**Dynamic Profiling of the Sinonasal Microbiome Using Nanopore Sequencing in the Diagnosis of Chronic Rhinosinusitis**

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{*s.bozek,t.kosciolek*}@*sanoscience.org*, {*joanna.szaleniec*}@*uj.edu.pl***Keywords**: microbiome, antibiotic resistance, bioinformatics, rhinosinusitis, full-length 16S rRNA sequencing, nanopore, longitudinal analysis, personalized medicine

1. Introduction

The sinonasal microbiome is a complex community of microorganisms. In Poland, standard diagnosis for chronic rhinosinusitis primarily relies on microbiological cultures, which are often insufficient for detecting the full spectrum of microorganisms. In response to these challenges, the dynamic development of modern molecular techniques, including third-generation sequencing and long-read technologies, enables profiling the microbiome with species-level precision.

1. Description of the problem

Many bacteria don't grow in standard culture conditions or grow very slowly, which can lead to an incomplete diagnosis. By comparing data obtained through sequencing with the results of classic cultures, it's possible to identify discrepancies, complementary information, and detect clinically significant patterns that remain invisible in standard diagnostic practice. Additionally, understanding the dynamics of human-associated microbial communities allows us to track changes in the microbiome in response to internal factors (e.g., health status) and external factors (e.g., treatment, environment). As recent studies have shown, sampling from a single site may not be universally representative of the entire sinus microbiome due to significant spatial variability [1]. In this study, we use full-length 16S rRNA gene sequencing with Oxford Nanopore Technologies, which allows for microorganism identification with species-level resolution.

1. Related work

Prior research on the sinonasal microbiome has predominantly utilized short-read sequencing, limiting the taxonomic resolution of microorganism identification to the genus level.   
Third-generation nanopore sequencing enables species-level microbial community profiling [2]. In the sinuses, species-level differentiation is particularly important because microorganisms from the same genus can exhibit extremely different behavior. For example, *Staphylococcus aureus* has a wide variety of proinflammatory virulence factors, and its presence is related to refractory rhinosinusitis while *Staphylococcus epidermidis* is supposedly a commensal that may protect from *S. aureus* colonization [3-5].

1. Solution to the problem

To address the limitations of traditional diagnostic methods and single time-point analyses, our study focuses on the advanced analysis of long-read data. Because Oxford Nanopore Technology is characterized by a higher error rate than other sequencing methods, we use the PRONAME pipeline [6] for data processing. This pipeline integrates quality and length filtering, clustering, and advanced error correction to produce high-quality consensus sequences. We also use the precompiled Greengenes2 database [7], which enables precise species-level taxonomic classification. The processed microbiome data is then analyzed for changes over time to detect patterns and trends that are invisible in single time-point analyses. Our approach not only allows for longitudinal microbiome profiling but also enables a direct comparison with the results of classical microbiological cultures. This confrontation provides information about bacteria that don't grow in laboratory conditions and may play a significant role in disease pathogenesis. By comparing sequencing and culture data, we verify the accuracy and sensitivity of both methods, revealing how full-length 16S rRNA gene sequencing can provide a much more complete picture of the microbiome and detect potentially overlooked microorganisms.

1. Conclusions and future work

Our analysis of the current study group has shown that the composition of the sinonasal microbiome in patients with chronic rhinosinusitis and in healthy individuals does not show consistent patterns but is instead highly individual. In the future, we plan to expand the study group. This will allow us to continue our longitudinal analyses and thoroughly investigate temporal trends in the microbiome's composition. Additionally, we aim to evaluate the impact of systemic antibiotic therapy on the sinonasal microbiome, including changes in its taxonomic composition and the presence of antibiotic-resistant bacteria. In the long term, we will seek to integrate microbiome data with other clinical data. This is crucial for creating a patient's digital twin and applying personalized medicine in routine clinical practice.

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