assess the difference between healthy and CRS sinuses, a systemic review published by Anderson et al, found that similar phyla were identified in both healthy and diseased groups, and they were not able to identify a single causative microbiome [38]. Mackenzie et al, published an interesting Meta-analysis in 2016 and identified *Staphylococcus*, *Corynebacterium*,

Propionibacterium, *Streptococcus* and an unclassified lineage of Actinobacteria as the most abundant genera among both groups and linked an increase in the members of the *Corynebacterium* genera to CRS. Furthermore, they highlighted the importance of the *Burkholderia* and *Propionibacterium* as gatekeepers to maintain a stable bacterial community [39].

A few years later, Paramasivan et al published the largest study to date with 410 patients to describe the sinus microbiome and concluded that *Staphylococcus*, *Corynebacterium*, *Streptococcus*, *Haemophilus* and *Moraxella* genera were the core microbiome in both healthy and diseased sinuses with *Corynebacterium* and *Staphylococcus* being the most prevalent genera (the mean relative abundance 44.02 % and 27.34 % respectively). However, in the CRSwNP group a significant drop in the prevalence of *Corynebacterium* and an increase in *Streptococcus* was noted [40].

2.4 Systemic antibiotics and corticosteroids in recent CRSwNP management guidelines:

The American Academy of Otolaryngology & Head and Neck Surgery (AAO-HNS) CRS guidelines recommended the use of nasal saline irrigation or intranasal corticosteroids (INCS) or both as a first line treatment for symptoms relief in CRS patients with Level A evidence, but the use of systemic corticosteroids and antibiotics was not discussed [6].

Furthermore, the Canadian guidelines moderately recommended INCS and a short-term systemic corticosteroid as the mainstay treatment for CRSwNP, with the recommendation to use oral antibiotics if there are any signs of infection (presence of symptoms suggesting infection as an

example: pain or recurrent episodes of sinusitis, or when purulence is documented on rhinoscopy/endoscopy), the level of evidence was moderate [1].

Finally, the European guidelines strongly recommend (Level A recommendation) the use of INCS and systemic corticosteroids for CRSwNP with Level 1A evidence. The use of short-term oral antibiotics for three weeks in CRSwNP is a level C recommendation with a level 1b evidence. However, the long-term use of oral antibiotics requires further study [41].

2.5 The effect of medical management on microbiota:

Only a few studies have assessed the effect of medical treatment on microbiota. A study published in 2015 found a notable change in the sinonasal microbiota in CRSwNP patients post-surgically who used intranasal saline irrigation and corticosteroid spray, and a distinct sinonasal microbiota in controls [42].

Feazal et al, in their cross-sectional study that examined the factors influencing the biodiversity in CRS patients, concluded that antibiotics decrease biodiversity and increase the abundance of *Staphylococcus aureus* [10]. Similar findings were described by Liu et al, in his small cohort where they examined the effect of pre and post maximal medical therapy on sinus microbiota in CRS patients and found a decrease in the biodiversity (richness and evenness) [43]. Another study assessing the effect of antibiotics on the sinus microbiome in CRS patients with an acute exacerbation showed an increase in biodiversity and a decrease in the bacterial abundance, and the

predominant organism identified by 16S sequencing was correlated with bacteria identified by culture in each sample [44].

A study assessing the association between CRS clinical variants (such as: asthma and cystic fibrosis) and sinus microbiota changes, found it to be not influenced by the use of antibiotics or corticosteroids [11].

Furthermore, a recent longitudinal study published by Jaid et al in 2018 showed a variable effect of 7 days of medical therapy on the sinus bacterial community with no clear pattern observed. They observed an increase in the *Propionibacterium* average relative abundance in the group that received doxycycline as a medical therapy, and a decrease in the *Corynebacterium* genera in the prednisone group. This study also recommended assessing the effect systemic therapies have on the sinus microbiota over a longer duration [12].

Study	Treatment	Study group	No. Subjects Controls/CRS	Identification method	Findings
Liu et al.[42]	Saline nasal irrigation and topical nasal corticosteroids	Post-surgery	28/14(CRSwNP)	Nuclear acid testing	Sex-specific differences: male with CRSwNP showed higher abundances of Corynebacterium, Serratia and Finegoldia. Intranasal corticosteroids spray was associated distinct sinonasal microbiota
Feazel et al.[10]	Antibiotics	Intraoperative ESS	5/15	Nuclear acid testing	Antibiotics use decreased microbiome diversity (both richness and evenness) and increase Staphylococcus Aureus
Liu et al.[43]	Antibiotics and Corticosteroids	Pre and Post medical treatment	0/6 (4 CRSwNP)	Nuclear acid testing	Significant decrease in microbiome diversity (richness and evenness), with highly individualized microbiota profile
Merkley et al.[44]	Antibiotics	Pre and post treatment	0/8 (CRSwNP)	Nuclear acid testing + Culture	Decrease in biodiversity and an increase in the bacterial abundance
Hoggard et al.[11]	Antibiotics and Corticosteroids	Intraoperative ESS	29/94 (51 CRSwNP)	Nuclear acid testing	Microbiota dysbiosis was not explained by Antibiotics use or corticosteroids.
Jain et al [12]	Doxycycline or Prednisone	Pre and Post medical treatment	6/20 (13 CRSwNP)	Nuclear acid testing	Patient treated with doxycycline or prednisone had a variable sinus microbiota unpredictable, with increase in the *Propionibacterium* average relative abundance in the doxycycline group and decrease in the Corynebacterium in the prednisone group.

Table 2-1 *Summary of studies discussing the effect of medical treatment on sinus microbiota*

Chapter Three: METHODS

3.1 Study design and Population:

A longitudinal prospective case-control study was performed. The case group was composed of patients diagnosed with CRSwNP, and control subjects were patients undergoing either a transsphenoidal resection of pituitary tumors or a septoplasty. CRSwNP were assigned to one of the two treatment groups using simple randomization. The McGill University Health Centre ethics committee approved the study. Informed consent was obtained from all subjects.

The definition of the Canadian clinical practice guidelines for acute and chronic rhinosinusitis was used to select patients with CRSwNP [1]. This definition specifies that the diagnosis of CRS requires at least 8 to 12 weeks of two or more major symptoms, in addition to documented inflammation of the paranasal sinuses or nasal mucosa with computed tomography or endoscopy. Symptoms are facial congestion, facial pain/pressure, nasal obstruction, nasal discharge and hyposmia/anosmia.

To recruit patients the following inclusion criteria was used: 1) Documented diagnosis of bilateral CRSwNP; 2) Eighteen years of age or older; 3) No antibiotics and/or oral corticosteroids for at least one month prior to recruitment. Patients were excluded from this study if they were found to have: 1) documented diagnosis of fungal sinusitis; 2) pregnancy, 3) diagnosed crohn's disease or ulcerative colitis; 4) diagnosed immotile cilia syndrome; 5) diagnosed cystic fibrosis; 6) diagnosed immunodeficiency syndrome; 7) diagnosed sinonasal tumor; or 8) patients on immunomodulatory medications.

3.2 Sample size:

This thesis aimed to be a pilot study allowing exploration of a new research question: whether corticosteroid therapy has an effect on sinus microbiota in CRSwNP patients. As a result, based on prior similar pilot research studies, a sample size of 10 or more subjects per group was chosen, and considered to be satisfactory [12, 43].

3.3 Intervention:

CRSwNP patients were divided into two subgroups. The first subgroup was the **maximal medical therapy group** (CRswNPM): They received corticosteroids (Prednisone tapering dose once daily for 21 days, first week: 30 mg, second week: 15 mg and the third week: 5 mg) in addition to antibiotics (Amoxicillin Clavulanate 875 mg, by mouth twice a day for 14 days). The second subgroup was the **corticosteroid therapy group** (CRSwNPS): they received only corticosteroids (Prednisone tapering dose once daily for 21 days, first week: 30 mg, second week: 15 mg and the third week: 5 mg).

3.4 Sample collection:

In an outpatient setting, endoscopically guided flocked nasal swabs (Copan Italia S.p.A., Brescia, Italy) were collected from the middle meatus using a guarded technique to minimize the contamination with rotating it at least 5 times clockwise and counter clockwise.

From each patient with CRSwNP, three different nasal swabs from the same side were collected at three different time points. The first was collected during the time of recruitment and completion the study entry questionnaire (time point 1). The second nasal swab was collected after a month, soon after finishing the course of treatment based on the assigned group (time point 2). Finally, the third nasal swab was collected three months after completing the treatment (time point 3). During each visit, every subject completed a questionnaire to track the change in disease status using SNOT-22[45]. The nasal swabs were placed on ice as soon as they were collected and transferred within four hours to the Meakins-Christie lab (McGill University) for processing.

3.5 Sample processing:

Each sample was cultured by striking the swab onto four different media: blood agar, chocolate agar, mannitol salt agar and methylene blue agar. The striking was done in standardised fashion by holding the swab from the shaft with continuous rotation and striking in two different directions with gentle pressure under the flame to avoid contamination. Plates were then cultured in an incubator at 37 C under 5% CO2 - 20% O2 atmosphere for 72 hours.

Bacterial colonies were differentiated by color, size, shape, consistency and hemolysis. A 10 μl loop was used to reculture the identified colonies in the same media with the same technique described earlier and incubated for another 72 hours in the same atmosphere. Furthermore, the colonies were isolated in a 1 ml of 80% glycerol tube and stored in a –80 C fridge until further processing.

3.6 Microbial Extraction:

In preparation for microbial identification, isolates stored in –80 C fridge were stroked on a Lysogeny broth (LB) media using the same striking technique described above and cultured in an incubator at 37 C under 5% CO2 - 20% O2 atmosphere for 72 hours.

From the cultured isolate a scraped sample of pure cells was taken and added on in a 1.5 ml eppendorf tube filled with 300 μl of mass spectroumay water and vortexed for 10 seconds to suspend cells in water. Furthermore, 900 μl of 100% ethanol was added and vortexed for 10 seconds as well. The sample was spun for 2 minutes at 15000 RPM, and then supernatant was decant and the sample was respun for 2 minutes with same settings used before. Using a 200 μl pipette the remnant of the supernatant was removed, and the samples were left to dry for a minimum of 5 minutes. A Twenty-five μl of 70 % formic acid was added and the sample was then vortexed for 10 seconds. Finally, 25 μl of acetonitrile was added with mixing the sample gently to avoid introducing bubbles and the last spin was done at this point for 2 minutes at 15000 RPM. After removing 10 μl into a 0.2 ml PCR eppondorf tube avoiding the pellet, the sample was ready to be spotted on the microbial identification plate.

3.7 Microbial Identification:

For microbial identification, 1μl extracted bacterial isolates were placed on a 96-spot target steel plate (Bruker Daltonics, Solna, Sweden) and left to dry at room temperature. The bacterial spots were then coated with 1 μl alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix solution and kept for crystallization. Samples were processed for identification in the MALDI-TOF MS, using the flexcontrol software (FlexControl 3.3, Bruker Daltonics) in linear positive mode in a range of 2000 – 20000 DA. Using MALDI Biotyper software 3.1 (Bruker Daltonics) and mass spectra were analyzed using the reference database MBT-BDAL-5627.

The microbial identification criteria was as follows: a score of >=2.0 indicates an identification at the species level and a score between 1.70 and 1.99 was considered as identification at the genus level, however, a score < 1.70 was a read as no identification [46].

3.8 Statistical Analysis:

Statistical analysis was done using GraphPad Prism: Version 8.1 (GraphPad, La Jolla, CA). Data obtained at the 3 sampled times were summarised descriptively. Continuous data were summarized using Mean ± Standard deviation. Then, comparisons between two time points and the two different groups were done using t-tests. Finally, comparisons between the three different time points were done using one-way ANOVA test.

Categorical data were summarized as percentages; differences between two time points or groups were assessed using Fisher's exact test. Changes between three time points were assessed for significance using the Pearson Chi square test.

Multivariant regression analysis was done to assess the effect of number of organisms isolated, gender, age of patients, asthma, aspirin sensitivity, previous endoscopic sinus surgery, smoking and the most prevalent genera on the response to treatment in each case group. P value < 0.05 was considered statistically significant.

Chapter Four: Results

4.1 Patient demographics and clinical characteristics:

A total of 15 controls and 29 CRSwNP patients were recruited. The 29 CRSwNP patients were subdivided into 16 CRSwNPM and 13 CRSwNPS patients. The mean age for the control cohort was 43.07 (± 13.72); 52.13 (± 14.39) for CRSwNPM, and 47.54 (± 12.39) for CRSwNPS. Male to female ratio (M:F) was (11:4) in the control cohort, (8:7) and (6:7) in the CRSwNPM and CRSwNPS cohorts respectively. There was no significant difference between the baseline characteristics. Furthermore, several variables such as smoking, aspirin sensitivity, asthma and previous endoscopic sinus surgery (ESS) were assessed between CRSwNPM and the CRSwNPS cohorts and showed no statistical significance (Table 4-1).

Characteristics	Control Cohort (n=15)	CRSwNPM Cohort (n=16)	CRSwNPS Cohort (n=13)	P Value
Age, mean (± SD), y	43.07 (±13.72)	52.13 (±14.39)	47.54 (±12.39)	0.19
Gender M: F	11: 4	8: 7	6: 7	0.33
Smoking (%)	5(33.3)	5(31.25)	4(30)	0.99
Aspirin sensitivity (%)		5(31.25)	6(46.15)	0.99
Asthma (%)		8(50)	10(76.9)	0.24
Previous ESS (%)		6(37.5)	9(69.2)	0.13

Table 4 -1: *Patient demographics and clinical characteristics. ESS; Endoscopic sinus surgery.*

4.2 Control Cohort

The growth rate in the control cohort was 76.2 %, while the identification rate was 95.24%. Eighteen different organisms were isolated in this cohort, 14 gram-positive and 4 gram-negative. *Staphylococcus* was the most prevalent genera (73.3 %) and *Micrococcus* (13.3 %). On a species level, the most prevalent species in the control group was *Staphylococcus aureus* (Figure 4-1).

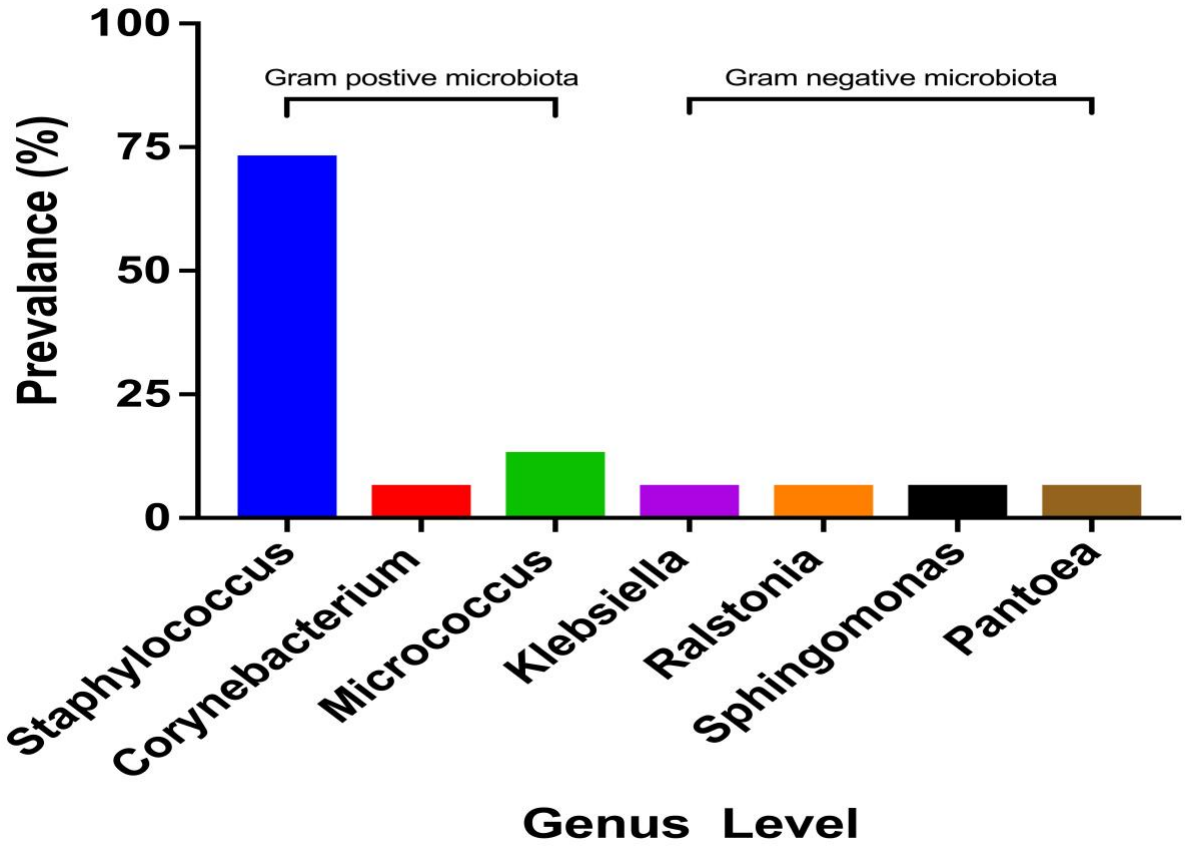

Figure 4 -1. *The prevalence of microbiota in the control cohort on a genus level.*

4.3 Control vs CRSwNP cohort

The difference in the number of isolated organisms and the difference between gram-positive and gram-negative isolates were not statistically significant. In CRSwNP, the most prevalent organism *Staphylococcus* tended to be higher than the controls (P = 0.46). Also, the number of *Corynebacterium* isolates tended to be higher once compared to the control group, but again it was not statistically significant (P = 0.39) (Figure 4-2).

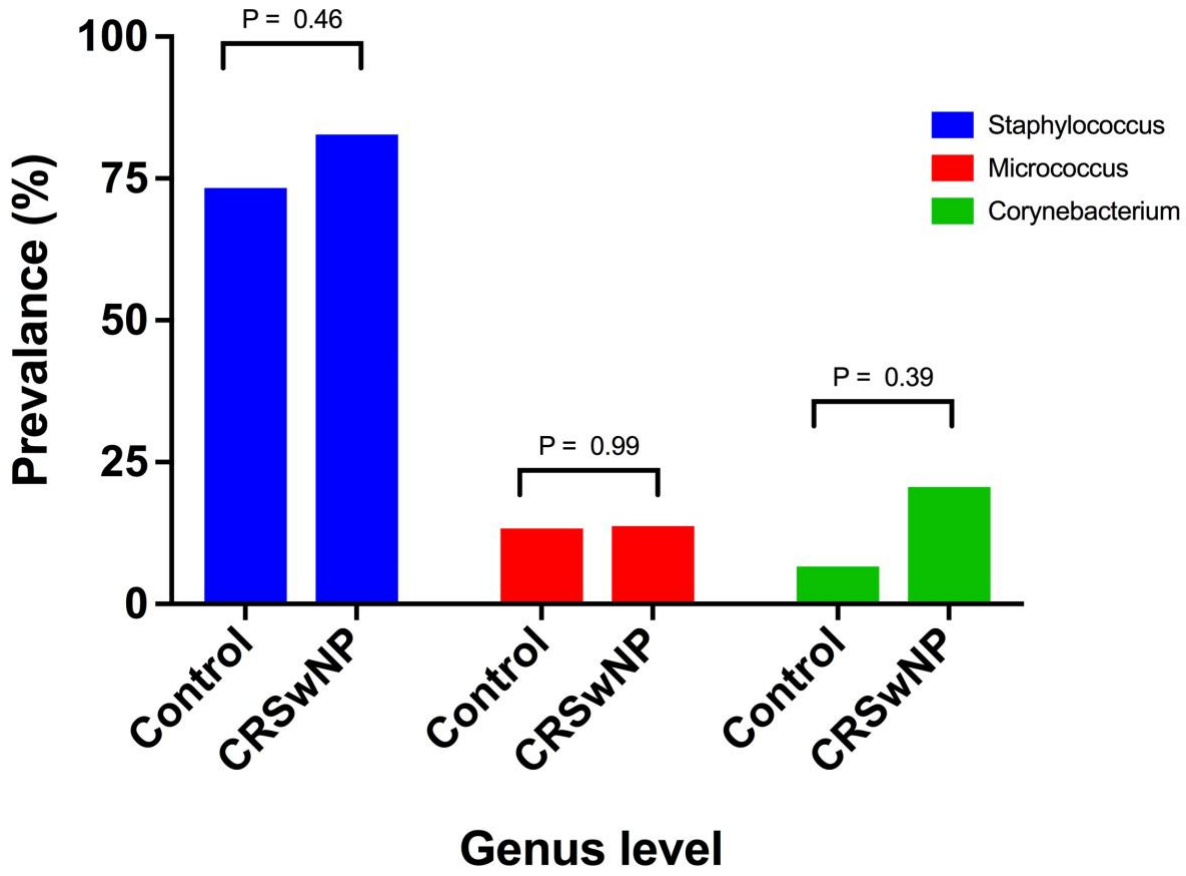

Figure 4 -2. *Comparison of the most prevalent microbiota between the control cohort and the CRSwNP cohort (case group).*

4.4 CRSwNP Maximal medical therapy cohort (CRSwNPM)

In this group, differences in the number of organisms isolated between the three different time points was insignificant (p = 0.92). As well, there was no change in the gram-positive microbiome or the gram-negative microbiome. However, when comparing the three time points using the one- way ANOVA test, the SNOT-22 score showed a significant improvement (**P < 0.0001**). The main improvement was between CRSwNPM-1 and CRSwNPM-2 (**P = 0.019**) (Table 4-2) (Figure 4-3).

Characteristics	CRSwNPM-1	CRSwNPM-2	CRSwNPM-3	P Value
Growth rate	93.75%	100%	100%	
Identification rate	100%	93.75%	87.5%	
Species no., (Mean, ± SD)	24 (1.50,± 0.89)	22 (1.37, ±0.71)	25 (1.66, ±1.39)	0.92
Gram positive (%)	23(95.83)	21(95.45)	24(96)	0.78
Gram negative (%)	2(4.17)	1(4.5)	1	
SNOT-22 Mean (± SD)	39.13 (± 30.77)	32.44 (±27.85)	36.31 (±29.80)	**P<0.0001**

Table 4-2: *Comparing CRSwNP Maximal medical therapy cohort characteristics over the three different time points.*

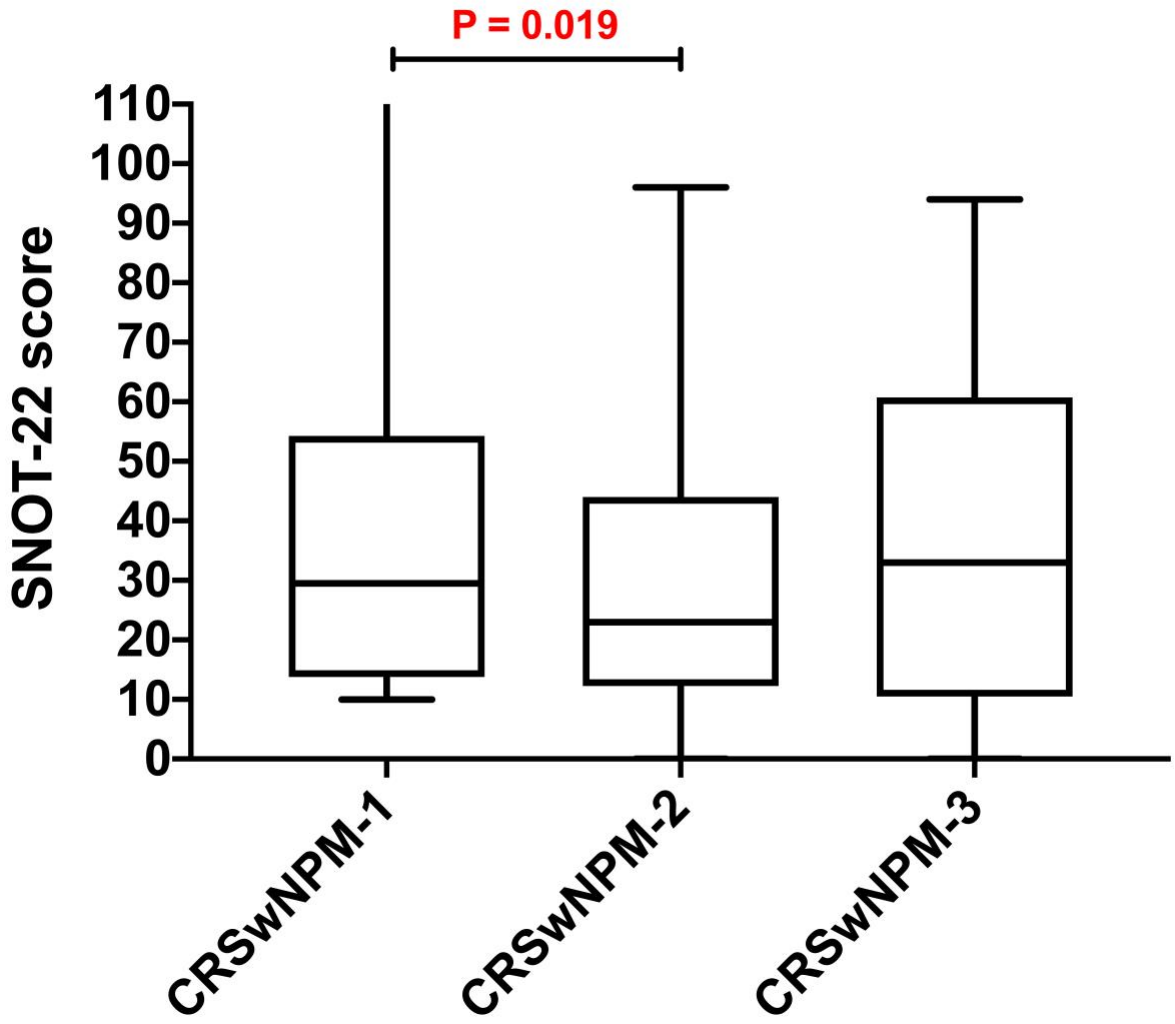

Figure 4 -3. *Comparing SNOT-22 scoring between the three different time points in the Chronic Rhinosinusitis with nasal polyposis group who received maximal medical therapy (CRSwNPM).*

The most prevalent genera in CRSwNPM observed at the three different time points was *Staphylococcus,* with slight tendency to drop after receiving the treatment. However, this change was not statistically significant (P = 0.65). *Corynebacterium* was the second most prevalent genera with an increase between CRSwNPM-2 and CRSwNPM-3 (17.5 %). However, this slight difference was not significant (P= 0.66) (Figure 4-4).

With a deeper look at the species level *Staphylococcus epidermis* and *Staphylococcus aureus* were communally isolated at the three different time points.

Finally, a Multivariant regression analysis was done to assess the effect of several variables on the response to the maximal medical treatment. Only smoking showed a significant impact on CRSwNPM-2 (**p = 0.04**).

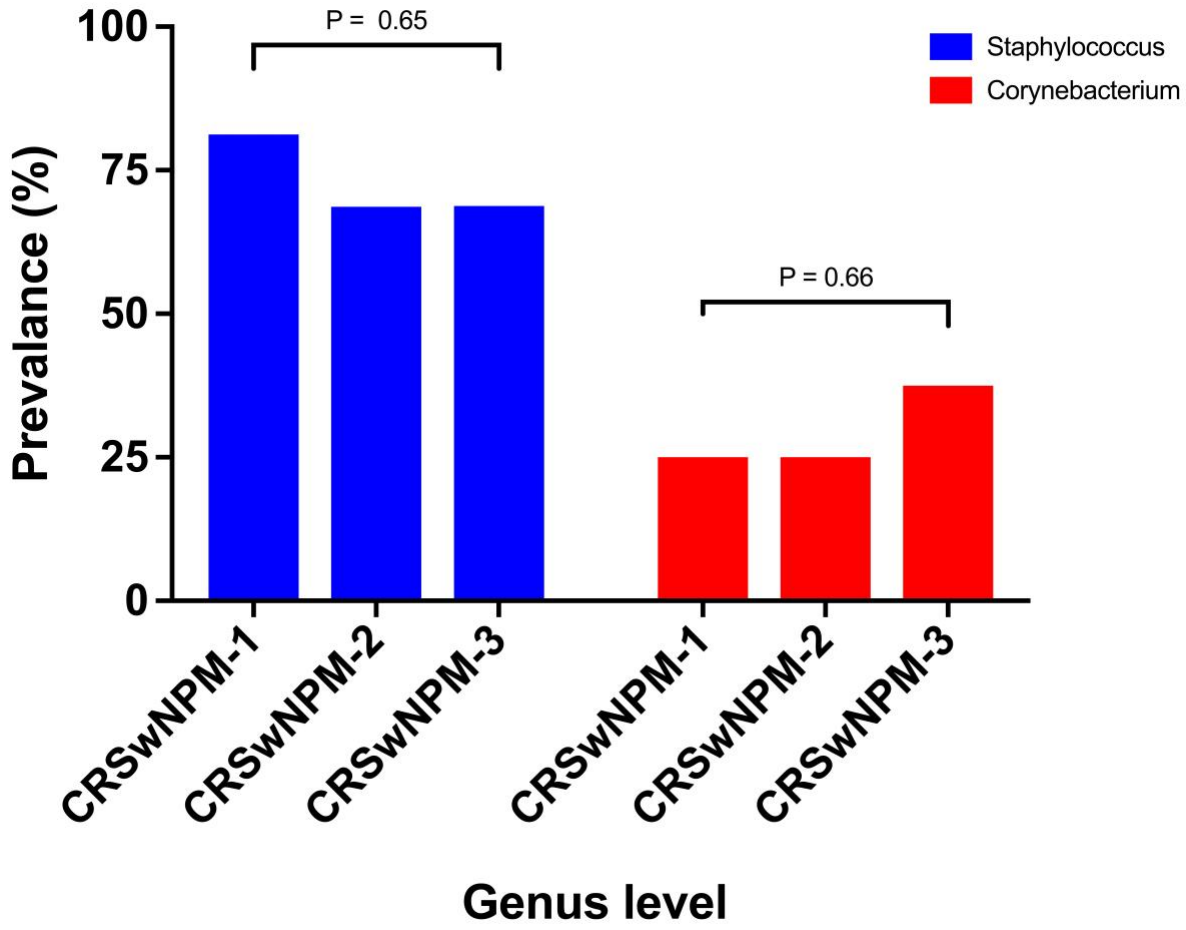

Figure 4 -4. *Comparing the most prevalent genera between the three different time points in the Chronic Rhinosinusitis with nasal polyposis group who received maximal medical therapy (CRSwNPM) .*

4.5 CRSwNP Corticosteroid therapy cohort (CRSwNPS)

The number of organisms isolated did not show a statistically significant change between the three different time points (P = 0.8). Although there was no significant difference between the gram-positive and gram-negative microbiota, there was a tendency for the number of gram-negatives isolates to increase at the 3-month time point (CRSwNPS-3) (≈ 24%). The SNOT-22 score showed a significant improvement when comparing the three measured time points (**P = 0.03**). The main improvement was between CRSwNPS-1 and CRSwNPS-2 (**P = 0.004)** (Table 4-3) (Figure 4-5).

Characteristics	**CRSwNPS-1**	**CRSwNPS-2**	**CRSwNPS-3**	**P Value**
Growth rate	86.7 %	100 %	93.4%	
Identification rate	100 %	93.4 %	100 %	
Species no., (Mean ± SD)	21(1.61 ± 1.12)	21(1.61 ± 0.86)	21(1.61 ± 0.86)	0.803
Gram positive (%)	20 (95.2 %)	19 (90.4%)	16(76.1%)	0.16
Gram negative (%)	1(4.8 %)	2(9.6%)	5(23.9%)	
SNOT-22 Mean (± SD)	54.08 (±31.40)	29.85 (±24.98)	34.62 (±31.25)	**0.033**

Table 4-3: *Comparing CRSwNP corticosteroid therapy cohort characteristics over the three different time points.*

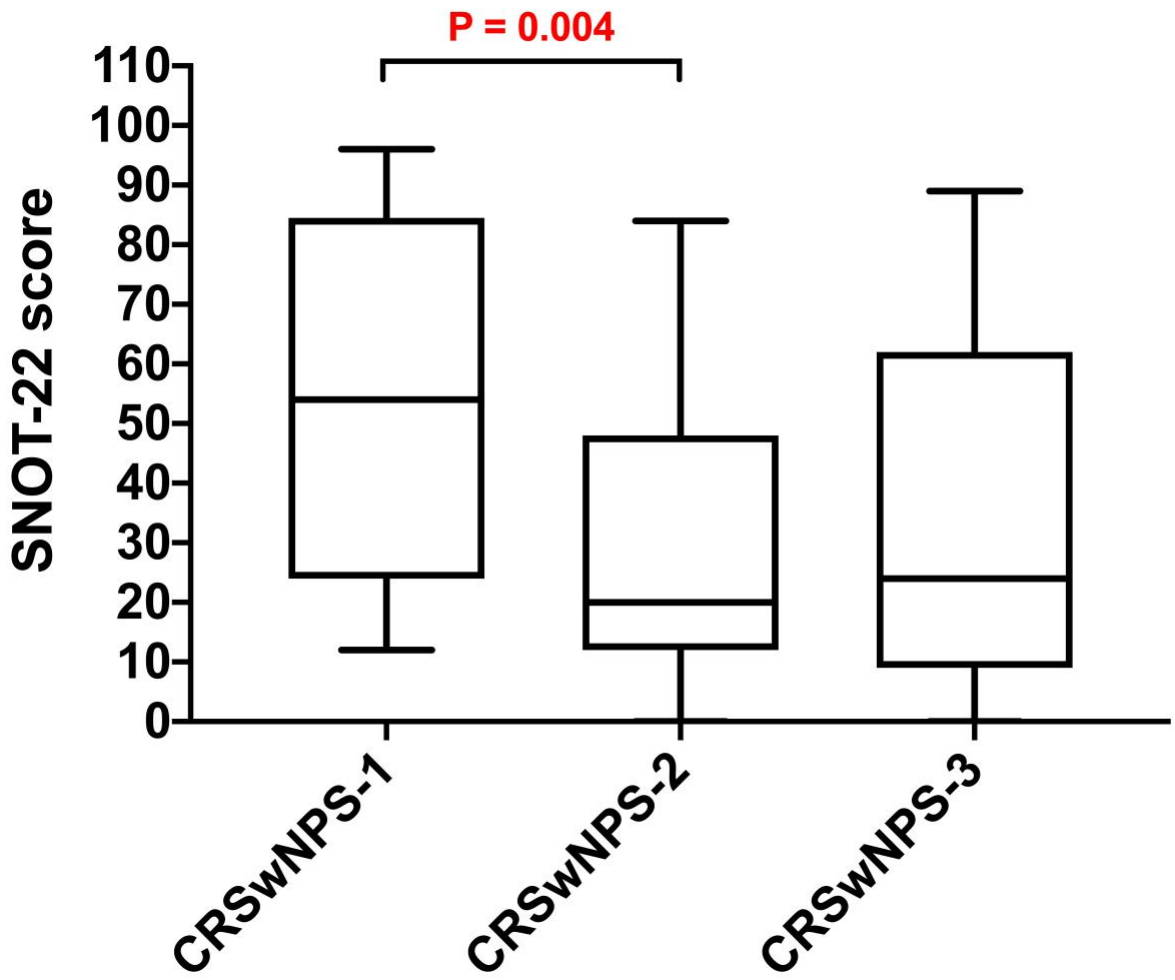

Figure 4 -5. *Comparing SNOT-22 scoring between the three different time points in the Chronic Rhinosinusitis with nasal polyposis group who received corticosteroid therapy (CRSwNPS).*

The most prevalent genera in CRSwNPS observed at the three different time points was *Staphylococcus*, with a slight drop after receiving the treatment and a noticeable increase after three months of receiving the treatment. However, this pattern was not statistically significant (P = 0.5). *Micrococcus* was the second most prevalent organism in CRSwNPS-1, but it was not isolated in the other measurement points after treatment. This change was statistically significant (**P = 0.01**). *Corynebacterium* was the second most prevalent genera in CRSwNPS2. As well, *Pseudomonas* was identified as the second most prevalent genera in CRSwNPS-3, with a noticeable increase also observed (Figure 4-6).

At the species level, while *Staphylococcus epidermis* and *Micrococcus luteus* were the most prevalent before treatment, *Staphylococcus aureus* and *Staphylococcus epidermis* were communally isolated at the measurement points after treatment.

A multivariable regression analysis was done to assess the effect of several variables on the response to the corticosteroid treatment. It was found that smoking, previous endoscopic sinus surgery and aspirin sensitivity had a significant impact (**P = 0.04, P = 0.04, P = 0.02** respectively) on clinical improvement in CRSwNPSM-3.

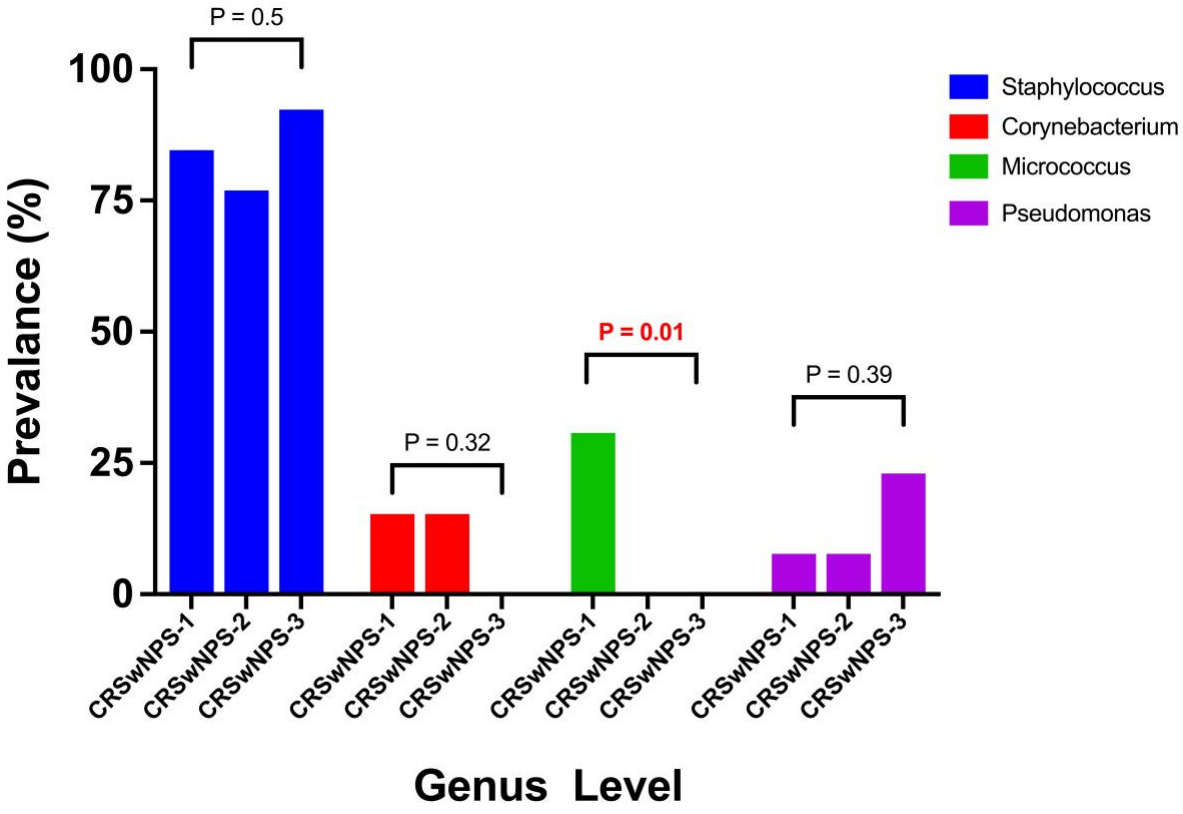

Figure 4 -6. *Comparing the most prevalent genera between the three different time points in the Chronic Rhinosinusitis with nasal polyposis group who received corticosteroid therapy (CRSwNPS).*

4.5 Comparing CRSwNP maximal medical therapy cohort to corticosteroid medical therapy cohort

The difference between CRSwNPM-2 and CRSwNPS-2 in the number of species isolated was not statistically significant (P = 0.61). Neither was the difference between gram-positive and gram-negative isolates (P = 0.6). Also, the response to treatment that was measured by the SNOT-22 scoring system was similar in the both groups (P = 0.79). In the two cohorts, the most prevalent microbiome isolates were *Staphylococcus* and *Corynebacterium*, with no significant difference (Figure 4-7).

On the other hand, when comparing CRSwNPM-3 and CRSwNPS-3 patients, the number of species isolated was similar. In CRSwNPS-3, the prevalence of gram-negatives showed tendency to increase (38.4%) while the percentage in CRSwNPM-3 was (6.25%). However, the difference had not yet reached significance (P = 0.06).

At time point 3, *Staphylococcus* was the most prevalent in both groups, with an almost one third increase in CRSwNPS-3 compared to CRSwNPM-3. This observation of the change of prevalence between the two group was not statistically significant. With the increase of *Staphylococcus* prevalence in CRSwNPS-3, there was a clear drop in the prevalence of *Corynebacterium*, which led to a significant statistical difference when compared to CRSwNPM-3 (**P = 0.02**). Moreover in CRSwNPS-3, the prevalence of gram-negative isolates reached a peak and *Pseudomonas* organisms were isolated in 23% of the participants. However, this change did not reach statistical significance (P = 0.07) (Figure 4-7).

Finally, there was no clear bacterial pattern explaining the worsening of clinical status after three months from treatment in both groups. However, with the tendency of *Corynebacterium* genera to increase in the CRSwNPM-3 group, and the tendency of *Staphylococcus* and *Pseudomonas* to increase in the CRSwNPS-3 group, these observations may provide a possible explanation. This should be investigated further in a larger study with more patients.

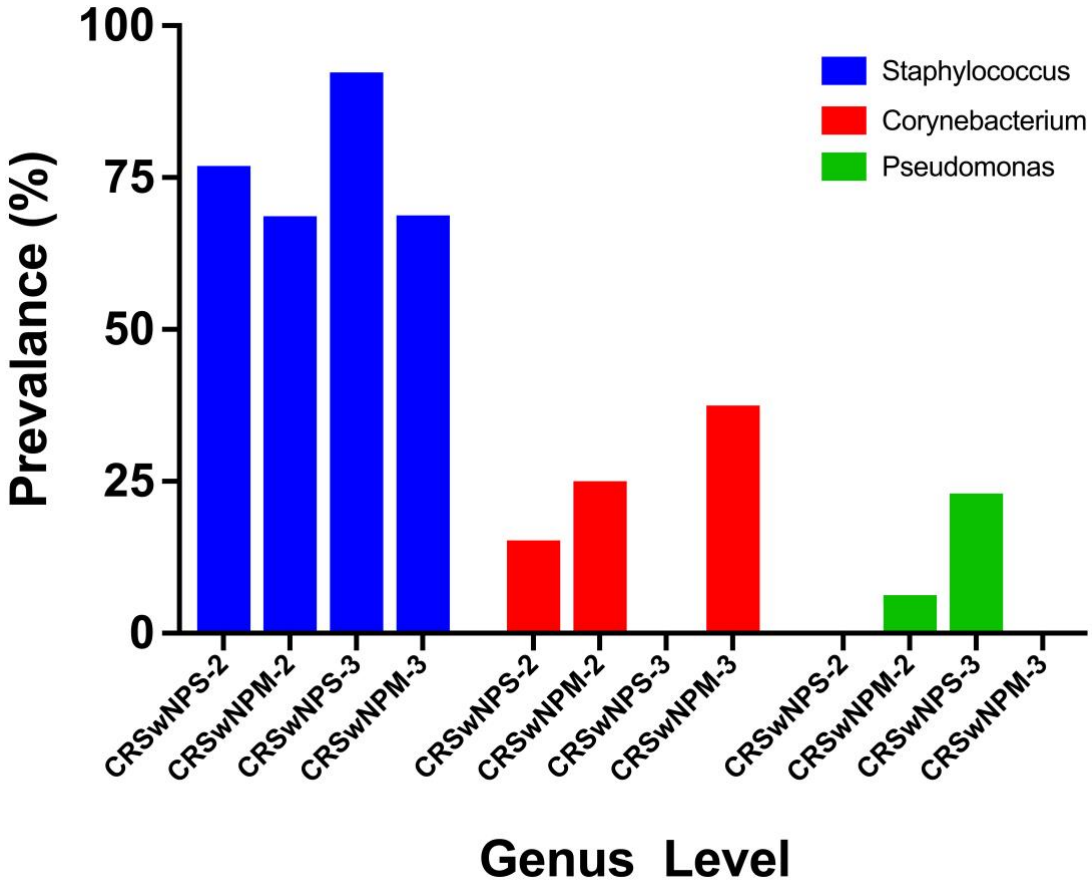

Figure 4-7. *Comparing the most prevalent genera between the two different treatment groups; Chronic Rhinosinusitis with nasal polyposis who received maximal medical therapy (CRSwNPM) and Chronic Rhinosinusitis with nasal polyposis who received corticosteroid therapy after (CRSwNPS) at the one month and three months' time points.*

Chapter Five: Discussion

Although the effect of systemic corticosteroids and antibiotics on the sinus microbiota is poorly understood, they are still the main treatment for CRS. This thesis, a longitudinal study, aimed to investigate the effect of systemic corticosteroid therapy with and without antibiotics (maximal medical therapy) on the sinus microbiota in CRSwNP patients and compare it with controls. This is apparently the first study to assess the long-term effect of corticosteroid therapy on sinus microbiota in CRSwNP patients. However, there was not a significant sinus microbiota change seen, but in the CRSwNPS (corticosteroids-only group) a slight decrease in *Staphylococcus* genera was noted immediately posttreatment, with an increase in both *Staphylococcus* and gram-negative genera at the long-term posttreatment period associated with worsening of clinical symptoms.

This study was able to identify *Staphylococcus* and *Corynebacterium* as the most prevalent microbiota in patients with CRSwNP. This finding was compatible with Paramasivan et al, the largest international study to date describing the sinus microbiota [40]. In addition, there was no significant difference in the sinus microbiota prevalence between CRSwNP patients and normal subjects, although recent reports discussed a significant drop in the prevalence of *Corynebacterium* genera and an increase in *Streptococcus* genera associated with CRSwNP [40].

Moreover, both treatment options used in this study showed significant short-term clinical improvement when assessed using SNOT-22 at the second time point. In both groups, there was a slight decrease in *Staphylococcus* prevalence but no difference in *Corynebacterium* genera. This might explain this improvement with no notable change in the number of the

bacteria isolated. An additional change was seen in the CRSwNPS (corticosteroids only group): the *Micrococcus* genera was not isolated post-treatment. Moreover, *Micrococcus* is not a common organism isolated in the CRS literature. Thus, further investigation is required to clarify this finding. Jain et al recently compared the effect of antibiotics and corticosteroids on sinus microbiota. They found a decrease in relative abundance in *Corynebacterium* genera after corticosteroid treatment and an increase of relative abundance in the *Propionibacterium* genera after antibiotics treatment. However, this change was not assessed in this study due to the difference in the methodologies used [12].

Three months after completing the medical treatment, there was worsening of clinical symptoms in both groups. In CRSwNPM-3 (corticosteroids and antibiotics group), *Corynebacterium* genera showed a notable tendency to increase in prevalence, which could be linked to the deterioration in clinical symptoms seen in this group. A meta-analysis published recently found *Corynebacterium* genus was linked to CRS and worsening of the disease as well [39].

In CRSwNPS-3 (corticosteroids only group), there was an interesting increase in the gram-negative-genera prevalence with around 24 % mostly *Pseudomonas* and an increase as well in *Staphylococcus* genera. In the past, *Staphylococcus* and *Pseudomonas* have been the most commonly cultured microbiota in CRS. Several reports linked disease recalcitrance postoperatively to positive cultures for *Staphylococcus* and *Pseudomonas* [47,48,49,50]. It would be interesting to investigate this pattern further, which might improve the understanding of *Staphylococcus* and *Pseudomonas* effect on medical treatment response in CRS.

A Multivariant analysis was done to assess the different cofactors that might impact the response to corticosteroid therapy in CRSwNP patient after three months from receiving the treatment. It was found that smoking, previous endoscopic sinus surgery, and aspirin sensitivity were all significantly associated with the responses. In 2013, a study discussing the factors effecting the sinus microbiota found distribution of species in smokers differed qualitatively with significant increase in the species *Staphylococcus aureus*, where *Propionibacterium acnes* and *Corynebacteria* tended toward decreased levels [37].

MALDI-TOF-MS provides an effective method of microbial identification in the sinuses of patients with CRSwNP and in normals; in the current study, the most prevalent bacteria were readily identified. In addition, it was possible to better understand the effect of corticosteroid therapy on sinus microbiota longitudinally and to compare it with the additional effect of antibiotics. Although, there was not a significant difference in clinical response between the two treatment groups. This agrees with results from other studies and stresses the benefits of decreasing antibiotics use such as decrease in cost, medication side effects, drug-resistance and morbidity. A clear advantage in the current study (in addition to the longitudinal design) is the standardized treatment between participants, which limits the intervention bias.

Several limitations have been identified in this thesis. First, this study examined the effect of corticosteroid therapy and maximal medical therapy on a relatively small number of patients. As a result, some of the notable trends in the bacterial prevalence between treatment groups and the clinical scores did not reach statistical significance. A further study with a larger sample size should better clarify changes in the sinus microbiota in response to medical treatment.

A second limitation of this study was that the microbiota identification method used focused on anaerobic bacteria. Thus, corticosteroid effects on the aerobic bacteria, and the associated changes, were not investigated.

However, the microbiota identification method used in this study had multiple advantages. It was cost effective, fast, accurate, did not require trained laboratory personnel, yet it gave sufficient microbiome information at strain level. However, a DNA sequencing identification method can provide more information to better understand the change in the sinus microbiota by investigating the microbiome abundance and diversity.

Finally, while this study focused on examining the effect of corticosteroid therapy on sinus microbiota in CRSwNP, other subtypes of CRS such as CRSsNP (Chronic Rhinosinusitis without nasal polyposis) and cystic fibrosis still requires further study to clarify the relation between these varies disease subtypes, response to treatment and the microbiota.

Chapter Six: Summary and Conclusion

This is the first pilot study to assess the long-term effect (3-months) of corticosteroid therapy on chronic rhinosinusitis patients with nasal polyposis (CRSwNP). Patients reported significant symptom improvement initially (1-month), but not in the long-term (3-months). This result was found as a response to both treatment groups, whether or not antibiotics were used.

There was no significant difference in the sinus microbiota prevalence between CRSwNP patients and normal subjects. Preliminary observation after 1 month from treatment showed that *Staphylococcus* genera prevalence tended to decline generally. However, after three months from treatment, *Corynebacterium* genera tended to increase in the maximal medical therapy group where patients received both corticosteroids and antibiotics, while *Staphylococcus* and gram-negative genera (*Pseudomonas)* tended to increase after corticosteroids treatment.

Other co-factors were found to be significantly associated with the response to systemic corticosteroid therapy. These were smoking, aspirin sensitivity and previous endoscopic sinus surgery.

Both treatment options were effective in the short-term, but not in the long-term with no clear sinus microbiota response linked. As a result, this study agrees with previous reports that discourage the use of systemic antibiotics without active infection. It also highlights a potential role of *Staphylococcus* and *Pseudomonas* in the disease response to medical treatment.

Finally, as a future direction, a continuation project using one of the nucleic acid bacterial identification methods to compare with a larger study group will add a better understanding of long-term medical treatment effects on sinus bacterial communities.

Chapter Seven: References

1- Desrosiers, M., et al. (2011). "Canadian clinical practice guidelines for acute and chronic rhinosinusitis." Allergy Asthma Clin Immunol **7**(1): 2.

2- Blackwell, D. L., et al. (2002). "Summary health statistics for U.S. adults: National Health Interview Survey, 1997." Vital Health Stat **10**(205): 1-109.

3- Hastan, D., et al. (2011). "Chronic rhinosinusitis in Europe—an underestimated disease. A GA(2)LEN study. "Allergy **66**(9):1216-1223.

4- Chen, Y., et al. (2003). "The epidemiology of chronic rhinosinusitis in Canadians." Laryngoscope **113**(7): 1199-1205.

5- IMS Health: Canadian Disease and Therapeutic Index (CDTI) database.2006.

6- Rosenfeld, R. M., et al. (2015). "Clinical practice guideline (update): Adult Sinusitis Executive Summary." Otolaryngol Head Neck Surg **152**(4): 598-609.

7- Van Crombruggen, K., et al. (2011). "Pathogenesis of chronic rhinosinusitis: inflammation." J Allergy Clin Immunol **128**(4): 728-732.

8- Hamilos, D. L. (2007). "Chronic rhinosinusitis patterns of illness." Clin Allergy Immunol **20**: 1-13.

9- Lam, K., et al. (2015). "The Etiology and Pathogenesis of Chronic Rhinosinusitis: a Review of Current Hypotheses." Curr Allergy Asthma Rep **15**(7): 41.

10- Feazel, L. M., et al. (2012). "Microbiome complexity and Staphylococcus aureus in chronic rhinosinusitis." Laryngoscope **122**(2): 467-472.

11- Hoggard, M., et al. (2017). "Evidence of microbiota dysbiosis in chronic rhinosinusitis." Int Forum Allergy Rhinol **7**(3): 230-239.

12- Jain, R., et al. (2018). "The effect of medical treatments on the bacterial microbiome in patients with chronic rhinosinusitis: a pilot study." Int Forum Allergy Rhinol.

13- Biswas, K., et al. (2015). The nasal microbiota in health and disease: variation within and between subjects. *Frontiers in Microbiology*, *6*, 134.

14- Schwartz, J. S., et al. (2017). "Medical management of chronic rhinosinusitis - a review of traditional and novel medical therapies." Expert Opin Investig Drugs **26**(10): 1123-1130.

15- Meltzer, E. O., et al. (2004). "Rhinosinusitis: establishing definitions for clinical research and patient care." J Allergy Clin Immunol **114**(6 Suppl): 155-212.

16- Klossek, J. M., et al. (2005). "Prevalence of nasal polyposis in France: a cross-sectional, case-control study." Allergy **60**(2): 233-237.

17- Newton, J. R. and K. W. Ah-See (2008). "A review of nasal polyposis." Ther Clin Risk Manag **4**(2): 507-512.

18- Staikuniene, J., et al. (2008). "Association of chronic rhinosinusitis with nasal polyposis and asthma: clinical and radiological features, allergy and inflammation markers." Medicina (Kaunas) **44**(4): 257-265.

19- Chang, J. E., et al. (2012). "Aspirin-exacerbated respiratory disease: burden of disease." Allergy Asthma Proc **33**(2): 117-121.

20- Baroody, F.M. et al. (2008). "Nasal challenge with allergen leads to maxillary sinus inflammation."J Allergy Clin Immunol **121**(5):1126 – 132.

21- Platt, M. P., et al. (2011). "Pathways analysis of molecular markers in chronic sinusitis with polyposis." Otolaryngol Head Neck Surg **144**(5): 802-808.

22- Fnais, N., et al. (2017) "Differential innate immunity gene expression between corticosteroids responsive and non-responsive patients with chronic rhinosinusitis and nasal polyposis" Thesis submitted for publication.

23- Bachert, C., et al. (2008). "Role of staphylococcal superantigens in upper airway disease." Curr Opin Allergy Clin Immunol **8**(1): 34-38.

24- Sasama, J., et al. (2005). "New paradigm for the roles of fungi and eosinophils in chronic rhinosinusitis." Curr Opin Otolaryngol Head Neck Surg **13**(1): 2-8.

25- Psaltis, A. J., et al. (2007). "Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis." Laryngoscope **117**(7): 1302-1306.

26- Psaltis, A. J. and P. J. Wormald (2017). "Therapy of Sinonasal Microbiome in CRS: A Critical Approach." Curr Allergy Asthma Rep **17**(9): 59.

27- Ramakrishnan, V. R. and D. N. Frank (2015). "Impact of cigarette smoking on the middle meatus microbiome in health and chronic rhinosinusitis." Int Forum Allergy Rhinol **5**(11): 981-989.

28- Ramakrishnan, V.R., et al. (2015). "Sinus microbiota varies among chronic rhinosinusitis phenotypes and predicts surgical outcome. "J Allergy Clin Immunol. **136**(2):334–342.

29- Feazel, L. M., et al. (2011). "Update on bacterial detection methods in chronic rhinosinusitis: implications for clinicians and research scientists." Int Forum Allergy Rhinol **1**(6): 451-459.

30- Singhal, N., et al. (2015). "MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis." Front Microbiol **6**: 791.

31- Martensson, A., et al. (2017). “Clinical efficacy of a topical lactic acid bacterial microbiome in chronic rhinosinusitis: A randomized controlled trial.” Laryngoscope Investig Otolaryngol **2**(6): 410-416.

32- Kaspar, U., et al. (2016). "The culturome of the human nose habitats reveals individual bacterial fingerprint patterns." Environ Microbiol **18**(7): 2130-2142.

33- Koeller, K., et al. (2018). "Microbiome and Culture Based Analysis of Chronic Rhinosinusitis Compared to Healthy Sinus Mucosa." Front Microbiol **9**: 643.

34- Nadel, D. M., et al. (1999). "Endoscopically guided sinus cultures in normal subjects." Am J Rhinol **13**(2): 87-90.

35- Abou-Hamad, W., et al. (2009). "Bacterial flora in normal adult maxillary sinuses." Am J Rhinol Allergy **23**(3): 261-263.

36- Lal, D., et al. (2017). "Mapping and comparing bacterial microbiota in the sinonasal cavity of healthy, allergic rhinitis, and chronic rhinosinusitis subjects." Int Forum Allergy Rhinol **7**(6): 561-569.

37- Ramakrishnan, V. R., et al. (2013). "The microbiome of the middle meatus in healthy adults." PLoS One **8**(12): e85507.

38- Anderson, M., et al. (2016). "A systemic review of the sinonasal microbiome in chronic rhinosinusitis." Am J Rhinol Allergy **30**(3): 161-166.

39- Wagner Mackenzie, B., et al. (2017). "Bacterial community collapse: a meta-analysis of the sinonasal microbiota in chronic rhinosinusitis." Environ Microbiol **19**(1): 381-392.

40- Paramasivan, S., et al. (2019). "The international sinonasal microbiome study (ISMS): a multi-centre, international characterization of sinonasal bacterial ecology." Retrieved from https://www.biorxiv.org.

41- Fokkens, W. J., et al. (2012). "European Position Paper on Rhinosinusitis and Nasal Polyposis 2012." Rhinol Suppl **23**: 3 p preceding table of contents, 1-298.

42- Liu, C. M., et al. (2015). "Impact of saline irrigation and topical corticosteroids on the postsurgical sinonasal microbiota." Int Forum Allergy Rhinol **5**(3): 185-190.

43- Liu, C. M., et al. (2013). "Medical therapy reduces microbiota diversity and evenness in surgically recalcitrant chronic rhinosinusitis." Int Forum Allergy Rhinol **3**(10): 775-781.

44- Merkley, M. A., et al. (2015). "The effect of antibiotics on the microbiome in acute exacerbations of chronic rhinosinusitis." Int Forum Allergy Rhinol **5**(10): 884-893.

45- Hopkins, C., et al. (2009). "Psychometric validity of the 22-item Sinonasal Outcome Test." Clin Otolaryngol **34**(5): 447-454.

46- Martensson, A., et al. (2017). "Clinical efficacy of a topical lactic acid bacterial microbiome in chronic rhinosinusitis: A randomized controlled trial." Laryngoscope Investig Otolaryngol **2**(6): 410-416.

47- Bendouah, Z., et al. (2006). "Biofilm formation by Staphylococcus aureus and Pseudomonas aeruginosa is associated with an unfavorable evolution after surgery for chronic sinusitis and nasal polyposis. " Otolaryngol Head Neck Surg **134**(6):991–996.

48- Coffey, C. S., et al. (2006). "Endoscopically guided aerobic cultures in postsurgical patients with chronic rhinosinusitis." Am J Rhinol **20**(1): 72-76.

49- Jervis-Bardy, J., et al. (2009). "Impaired mucosal healing and infection associated with Staphylococcus aureus after endoscopic sinus surgery." Am J Rhinol Allergy **23**(5): 549-552.

50- Singhal, D., et al. (2011). "Staphylococcus aureus biofilms: Nemesis of endoscopic sinus surgery." Laryngoscope **121**(7): 1578-1583.

51- Sivasubramaniam, R. and R. Douglas (2018). "The microbiome and chronic rhinosinusitis." World J Otorhinolaryngol Head Neck Surg **4**(3): 216-221.