Organism and Microbiome Analysis

Techniques and Implications for Chronic Rhinosinusitis

Ashleigh A. Halderman, MD[a], Andrew P. Lane, MD[b,*]

KEYWORDS

• Chronic rhinosinusitis • Microbiota • Metagenome • Sequence analysis, DNA
• RNA, Ribosomal, 16S

KEY POINTS

- Next-generation DNA sequencing systems are becoming increasingly accessible and affordable.
- Molecular culture-independent techniques are complementary to standard cultures and can identify a significantly greater number of bacterial organisms.
- Whole metagenome shotgun and metatranscriptomic sequencing have provided insight into complex bacterial communities and their host interactions.

INTRODUCTION

Paralleling modern advances in microbiology, our understanding of the disease process of chronic rhinosinusitis (CRS) has shifted with time as technology allows more in-depth assessments of the paranasal sinus microenvironment. Not long ago, the sinuses were thought to be sterile. The advent of culture-independent techniques has shed light onto the complex bacterial communities present in healthy paranasal sinuses, exposing limitations of standard culture in terms of the range of organisms that can be detected and grown in the laboratory setting.[1,2] Information based on morphologic characteristics of colonies grown on culture, their metabolic production or consumption, and physiologic characteristics identified by stains are all nonspecific at lower taxonomic levels.[2] With culture-dependent techniques, it is likely that microbiological results for sinonasal samples reflect in part the laboratory growth conditions rather than accurately depicting the breadth and relative abundances of resident microbes and potential pathogens. Multiple studies have shown that

[a] Department of Otolaryngology–Head and Neck Surgery, University of Southwestern Medical Center, 5303 Harry Hines Blvd, Dallas, TX 75390-9035, USA; [b] Department of Otolaryngology–Head and Neck Surgery, Johns Hopkins School of Medicine, 6th Floor, 601 North Caroline Street, Baltimore, MD 21287-0910, USA
* Corresponding author.
E-mail address: alane3@jhmi.edu

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culture-independent techniques identify significantly more organisms than do standard cultures.[3–5] Furthermore, our understanding of the role of bacteria in driving CRS has begun to change as more studies have shown compelling evidence against a direct bacterial cause of the disease. This review focuses on the current technology and emerging advancements in modern microbiology.

A HISTORY OF MICROBIOLOGIC TECHNOLOGY

The microscope was known to exist as far back as the mid-1600s when Robert Hooke and later Anton van Leeuwenhoek made the first notable observations of microorganisms. However, it would not be until 200 years later when Louis Pasteur disproved the widely accepted "spontaneous generation" theory and postulated the germ theory of disease, before the field of microbiology would experience a renaissance. The 1880s proved an essential decade for techniques surrounding microbial cultivation and growth and identification that would allow the field to grow by leaps and bounds. During that 1 decade, solid media, the Petri dish, enriched culture media, plating techniques, and acid-fast and Gram stains were all invented.[6]

The late nineteenth and early twentieth centuries are sometimes referred to as "The Golden Age of Microbiology," as countless causative agents of infectious disease were identified during this time and the knowledge base grew exponentially.[6] New insights made in the 1940s and 1950s would ultimately give birth to a more modern era of microbiology and our current technology. Watson and Crick famously described the double helix structure of DNA in 1953 and the 1960s would prove a critical time for scientific advancements that would break and decipher the genetic code. A pivotal point for the field of molecular biology came in 1965, when Nobel laureate Linus Pauling introduced the concept of molecular systematics using proteins and nucleic acids to identify microorganisms.[7] The 1970s saw the development of recombinant DNA technology and the techniques to sequence DNA. Polymerase chain reaction (PCR) technology was developed in the 1980s, and the 1990s would see the achievement of the first complete genome sequence of a microorganism, *Haemophilus influenzae*. From here, we enter the modern era.

All modern methods for investigating DNA rely on the same basic steps:

1. Lyse tissue/cells
2. Isolate DNA
3. Fragment DNA
4. Sequence DNA fragments

Several methods exist for sequencing DNA. The first was described by Sanger and colleagues in the 1970s.[8,9] In this method, a single strand of DNA is divided into 4 separate sequencing reactions. Each reaction contains all 4 deoxynucleotides (dATP, dCTP, dGTP, and dTTP), DNA polymerase, and only 1 of 4 dideoxynucleotides (ddATP, ddCTP, ddGTP, or ddTTP). Starting at a specific primer, the DNA polymerase adds deoxynucleotides corresponding to the DNA template until a dideoxynucleotide is added, resulting in chain termination.[9] Originally, the fragments generated by this process were denatured into single strands and separated based on size by using gel electrophoresis. They were then visualized by autoradiography and the DNA sequence read off autoradiographs.[9] In the 1990s, the Sanger technique received updates in the form of fluorescent labeling, capillary electrophoresis, and general automation. These modifications lowered costs and improved efficiency. Yet it would be some years before DNA sequencing became feasible from a logistics, time, and expense standpoint for it to be widely accessible.