REVIEW OF TECHNIQUES TO DETECT GUT MICROBIOME AND DETERMINATION OF SENSITIVITY AND SPECIFICITY OF THE TESTS USED AT A TERTIARY CARE HOSPITAL IN PAKISTAN

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Abstract:
Introduction: Gut microbiome and microbiota play a vital role in human health and influence various physiological processes. Alterations in the composition of the gut microbiota are associated with various diseases. Understanding the gut microbiome is crucial for personalized nutrition and health care. This study reviewed various techniques for detecting the gut microbiome and evaluated the sensitivity and specificity of selected tests used in a tertiary care hospital in Pakistan.
Methodology: Data from patients with gastrointestinal issues were retrospectively analyzed considering demographic information, symptoms, and test results. The sensitivity and specificity were determined for three techniques: Shotgun Metagenomics, Breath Tests, and Single-Cell Sequencing.
Results: The study included 57.3% males, with most patients aged 40–50 years. Shotgun Metagenomics demonstrated the highest sensitivity (0.936) and specificity (0.667), followed by Single-Cell Sequencing with sensitivity (0.901) and specificity (0.762), whereas Breath Tests had lower sensitivity (0.794) and specificity (0.483). The area under the ROC curve was the highest for Shotgun Metagenomics (0.675), indicating its potential as a diagnostic tool for gut microbes.
Conclusion: Understanding the impact of the gut microbiome on human health is a rapidly evolving field, with promising diagnostic techniques, such as Shotgun Metagenomics, demonstrating high sensitivity and specificity. Future studies should continue to explore and refine these methods to improve healthcare and personalize nutritional interventions.

Keywords: gut microbiome, sensitivity, specificity, novel techniques

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Introduction

Human health is greatly influenced by the microbiome, which comprises the collective genomes of microorganisms in an environment, and the microbiota, which is the actual community of microorganisms, especially in the gastrointestinal tract of humans. The microbiome, which is sometimes referred to as a virtual organ, contains millions of genes that generate metabolites that influence host health and fitness. Studies have shown that microbiota plays an important role in immunological, metabolic, and neurobehavioral processes. Environmental factors such as food, medications, and anthropometric measurements are important drivers of microbiota composition. Reduced diversity has been linked to autoimmune diseases, obesity, and cardiometabolic disorders. Gut microbiota diversity, determined by the quantity and distribution of species, is related to health. It is possible to improve health by modifying the gut microbiota through nutrition, which includes dietary fiber and prebiotic foods. Probiotics are live microorganisms that provide health benefits when ingested in sufficient quantities. They have demonstrated the potential for the prevention of several illnesses. Dietary advice can be revolutionized by an emerging area called personalized nutrition, which is based on an individual's gut microbiota makeup. Ultimately, a fascinating area of research in nutrition and healthcare is the exploitation of the impact of gut microbiota on human health (1).

Review about all the available techniques: Targeting a specific gene (16S rRNA) unique to bacteria, 16S rRNA sequencing is an additional technique that can be used to determine the type of bacteria present in a sample. It is widely used to assess changes associated with diseases or treatments as well as the composition of bacterial communities in the gastrointestinal tract. By focusing on no single gene, shotgun metagenomic sequences of every gram of genetic material were present in a sample. This method provides a more objective and comprehensive image of the microbiome by providing information on functional genes and likely pathogenic factors. Functional metagenomics analyzes the genes responsible for several functions, including digestion and antibiotic resistance, to assess the metabolic capacity of GIT bacteria. This can help determine the potential functions of the microbiome and how health and illness are affected by it. Understanding how a patient's immune system or intestinal lining interacts with GIT bacteria is the goal of microbiota-host interaction investigations. Strategies including transcriptomics, proteomics, and metabolomics can be used to study the host response to microbial populations or metabolites (2).

Using a process called metagenomic sequencing, all the genetic material in a sample, including DNA and RNA, can be analyzed to identify every type of bacteria present in the gastrointestinal tract. Information on the relative abundances of different species and the entire microbiome, which consists of bacteria, viruses, fungi, and archaea, can be obtained using this technique. Its aid allows for the prediction of infections, dysbiosis, and even outcomes of nutritional therapy or interventions (2–4).

Artificial intelligence (AI)-based techniques use machine learning algorithms to assess complex microbiome data, identify patterns, and predict the likelihood of a disease or its prognosis (5). Breath tests can identify methane or hydrogen gas in the breath-borne emissions of specific GIT microorganisms, and can be used to diagnose diseases such as small intestinal bacterial overgrowth (SIBO) (6). The diversity and makeup of donor fecal material can be assessed using novel assays to ensure the efficacy and safety of FMT, a treatment for specific GIT disorders (7). By focusing on individual microbial cells within a sample, single-cell sequencing provides more detailed insight into microbial diversity, functional capacities, and interactions. With a focus on the viral component of the GIT microbiome, gut virome profiling aids in the identification and characterization of viruses, such as enteric and bacteriophages, that may have an impact on the health or illness of the GIT. In proteomic analysis, each protein produced by the bacteria was examined to gain insight into the functions and relationships of GIT microorganisms with their host.

By analyzing the RNA transcripts of GIT microorganisms, a technique known as meta-transcriptomics can be used to identify genes that are actively expressed and provide insights into microbial activity and responses to environmental changes (8). The approach known as Stable Isotope Probing (SIP) tracks the integration of stable isotope labels onto specific substrates into the microbial biomass, thereby facilitating the identification of the GIT bacteria responsible for the metabolism of certain substances (9). Using microfluidic devices, microbiome on-a-chip technologies, also referred to as "microbiome-on-a-chip" technologies, can mimic GIT settings and allow researchers to study the interactions between microbes, host cells, and food components in a controlled environment (10).

Table 1: Quality assessment of tests according to existing literature (1–10).

Technique	Specificity	Sensitivity	Turnover Time
Metagenomic Sequencing	High	High	Moderate to Long
16S rRNA Sequencing	Moderate	Moderate	Short to Moderate
Shotgun Metagenomics	High	High	Moderate to Long
Functional Metagenomics	Moderate	Moderate	Moderate
Microbiota-Host Interaction Studies	Moderate	Moderate	Variable
Microbiome Profiling with AI	Variable	Variable	Variable
Breath Tests	Moderate	Moderate	Short to Moderate
FMT Evaluation	Moderate	Moderate	Short to Moderate
Single-Cell Sequencing	High	Moderate	Moderate to Long
Gut Virome Profiling	Moderate	Moderate	Moderate
Proteomics	High	Moderate	Moderate to Long
Meta Transcriptomics	Moderate	Moderate	Moderate
Stable Isotope Probing (SIP)	Moderate	Moderate	Moderate
Microbiome On-a-Chip	Moderate	Moderate	Short to Moderate
Artificial Gut Models	Moderate	Moderate	Short to Moderate
Multiple Omics Integration	Variable	Variable	Variable
Predictive Diagnostics through ML	Variable	Variable	Variable
CRISPR-Cas Technology	Variable	Variable	Variable
Microbiome Therapeutic Monitoring	Variable	Variable	Variable
Nanopores Sequencing	Variable	Variable	Short to Moderate

Researchers have investigated the relationships between gut bacteria and intestinal cells and how they impact health and disease using artificial gut models such as organoids and three-dimensional cell cultures (5). To obtain a comprehensive understanding of the composition and activities of the GIT microbiome, an approach known as "multiple omics integration" merges data from several omics methodologies, such as transcriptomics, metagenomics, proteomics, and metabolomics. To predict sickness risk, medication response, or nutritional advice specific to everyone is unique microbiome, machine learning algorithms can be employed to analyze complex GIT microbiome data. Using CRISPR-based technology, one can be edited to treat GIT issues or target infections that are damaging to the microbiota. To assess the effectiveness of microbiome-targeted medications such as probiotics, prebiotics, and dietary treatments, Microbiome Therapeutic Monitoring entails the use of advanced testing techniques to track changes in the GIT microbiome over time. Nanopore sequencing technology is a viable method for the rapid and portable characterization of the microbiome in clinical settings.

Methodology

This study retrospectively analyzed the data of patients who presented with gastrointestinal issues and underwent extensive examination of the gut microbiome using the latest technologies. The demographic details of the patients were noted, including age, sex, and symptoms. The duration of the symptoms is also important in such cases. All essential details were noted on an Excel sheet and analyzed. The sensitivity and specificity of the tests were determined. Not all the tests mentioned in the literature are used in Pakistan. Techniques such as Shotgun Metagenomics, Breath Tests, and Single-Cell Sequencing have been used to test the sensitivity and specificity of these techniques. The ideal values for the sensitivity and specificity were 1.

Results

The study included 57.3% males and the remaining females in this study. Of the participants, 24.6% were under the age of 35. Most patients were aged between 40 and 50 years.

Sensitivity is the ability to detect the presence of a particular microorganism, and specificity is the ability of the test to determine if the organism is not present (absence of microorganisms).

Table 2: Sensitivity and specificity

Technique	Specificity	Sensitivity	Turnover Time
Shotgun Metagenomics	0.667	0.936	Moderate to Long
Breath Tests	0.483	0.794	Short to Moderate
Single-Cell Sequencing	0.762	0.901	Moderate to Long

The sensitivity of Shotgun Metagenomics and Single-Cell Sequencing is near 1, so they are sensitive tests, but the specificity values are not good. When the receiver operating characteristics (ROC) curve was constructed, the area under the curve was less than 1, the test was only applied for Shotgun Metagenomics and Single-Cell Sequencing, and the breath test was excluded owing to its poor sensitivity and specificity. The area Under the Curve for Single-Cell Sequencing was 0.609 and that for Shotgun Metagenomics was 0.675.

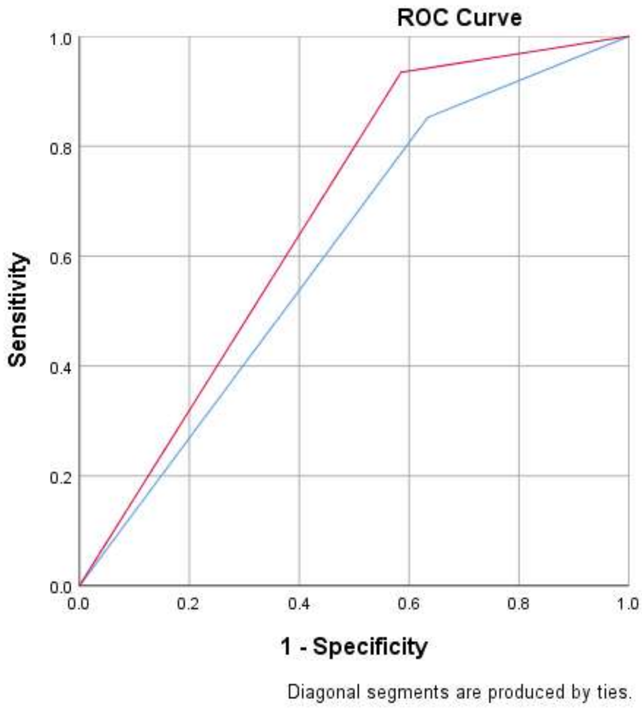

Figure 1: ROC curve for Single-Cell Sequencing (blue) and Shotgun Metagenomics (Pink)

Discussion

Shotgun Metagenomics had the highest specificity at 0.667, followed by Single-Cell Sequencing at 0.762, and Breath Tests at 0.483. This suggests that Shotgun Metagenomics and Single-Cell Sequencing are better at avoiding false positives than Breath Tests. Shotgun Metagenomics had the highest sensitivity at 0.936, followed by Single-Cell Sequencing at 0.901, and Breath Tests at 0.794. This suggests that Shotgun Metagenomics and Single-Cell Sequencing are better at detecting true positives than Breath Tests. The area under the ROC curve was greater for Shotgun metagenomics. Therefore, it may be a useful diagnostic tool for GIT microbes. Shotgun metagenomic DNA sequencing is a relatively new and effective environmental sequencing technique that can shed light on the biodiversity and function of communities. However, the intricate nature of these data makes metagenomic sequence analysis challenging. New methods and information sources have emerged to avoid these difficulties and enable scientists to identify the bacteria present in the community and their potential activities (11).

By cataloguing the bacterial, fungal, and viral gene contents inside a sample, shotgun sequencing of microbial communities offers a comprehensive understanding of the microbiome. This contrasts with amplicon sequencing methodologies, which are taxonomically constrained and only assess taxonomy without considering functions (12). The development of next-generation sequencing (NGS) has made it possible to study the gut microbiota at a resolution that has never been possible before. As a result, advanced bioinformatics tools have been developed to analyze the vast volumes of data produced. Therefore, researchers must have a thorough understanding of the fundamental ideas involved in the planning, carrying out, and interpretation of NGS studies of microbiomes. Libraries were analyzed to demonstrate the advantages and disadvantages of two primary methods for studying the microbiome: shotgun metagenomics and 16S ribosomal RNA (rRNA) gene amplicons (13). In this study, the most sensitive and specific method was Shotgun Metagenomics. Further research should be performed to evaluate the diagnostic tests.

Conclusion:

Gut microbiota, comprising an array of microorganisms residing in the gastrointestinal tract, has a profound impact on human health. By retrospectively analyzing patient data, this research not only provided valuable insights into the demographics and symptoms of individuals seeking gastrointestinal care but also evaluated the performance of advanced diagnostic techniques. Shotgun Metagenomics and Single-Cell Sequencing have emerged as standout methods, demonstrating both high sensitivity and specificity for detecting gut microbes. These findings highlight the potential of these cutting-edge approaches as powerful diagnostic tools for gut microbiome-related disorders. With the promise of personalized nutrition and healthcare, this study highlights the importance of ongoing research to further refine and expand our understanding of the role of the gut microbiome in human health.

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