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Advances in metagenomic sequencing for infectious disease management: From pathogen profiling to antibiotic resistance

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ABSTRACT

Metagenomics has revolutionized the study of infectious diseases by enabling the analysis of microbial communities without the need for cultivation. This approach, powered by high-throughput sequencing technologies like 16S rRNA sequencing and shotgun metagenomics, allows the identification of both culturable and unculturable microbes, providing a comprehensive view of pathogens. Metagenomics offers critical insights into microbial diversity and interactions, particularly in polymicrobial infections, respiratory and gastrointestinal infections, and bloodstream infections. Advancements in bioinformatics, artificial intelligence (AI), and machine learning have enhanced the accuracy and speed of metagenomic data interpretation, aiding pathogen detection, antibiotic resistance surveillance, and personalized treatment strategies. Despite challenges such as sample complexity, host DNA interference, and data interpretation difficulties, metagenomics continues to evolve as a crucial tool for diagnostics and therapeutic development. Future research aims to address current gaps, including integrating AI into metagenomics for more precise pathogen profiling and developing targeted therapies based on microbial communities.Ultimately, metagenomics holds the potential to transform infectious disease management, offering real-time diagnostics, better global surveillance, and the promise of personalized medicine in the fight against emerging pathogens.

KEYWORDS

Metagenomics; Polymicrobial infections; Real-time diagnostics; Precision medicine; Antibiotic resistance

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Introduction

Metagenomics, a powerful approach to studying complex microbial communities, involves analyzing the collective genomes of microorganisms directly from environmental or clinical samples, bypassing the need for cultivation. This field has transformed microbial ecology and infectious disease research, enabling scientists to explore the diverse roles of pathogens and commensal microbes in health and disease. Historically, infectious disease diagnostics relied heavily on culture-based methods, which are limited by the inability to grow many microorganisms in the lab [1]. The advent of high-throughput sequencing technologies, such as next-generation sequencing (NGS), marked a turning point by allowing the identification of both culturable and unculturable microbes through DNA sequencing. This shift from traditional techniques to culture-independent methods has provided deeper insights into microbial diversity and pathogenicity, offering a more comprehensive view of disease-causing organisms and their interactions with the host [2].

Metagenomics is particularly important in the context of infectious diseases because many pathogens operate within microbial communities rather than in isolation. By examining these communities holistically, researchers can better understand how infections develop, how pathogens evade immune responses, and how microbial dysbiosis contributes to disease progression. In addition, metagenomic studies have expanded our knowledge of the microbiome's role in maintaining health and how its disruption can lead to infectious diseases, making it a valuable tool for advancing diagnostics, surveillance, and treatment strategies [3]. This growing field holds the promise of revolutionizing infectious disease management by offering pathogen-agnostic approaches to detect and characterize infections, ultimately leading to more effective therapeutic interventions and precision medicine [4].

Technological Advances in Metagenomics

Metagenomics has evolved significantly, with advancements in sequencing technologies and bioinformatics tools playing a critical role in microbial research. Two key sequencing approaches stand out: 16S rRNA gene sequencing and shotgun metagenomics [5].

16S rRNA sequencing

It is a popular amplicon-based method that targets the conserved 16S rRNA gene, a marker for bacterial taxonomy. This technique allows for rapid identification of bacteria and archaea in diverse environments by comparing sequences to known reference databases. Although cost-effective and widely used, 16S rRNA sequencing is limited in its ability to resolve organisms at the species level due to sequence similarity across taxa. It is also primarily focused on bacteria, excluding viruses and fungi from the analysis [6,7].

Shotgun metagenomics

This sequences the entire microbial community's DNA, offering a more comprehensive view by capturing bacterial, viral, and fungal genomes. This approach provides higher taxonomic resolution and can identify both functional genes

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and microbial diversity. However, it is more expensive and requires significant computational resources for data processing and analysis [8].

Advances in bioinformatics

This has further transformed the field. New software platforms like QIIME, MetaPhlAn, and Kraken allow researchers to process and analyze massive amounts of metagenomic data with increased speed and accuracy. These tools integrate taxonomy classification, functional annotation, and pathway analysis, thus providing insights into microbial functions in various environments. Machine learning algorithms are increasingly being used to improve the accuracy of metagenomic data interpretation, helping to distinguish between pathogenic and non-pathogenic organisms [9].

However, challenges persist in metagenomics. Sample preparation remains a critical bottleneck; environmental and clinical samples often contain low microbial DNA concentrations, requiring robust extraction methods to avoid bias. The presence of host DNA in clinical samples, such as blood or tissue, can also interfere with microbial detection, complicating downstream analysis [10].

Data complexity

It is another major challenge. The large volume of sequencing data generated by shotgun metagenomics requires substantial computational power for analysis. This is compounded by the presence of unknown organisms in many samples, for which reference genomes may not be available, complicating the accurate identification and classification of species. Additionally, errors introduced during sequencing, such as bias from PCR amplification or base-calling errors, can lead to misinterpretation of microbial diversity [11].

Finally, the interpretation of results poses significant hurdles. The dynamic and context-dependent nature of microbial communities makes it difficult to draw definitive conclusions about their roles in disease processes or environmental conditions. Integration of multiple layers of data, including genomic, transcriptomic, and proteomic information, is often required to fully understand microbial function and interactions [12].

Metagenomic Profiling of Pathogens in Infectious Diseases

Metagenomic approaches have transformed the profiling of pathogen diversity in infectious disease environments, allowing for the identification of microbes beyond traditional culture-based techniques. By sequencing all genetic material in a sample, metagenomics enables researchers to uncover both known and previously uncharacterized pathogens, significantly advancing our understanding of the complex microbial dynamics in various infections [14,15]. In respiratory infections, such as pneumonia, metagenomic sequencing has proven invaluable in detecting a wide array of bacterial, viral, and fungal pathogens. Unlike culture methods, which are limited to organisms that can grow in laboratory conditions, metagenomics can identify fastidious and unculturable microbes, contributing to a more accurate diagnosis. For instance, recent studies in lower respiratory tract infections (LRTIs) have highlighted how metagenomics not only identifies pathogens like Streptococcus pneumoniae and Mycoplasma pneumoniae but also uncovers co-infections that might otherwise be missed [16].

In gastrointestinal infections, metagenomics helps dissect the microbial composition of the gut, distinguishing between commensal microbes and harmful pathogens. This technique has been applied to cases of bacterial gastroenteritis, where pathogens such as Salmonella and Clostridium difficile have been profiled alongside less-expected viral or parasitic contributors. By providing a complete picture of the microbial ecosystem, metagenomics offers insights into the interactions between pathogens and the host gut microbiome, which can influence the severity and outcome of infections [17,18]. Similarly, in bloodstream infections (BSIs), metagenomic next-generation sequencing (mNGS) plays a critical role in pathogen detection, particularly in sepsis. This high-throughput technology can detect bacterial, viral, and fungal DNA in blood samples, speeding up diagnosis in critically ill patients. Studies have demonstrated the ability of metagenomics to identify rare or difficult-to-diagnose pathogens in bloodstream infections, such as Candida species and antibiotic-resistant bacteria, even when conventional tests fail [19].

One of the strengths of metagenomics is its ability to discern between pathogenic and commensal microbial populations in disease settings. This is particularly important in polymicrobial infections where pathogens coexist with harmless organisms, complicating diagnosis. For example, in respiratory infections, metagenomics can differentiate between normal flora and invasive pathogens by analyzing the relative abundance and virulence factors of the detected organisms. Such detailed profiling is critical in hospital-acquired infections, where distinguishing between colonization and infection can influence treatment decisions [20]. In conclusion, metagenomic profiling is revolutionizing pathogen detection in infectious diseases by providing comprehensive, unbiased insights into microbial communities across various infection types. As metagenomic technologies continue to advance, the ability to identify novel pathogens, track antimicrobial resistance, and enhance diagnostic precision will further improve clinical outcomes in infectious disease management [21].

Host-Microbe Interactions in Infectious Diseases

Host-microbe interactions play a critical role in shaping immune responses in infectious diseases. Metagenomics enables the detailed study of microbial communities and their influence on host immunity by revealing shifts in the microbiome. These shifts can drive immune dysregulation, leading to diseases like inflammatory bowel disease (IBD) and infections [22].

Dysbiosis, the imbalance in the microbiome, often compromises the immune system, leaving the host vulnerable to infections and inflammatory responses. Studies have shown a strong link between the gut microbiota and immune function. For example, reduced microbial diversity is associated with inflammatory diseases like IBD, where pathogens and immune triggers (e.g., Escherichia-Shigella) dominate overprotective,

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commensal microbes. Metagenomic analyses provide valuable insights into how microbial communities mediate immune responses, such as triggering inflammation, cytokine production, and even autoimmune reactions. For instance, beneficial microbes promote immune tolerance, while pathogenic ones initiate pro-inflammatory responses. Advanced sequencing techniques have shown how these interactions vary between healthy and diseased states, revealing crucial connections between microbial composition and immune health [23].

Through metagenomics, researchers gain insights into microbial factors that impact immune regulation, opening possibilities for microbiome-targeted therapies to restore immune balance in infections and immune-related diseases [24].

Antibiotic Resistance and Metagenomics

Detection using metagenomic approaches

Metagenomics has become an essential tool for identifying antibiotic resistance genes (ARGs) in microbial communities. Through techniques like shotgun sequencing, researchers can comprehensively assess the resistome, the collection of ARGs in an environment. This method allows the detection of ARGs across a broad range of microbes, including those not readily cultured in the laboratory. Studies have shown that metagenomics is highly effective in identifying resistance genes from hospital surfaces, sewage, and other clinical settings. For instance, beta-lactam, fosfomycin, and cephalosporin resistance genes are frequently detected in nosocomial environments, where these genes are often associated with virulent bacterial species like Staphylococcus aureus and Pseudomonas aeruginosa [25].

Role of horizontal gene transfer

Horizontal gene transfer (HGT) is a key mechanism by which antibiotic resistance spreads among bacteria, enabling even distantly related species to acquire resistance traits. Mobile genetic elements such as plasmids, transposons, and integrons play a vital role in this process, facilitating the transfer of ARGs between different bacterial populations [26]. Metagenomic studies have highlighted the widespread presence of HGT-mediated resistance in clinical settings, particularly in hospital-acquired infections (HAIs). The ability of pathogens like Acinetobacter baumannii and Enterococcus to share resistance traits via HGT complicates infection control, as these bacteria can rapidly disseminate ARGs within hospital environments [22,33].

Metagenomics of Healthcare-Associated Infections

Metagenomic profiling is increasingly being used in the surveillance of hospital-acquired infections. By analyzing environmental samples from hospitals, researchers can identify not only pathogens but also the ARGs that contribute to antimicrobial resistance. This approach has been instrumental in revealing the extent of resistance genes in high-risk areas such as surgical wards and intensive care units. In a global context, metagenomics has also been utilized to track the spread of ARGs in sewage systems and other environmental reservoirs, offering insights into resistance dynamics across different regions. Large-scale initiatives have shown a worrying increase in the abundance of ARGs linked to last-resort antibiotics like colistin, emphasizing the urgent need for global surveillance [18,20].

Overall, metagenomics provides an unparalleled level of detail regarding antibiotic resistance in both clinical and environmental settings. However, the complexity of data interpretation and the need for advanced bioinformatics tools remain ongoing challenges in leveraging its full diagnostic potential [21].

Clinical Applications and Diagnostic Potential

Meta genomics is revolutionizing infectious disease diagnostics by offering a comprehensive, unbiased approach to identifying pathogens in clinical samples. Unlike traditional diagnostic methods, which often require prior knowledge of the pathogen, metagenomics analyzes all microbial DNA or RNA present in a sample. This allows for the simultaneous detection of known and unknown pathogens, including bacteria, viruses, fungi, and parasites. It is especially useful in cases where pathogens are difficult to culture, helping clinicians uncover the etiology of complex or unknown infections. This ability to capture the microbial diversity in a sample makes it ideal for diagnosing emerging or unexpected infections [13,19].

Real-Time Diagnostics for Personalized Treatment

One of the most promising applications of metagenomics is its potential for real-time diagnostics. During outbreaks, such as the Ebola virus epidemic, metagenomics provided valuable data that helped identify the virus quickly and track its spread. The rapid sequencing capabilities of high-throughput technologies enable faster detection and classification of pathogens, improving response times for treatment and containment. Beyond outbreak settings, metagenomics can be applied in clinical settings to tailor treatment plans for individual patients. By identifying not just the pathogen but also the patient's microbiome, clinicians can design personalized therapies that account for the interactions between pathogens and the host's microbiota. This can inform the use of specific antimicrobials or probiotics to restore microbial balance, offering a more targeted therapeutic approach [26].

Limitations

Despite its potential, the clinical adoption of metagenomics faces several challenges. One major limitation is the complexity of sample preparation, which requires careful handling to avoid contamination and ensure accurate results. Another challenge lies in data analysis, where interpreting vast amounts of genetic information can be computationally demanding. Advances in bioinformatics are critical for making metagenomic diagnostics more accessible by improving the accuracy and speed of analysis [27].

Furthermore, the cost of metagenomic sequencing remains high compared to traditional methods, which limits its widespread use in clinical settings. Efforts are underway to standardize protocols for sample collection, sequencing, and analysis, which are essential to ensure the reproducibility of results and lower costs [28]. Moreover, there is a need for regulatory frameworks to establish the clinical validity of metagenomics, enabling it to become an integral part of routine

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diagnostics. Looking ahead, integrating artificial intelligence (AI) and machine learning into metagenomic data analysis holds great promise. These technologies can help manage the complexity of data interpretation, identify patterns in microbial communities, and predict the emergence of antibiotic resistance. Such advances could pave the way for metagenomics to become a cornerstone of personalized medicine and global pathogen surveillance [29].

Future Perspectives and Research Directions

The future of metagenomic research in infectious diseases holds immense potential but also faces several challenges that need to be addressed. Currently, there are gaps in the full understanding of microbial community dynamics and host-pathogen interactions. While metagenomic sequencing provides valuable insights into microbial diversity, the vast amount of data generated requires more refined interpretation techniques, which is where artificial intelligence (AI) and machine learning (ML) come into play. These tools can enhance the precision of metagenomic analyses, identifying patterns in complex datasets to detect pathogens more effectively and predict disease outcomes [30].

AI and ML algorithms are already proving useful in handling metagenomic data, from recognizing antibiotic resistance genes to predicting microbial interactions. Future developments in these technologies could further improve the accuracy of disease diagnostics and facilitate real-time pathogen surveillance. AI-based approaches will likely enhance personalized treatment plans, particularly in infectious diseases where the microbiome's role in health and disease is still under investigation. Looking ahead, the integration of metagenomics with therapeutic development offers exciting prospects. Researchers are exploring how metagenomic data could guide the creation of targeted therapies that modify microbial communities or bolster the immune system's response to infections. Functional metagenomics, driven by AI, may enable better prediction models for therapeutic interventions, shaping more effective treatments based on microbial profiles. However, for this potential to be fully realized, addressing data complexity, refining computational models, and securing greater cross-disciplinary collaboration will be essential [31,32]. The future of metagenomics in healthcare will depend on how effectively these challenges are met, especially in bridging the gap between research and practical clinical applications.

Conclusions

Metagenomics has emerged as a transformative tool in the field of infectious disease research, revolutionizing the way pathogens are identified and microbial communities are studied. By bypassing the need for traditional culture methods, metagenomics enables the comprehensive sequencing of all genetic material in a sample, allowing for the detection of pathogens that might otherwise be missed. This approach is particularly valuable for identifying novel or unexpected organisms, making it an indispensable tool for disease surveillance and diagnostics.

In recent years, the application of metagenomics has led to significant advancements in understanding the role of microbial diversity in infectious diseases. Through profiling microbial communities in different disease environments, metagenomics has highlighted the intricate relationships between host and pathogen, offering new insights into disease mechanisms. Importantly, it has also shed light on the dynamics between pathogenic and commensal microbes, which can influence disease outcomes. Looking ahead, the integration of artificial intelligence (AI) and machine learning into metagenomics promises to further enhance data interpretation, enabling faster and more accurate pathogen detection. As the technology continues to evolve, metagenomics is expected to play a key role in the development of personalized treatment strategies and the fight against emerging infectious diseases.

Disclosure statement

No potential conflict of interest was reported by the authors.

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