

Review

State-of-the-Art Adult Chronic Rhinosinusitis Microbiome: Perspective for Future Studies in Pediatrics

M. Asghar Pasha

Division of Allergy and Immunology, Albany Medical College, 176 Washington Avenue Extension, Suite 102, Albany, NY 12203, USA; pasham@amc.edu; Tel.: +1-518-264-2510; Fax: +1-518-264-2520

Received: 22 November 2017; Accepted: 30 January 2018; Published: 5 February 2018

Abstract: Chronic rhinosinusitis (CRS) is a prevalent disease that causes persistent mucosal inflammation and is associated with bacterial infection, which is thought to play a role in the inflammatory process. Microbiome analysis provides insight to host–microbial interactions. Disturbances in the host and commensal bacteria interaction may lead to CRS. Culture-based methods are useful to isolate some microorganisms but are unable to grow a majority of the bacteria. A review of the literature shows that several recent studies attempted to overcome this issue by using molecular techniques, such as microbial RNA sequencing, to describe the CRS microbiome. All of these studies were performed in adults, with no comparative studies reported in the pediatric population. Similar studies, utilizing molecular techniques, are needed to better understand the mechanism of CRS in children. Because valuable data from these adult studies may help to bridge the gap in our knowledge of the microbiome in pediatric CRS, we present an overview of the methodology and results behind the current microbiomic approach to adult CRS to set the stage for its use in the study of CRS in children.

Keywords: microbiome; pediatric chronic rhinosinusitis; molecular techniques

The field of the microbiological study of chronic rhinosinusitis (CRS) in adults has taken a quantum leap forward in only a few years. Unfortunately, similar approaches to the study of CRS in children have been totally lacking in the literature. A Medline search was performed for articles reporting on the microbiome in patients with CRS. Search terms included: 16S ribosomal RNA (rRNA) gene sequencing in chronic rhinosinusitis, chronic rhinosinusitis and microbiome, chronic rhinosinusitis and pyrosequencing, non-cultured molecular techniques in chronic rhinosinusitis, microbiome and 16S ribosome RNA. The following is an overview of the reported methodology and results behind the current microbiomic approach to adult CRS with the goal to set the stage for its use in the future studies of pediatric CRS.

Bacterial rhinosinusitis is one of the most common problems presented to the primary care physician's office and results in over \$5 billion in direct costs annually [1]. CRS symptoms lasting >12 weeks with or without exacerbations, affects more than 30 million Americans, which results in over \$2.4 billion in annual health care expenditures [2]. CRS is considered an inflammatory disorder of the paranasal sinuses. Analysis of direct microbial cultures, sinus secretions, and tissue samples has demonstrated the presence of bacteria. It has been speculated that the bacterial colonization in CRS plays a role in pathophysiology of the disease [3,4]. Despite these findings, the role of microbial stimuli causing inflammation in CRS remains controversial [5].

The normal flora 'microbiome', also called commensal bacteria, in the gastrointestinal tract, nasal cavity and oropharynx provide useful functions. Microbiome analysis provides insight to host–microbial interactions. In recent years, this has been done using molecular techniques based on

microbial RNA. Several published studies have shown that in the healthy state, sinuses are not completely sterile [6–10]. Disturbances in the host and commensal bacteria interaction may lead to disease, including upper airway disease such as CRS. The majority of the published studies used culture-based techniques as the mainstay of microbial diagnostics in CRS. The range of microbes detected by these techniques may not be representative of the actual diversity present, particularly in environmental samples [11]. Although culture-based methods are still useful to isolate and culture some microorganisms, it has been suggested that no more than 90% of bacteria can be cultured from most environments and that culture-positive results range from only 1 to 10% [12]. This has led to the development and use of molecular methodologies which include the following: phylogenetic oligonucleotide array, 16S ribosomal RNA gene clone libraries, analysis of functional gene arrays, next-generation sequencing technologies, sequencing by mass spectrometry, and random “shotgun” metagenomics [13,14]. These sophisticated methods help to identify both culturable and non-culturable organisms. For identification purposes, some techniques will selectively enhance or restrict growth of microorganisms. CRS has a polymicrobial community, and identification of every microorganism can be a monumental task [9]. Several recent studies attempted to overcome this issue by using molecular techniques to describe the CRS microbiome [5,7,9,10,15,16]. Here we review the available non-culture-based bacterial 16S rRNA gene sequencing data using pyrosequencing to describe bacterial diversity in patients with CRS and in healthy controls.

Abreu et al. report data regarding the healthy normal sinus microbiome in adults [7]. In this study, sinus brushing at the time of functional endoscopic sinus surgery (FESS) in patients with CRS was obtained and compared with that from a control group without CRS. A standardized phylogenetic microarray, the 16S rRNA PhyloChip, was used to analyze samples. This study showed significantly reduced bacterial diversity in patients with CRS compared with normal controls. Another finding in this study was that *Lactobacillus sakei* appeared to play a protective role in the normal sinus microbiome. The authors hypothesized that as the pathophysiology of CRS is multifactorial: it is co-dependent on a microbiome with increased relative abundance of *Corynebacterium tuberculostrictum* [7]. The study results could have been impacted by preoperative antibiotic use in some CRS subjects and by controls.

Ramakrishnan et al. analyzed sinus swabs collected during sinus surgery from a cohort of 56 patients with CRS and compared them with swabs from 20 controls using molecular phylogenetic analysis of 16S rDNA pyrosequences [17]. The authors attempted to determine whether a specific adult CRS phenotype shows an alteration in the sinus microbiome. The initial assessment of overall bacterial densities between groups showed the amount of total bacteria present was not statistically different between the groups. Bacteroidetes and Fusobacteria showed significant expansion with purulence in one half of the CRS study group. The isolation of anaerobes in CRS patients highlights the probable role and importance of these bacteria in the pathogenesis of CRS. The presence of nasal polyps was not independently associated with general bacterial community alterations. Baseline abundance of species of phylum Actinobacteria and genus *Corynebacterium* at the time of surgery were predictive of better surgical outcome in this study.

Feazel and colleagues compared conventional culture-based and culture-independent methodologies for the identification of microorganisms in CRS [10]. Middle meatus swab samples obtained during endoscopic sinus surgery from 15 CRS patients were compared with swabs from 5 controls. The samples were analyzed by using standard bacteriologic cultures and DNA pyrosequencing. Standard cultures were positive for all subjects, CRS patients as well as controls. The most common organisms isolated were coagulase-negative streptococci (75%), *Staphylococcus aureus* (50%), and *Corynebacterium acnes* (30%). The most prevalent species detected by pyrosequencing included coagulase-negative staphylococci (100%), *Corynebacterium* spp. (85.7%), *Propionibacterium acnes* (76.2%), and *S. aureus* (66.7%). Pyrosequencing was superior to standard culture technique for identifying significantly more diversity, particularly of anaerobes, in CRS patients relative to controls. The high prevalence of anaerobes in this study is an important finding, because earlier culture-based studies had reported a low prevalence, which could be due to

methodological errors. This study also highlights the importance of *S. aureus* species, which was highly prevalent and abundant in CRS patients compared to controls.

Stephenson et al. reported similar results in a prospective study of 18 patients undergoing endoscopic sinus surgery for CRS and 9 control patients with pituitary adenomas [5]. They utilized molecular culture (bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP)) for identification of bacterial species present on sinonasal mucosa and compared them with those identified using conventional standard cultures. Standard cultures showed mainly *S. aureus* and coagulase-negative *Staphylococcus*. Molecular cultures identified up to 20 organisms per sample, and anaerobic species (*Diaphorobacter* and *Peptoniphilus*) predominated. *S. aureus* was detected in 50% of samples. The authors concluded that molecular cultures such as bTEFAP are sensitive tools for bacterial identification in CRS. This study suggests anaerobic involvement in patients with CRS undergoing sinus surgery. This is in agreement with Feazel et al. [10] regarding the high prevalence of anaerobes in the microbiome of CRS patients.

Aurora and colleagues used deep sequencing of the bacterial 16S and fungal 18S ribosomes genes, a culture-independent method, to analyze lavage of adult patients with CRS and adult controls [16]. In addition to microbiome analysis, immune response in the lavage was measured for the number of cytokines. Peripheral blood leukocyte immune response was measured by quantifying various cytokines. A group of 30 patients with refractory CRS and 12 controls were recruited. Middle meatus lavage was obtained prior to surgical intervention, after induction of general anesthesia. *Corynebacterium accolens* was the most abundant species, with a statistically significant increase in CRS patients compared to controls. These findings are in accord with those of Abrue et al., as previously described [7]. Fungal microbiome analysis showed *Cryptococcus neoformans* was much higher in CRS patients than in controls. Cytokine response in the lavage of all CRS patients relative to controls showed significantly elevated levels of interleukin (IL)-4, IL-5, and IL-13. Peripheral leukocytes from CRS patients produced IL-5 in response to control lavage samples (commensals), indicating hyperresponsiveness to the normal microbiome. This study highlights the importance of *C. neoformans*, a fungus, as a major constituent of sinus fungal microbiome in addition to *Corynebacterium*.

In a small cross-sectional study, Ramakrishnan and colleagues analyzed middle meatus swabs in healthy subjects without CRS, utilizing quantitative PCR and 16 rRNA pyrosequencing [6]. The most prevalent and abundant species isolated included *S. aureus*, *Staphylococcus epidermidis*, and *P. acnes*. The study has potential limitation with a small sample size and possible contamination of nasal microorganisms into specimens during surgery. Also, middle meatus microorganisms may not be representative of the sinus microbiome.

Choi and colleagues performed analysis of nasal lavage fluid from healthy controls and CRS patients with and without nasal polyposis [18]. Bacterial 16S ribosomal RNA pyrosequencing showed increased bacterial abundance and lower diversity from both bacterial and EV (bacteria-derived extracellular vesicles) fractions among CRS patients compared to controls. The authors found higher *S. aureus* composition from both bacterial and EV fractions in CRS patients with polyps compared to CRS patients without polyps. *Prevotella*, a genus of gram-negative bacteria and a genus of Bacteroidetes phylum, significantly decreased in CRS patients compared to controls, suggesting that the reduction in the bacteria may contribute to CRS pathogenesis. The results of this study highlight the importance of *S. aureus* in the pathogenesis of CRS, particularly CRS with polyps, as reported previously [5,9,10]. The most important finding in this study was bacteria-derived EV in the nasal fluids, and the authors argue that it may serve as another useful biomarker of CRS in the future. However, the EV analysis was done on nasal fluids as opposed to sinus fluids. The study was also limited by small sample.

Studies of CRS patients have shown a close association between bacterial and fungal biofilms (19–21,23–26). In a prospective study, Cleland and colleagues examined the fungal microbiome in CRS patients undergoing endoscopic sinus surgery [27]. The control group consisted of patients undergoing endoscopic transsphenoidal resection of pituitary adenomas. Swabs were collected from the patients with CRS, intraoperatively and postoperatively, at 6 and 12 weeks. Fungal detection utilized 18S ribosomal DNA (rDNA) fungal tag-encoded FLX amplification pyrosequencing. The

authors did not find major differences in the fungal microbiome between controls and CRS patients intraoperatively. Postoperatively, there was a decline in richness and presence of genera *Fusarium* and *Neocosmospora*. The authors hypothesized that the presence of *Malassezia* in patients with CRS could potentially have a disease-modifying effect. *Malassezia* is considered part of the normal cutaneous flora and is found in areas such as the trunk and head [28], and therefore its presence in the sinonasal cavity could represent seeding from these sites rather than permanent sinus colonization. Moreover, surgery itself affects the microbiome, and the findings postsurgery may have shown this confounding effect.

The results of these studies show that the most common/abundant bacteria in all subjects, both CRS patients and healthy controls included *Corynebacterium*, *Staphylococcus*, *Propionibacterium*, and Actinobacteria. The relative abundance of *C. tuberculoearicum* and *Corynebacterium accolens* was associated with CRS inflammation [7,16]. Increased relative abundance and diversity—particularly of *Propionibacterium*, *Burkholderia*, and *L. sakei*—was associated with healthy sinuses. The first two bacteria are also called “gatekeepers” and are thought to play an important role in maintaining a stable sinonasal community [29]. It is important to note that the studies that examined the microbiome in healthy subjects demonstrated the presence of *Staphylococcus*, *Streptococcus*, and *Pseudomonas*, bacteria known to cause respiratory disease [6,7,16]. These studies also indicated that significant microbiome dysbiosis is associated with CRS [7,10]. The presence of certain bacteria in CRS does not provide information regarding functional capabilities that differentiate healthy people from disease-associated communities. Moreover, some of the conflicting results in these studies could be due to interpersonal variation of the sinus microbiota caused by environmental exposures and biogeography, i.e., sampling of sinus vs. middle meatus. Variation in sampling techniques used to obtain specimens, e.g., brush vs. swab, also may have significant impact. Most of the studies did not consider the impact of other factors that might influence the sinus microbiome, such as lower respiratory tract disease (asthma), smoking, use of antibiotics, and/or use of steroids (oral and/or topical).

The pathophysiology of CRS is likely multifactorial and may be dependent on the paranasal sinus microbiome composition. Our knowledge of microbiota in CRS is still evolving. Key findings from the microbiome studies in adult CRS patients show less richness, evenness, and diversity than in control groups, although the total microbial burden appears to be similar. All culture-independent CRS microbiome studies in the literature have been done in adults, with no comparative studies reported in the pediatric population. Advancement in the understanding of pathophysiology of pediatric CRS, particularly with respect to the microbiome, is limited. Although multiple studies have been published, they are limited to standard culture techniques [30–43]. The standard culture-based techniques are outdated, antiquated, and limited. Data on CRS microbiome analyses performed with the use of non-cultured molecular techniques utilizing microbial RNA are more sensitive. There is a need for culture-independent research methods for the identification of microbiomes as has been employed in other studies [5,10,44]. Valuable data from adult studies may bridge the gap in our knowledge and understanding of the microbiome in pediatric CRS. For now, we can only extrapolate the findings of studies in adults to their application in the pediatric population.

Microbiome dysbiosis is associated with CRS. Culture-independent technologies help to describe microbial diversity, composition, and functional changes, as well as specific immune responses. Future studies should examine larger, more diverse populations, particularly children, of CRS to characterize the microbiome of different CRS phenotypes in comparison with controls. Additionally, longitudinal studies evaluating the effects of antimicrobials, sinus surgery, and topical nasal treatments on microbial diversity and abundance in CRS will be invaluable.

Acknowledgments: Author would like to acknowledge Russell Hopp, Qi Yang, Paul Feustel, and Marcia Lamb for their manuscript review and helpful suggestions.

Conflicts of Interest: The author declares no conflicts of interest

References

1. Ray, N.F.; Baraniuk, J.N.; Thamer, M.; Rinehart, C.S.; Gergen, P.J.; Kaliner, M.; Josephs, S.; Pung, Y.H. Healthcare expenditures for sinusitis in 1996: Contributions of asthma, rhinitis, and other airway disorders. *J. Allergy Clin. Immunol.* **1999**, *103*, 408–414.
2. Wallace, D.V.; Dykewicz, M.S.; Bernstein, D.I.; Blessing-Moore, J.; Cox, L.; Khan, D.A.; Lang, D.M.; Nicklas, R.A.; Oppenheimer, J.; Portnoy, J.M.; et al. The diagnosis and management of rhinitis: An updated practice parameter. *J. Allergy Clin. Immunol.* **2008**, *122*, doi:10.1016/j.jaci.2008.06.003.
3. Biel, M.A.; Brown, C.A.; Levinson, R.M.; Garvis, G.E.; Paisner, H.M.; Sigel, M.E.; Tedford, T.M. Evaluation of the microbiology of chronic maxillary sinusitis. *Ann. Otol. Rhinol. Laryngol.* **1998**, *107*, 942–945.
4. Brook, I.; Frazier, E.H.; Gher, M.E., Jr. Microbiology of periapical abscesses and associated maxillary sinusitis. *J. Periodontol.* **1996**, *67*, 608–610.
5. Stephenson, M.F.; Mfuna, L.; Dowd, S.E.; Wolcott, R.D.; Barbeau, J.; Poisson, M.; James, G.; Desrosiers, M. Molecular characterization of the polymicrobial flora in chronic rhinosinusitis. *J. Otolaryngol. Head Neck Surg.* **2010**, *39*, 182–187.
6. Ramakrishnan, V.R.; Feazel, L.M.; Gitomer, S.A.; Ir, D.; Robertson, C.E.; Frank, D.N. The microbiome of the middle meatus in healthy adults. *PLoS ONE* **2013**, *8*, e85507.
7. Abreu, N.A.; Nagalingam, N.A.; Song, Y.; Roediger, F.C.; Pletcher, S.D.; Goldberg, A.N.; Lynch, S.V. Sinus microbiome diversity depletion and *Corynebacterium tuberculostrictum* enrichment mediates rhinosinusitis. *Sci. Transl. Med.* **2012**, *4*, 151ra124, doi:10.1126/scitranslmed.3003783.
8. Yan, M.; Pamp, S.J.; Fukuyama, J.; Hwang, P.H.; Cho, D.Y.; Holmes, S.; Relman, D.A. Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and *S. aureus* carriage. *Cell Host Microbe* **2013**, *14*, 631–640.
9. Boase, S.; Foreman, A.; Cleland, E.; Tan, L.; Melton-Kreft, R.; Pant, H.; Hu, F.Z.; Ehrlich, G.D.; Wormald, P.J. The microbiome of chronic rhinosinusitis: Culture, molecular diagnostics and biofilm detection. *BMC Infect. Dis.* **2013**, *13*, doi:10.1186/1471-2334-13-210.
10. Feazel, L.M.; Robertson, C.E.; Ramakrishnan, V.R.; Frank, D.N. Microbiome complexity and staphylococcus aureus in chronic rhinosinusitis. *Laryngoscope* **2012**, *122*, 467–472.
11. Dunbar, J.; White, S.; Forney, L. Genetic diversity through the looking glass: Effect of enrichment bias. *Appl. Environ. Microbiol.* **1997**, *63*, 1326–1331.
12. Sun, Y.; Wolcott, R.D.; Dowd, S.E. Tag-encoded flx amplicon pyrosequencing for the elucidation of microbial and functional gene diversity in any environment. *Methods Mol. Biol.* **2011**, *733*, 129–141.
13. Wilson, M.T.; Hamilos, D.L. The nasal and sinus microbiome in health and disease. *Curr. Allergy Asthma Rep.* **2014**, *14*, doi:10.1007/s11882-014-0485-x.
14. Dowd, S.E.; Sun, Y.; Secor, P.R.; Rhoads, D.D.; Wolcott, B.M.; James, G.A.; Wolcott, R.D. Survey of bacterial diversity in chronic wounds using pyrosequencing, dge, and full ribosome shotgun sequencing. *BMC Microbiol.* **2008**, *8*, doi:10.1186/1471-2180-8-43.
15. Stressmann, F.A.; Rogers, G.B.; Chan, S.W.; Howarth, P.H.; Harries, P.G.; Bruce, K.D.; Salib, R.J. Characterization of bacterial community diversity in chronic rhinosinusitis infections using novel culture-independent techniques. *Am. J. Rhinol. Allergy* **2011**, *25*, e133–e140.
16. Aurora, R.; Chatterjee, D.; Hentzleman, J.; Prasad, G.; Sindwani, R.; Sanford, T. Contrasting the microbiomes from healthy volunteers and patients with chronic rhinosinusitis. *JAMA Otolaryngol. Head Neck Surg.* **2013**, *139*, 1328–1338.
17. Ramakrishnan, V.R.; Hauser, L.J.; Feazel, L.M.; Ir, D.; Robertson, C.E.; Frank, D.N. Sinus microbiota varies among chronic rhinosinusitis phenotypes and predicts surgical outcome. *J. Allergy Clin. Immunol.* **2015**, *136*, 334–342.
18. Choi, E.B.; Hong, S.W.; Kim, D.K.; Jeon, S.G.; Kim, K.R.; Cho, S.H.; Gho, Y.S.; Jee, Y.K.; Kim, Y.K. Decreased diversity of nasal microbiota and their secreted extracellular vesicles in patients with chronic rhinosinusitis based on a metagenomic analysis. *Allergy* **2014**, *69*, 517–526.
19. Gosepath, J.; Brieger, J.; Vlachtsis, K.; Mann, W.J. Fungal DNA is present in tissue specimens of patients with chronic rhinosinusitis. *Am. J. Rhinol.* **2004**, *18*, 9–13.
20. Shin, S.H.; Ponikau, J.U.; Sherris, D.A.; Congdon, D.; Frigas, E.; Homburger, H.A.; Swanson, M.C.; Gleich, G.J.; Kita, H. Chronic rhinosinusitis: An enhanced immune response to ubiquitous airborne fungi. *J. Allergy Clin. Immunol.* **2004**, *114*, 1369–1375.
21. Inoue, Y.; Matsuwaki, Y.; Shin, S.H.; Ponikau, J.U.; Kita, H. Nonpathogenic, environmental fungi induce activation and degranulation of human eosinophils. *J. Immunol.* **2005**, *175*, 5439–5447.

22. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am. Rev. Respir. Dis.* **1987**, *136*, 225–244.
23. Ebbens, F.A.; Georgalas, C.; Fokkens, W.J. Fungus as the cause of chronic rhinosinusitis: The case remains unproven. *Curr. Opin. Otolaryngol. Head Neck Surg.* **2009**, *17*, 43–49.
24. Ponikau, J.U.; Sherris, D.A.; Kern, E.B.; Homburger, H.A.; Frigas, E.; Gaffey, T.A.; Roberts, G.D. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin. Proc.* **1999**, *74*, 877–884.
25. Braun, H.; Buzina, W.; Freudenschuss, K.; Beham, A.; Stammberger, H. ‘Eosinophilic fungal rhinosinusitis’: A common disorder in Europe? *Laryngoscope* **2003**, *113*, 264–269.
26. Tosun, F.; Hidir, Y.; Saracli, M.A.; Caliskaner, Z.; Sengul, A. Intranasal fungi and chronic rhinosinusitis: What is the relationship? *Ann. Otol. Rhinol. Laryngol.* **2007**, *116*, 425–429.
27. Cleland, E.J.; Bassiouni, A.; Boase, S.; Dowd, S.; Vreugde, S.; Wormald, P.J. The fungal microbiome in chronic rhinosinusitis: Richness, diversity, postoperative changes and patient outcomes. *Int. Forum Allergy Rhinol.* **2014**, *4*, 259–265.
28. Ashbee, H.R. Update on the genus *Malassezia*. *Med. Mycol.* **2007**, *45*, 287–303.
29. Wagner Mackenzie, B.; Waite, D.W.; Hoggard, M.; Douglas, R.G.; Taylor, M.W.; Biswas, K. Bacterial community collapse: A meta-analysis of the sinonasal microbiota in chronic rhinosinusitis. *Environ. Microbiol.* **2017**, *19*, 381–392.
30. Brook, I. Bacteriologic features of chronic sinusitis in children. *JAMA* **1981**, *246*, 967–969.
31. Brook, I.; Yocum, P.; Shah, K. Aerobic and anaerobic bacteriology of concurrent chronic otitis media with effusion and chronic sinusitis in children. *Arch. Otolaryngol. Head Neck Surg.* **2000**, *126*, 174–176.
32. Erkan, M.; Ozcan, M.; Arslan, S.; Soysal, V.; Bozdemir, K.; Haghighi, N. Bacteriology of antrum in children with chronic maxillary sinusitis. *Scand. J. Infect. Dis.* **1996**, *28*, 283–285.
33. Hsin, C.H.; Su, M.C.; Tsao, C.H.; Chuang, C.Y.; Liu, C.M. Bacteriology and antimicrobial susceptibility of pediatric chronic rhinosinusitis: A 6-year result of maxillary sinus punctures. *Am. J. Otolaryngol.* **2010**, *31*, 145–149.
34. Brook, I. Microbiology of sinusitis. *Proc. Am. Thorac. Soc.* **2011**, *8*, 90–100.
35. Brook, I.; Yocum, P. Antimicrobial management of chronic sinusitis in children. *J. Laryngol. Otol.* **1995**, *109*, 1159–1162.
36. Slack, C.L.; Dahn, K.A.; Abzug, M.J.; Chan, K.H. Antibiotic-resistant bacteria in pediatric chronic sinusitis. *Pediatr. Infect. Dis. J.* **2001**, *20*, 247–250.
37. Don, D.M.; Yellon, R.F.; Casselbrant, M.L.; Bluestone, C.D. Efficacy of a stepwise protocol that includes intravenous antibiotic therapy for the management of chronic sinusitis in children and adolescents. *Arch. Otolaryngol. Head Neck Surg.* **2001**, *127*, 1093–1098.
38. Goldenhersh, M.J.; Rachelefsky, G.S.; Dudley, J.; Brill, J.; Katz, R.M.; Rohr, A.S.; Spector, S.L.; Siegel, S.C.; Summanen, P.; Baron, E.J.; et al. The microbiology of chronic sinus disease in children with respiratory allergy. *J. Allergy Clin. Immunol.* **1990**, *85*, 1030–1039.
39. Muntz, H.R.; Lusk, R.P. Bacteriology of the ethmoid bullae in children with chronic sinusitis. *Arch. Otolaryngol. Head Neck Surg.* **1991**, *117*, 179–181.
40. Orobello, P.W., Jr.; Park, R.I.; Belcher, L.J.; Eggleston, P.; Lederman, H.M.; Banks, J.R.; Modlin, J.F.; Naclerio, R.M. Microbiology of chronic sinusitis in children. *Arch. Otolaryngol. Head Neck Surg.* **1991**, *117*, 980–983.
41. Otten, F.W. Conservative treatment of chronic maxillary sinusitis in children. Long-term follow-up. *Acta Oto-Rhino-Laryngol. Belg.* **1997**, *51*, 173–175.
42. Otten, F.W.; Grote, J.J. Treatment of chronic maxillary sinusitis in children. *Int. J. Pediatr. Otorhinolaryngol.* **1988**, *15*, 269–278.
43. Tinkelman, D.G.; Silk, H.J. Clinical and bacteriologic features of chronic sinusitis in children. *Am. J. Dis. Child.* **1989**, *143*, 938–941.
44. Feazel, L.M.; Frank, D.N.; Ramakrishnan, V.R. Update on bacterial detection methods in chronic rhinosinusitis: Implications for clinicians and research scientists. *Int. Forum Allergy Rhinol.* **2011**, *1*, 451–459.

