Modern Applied Medical Research

Volume 5,Issue 9, September, 2025,Page 01-14
ISSN: 2582-9181

Review Article Received: 12-08-2025| Accepted: 03-09-2025 | Published: 15-09-2025

Clinical Metagenomics Next-Generation Sequencing in Infectious Disease Diagnostics: Current Applications, Technological Advances, and Future Perspectives

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Abstract: Metagenomics next-generation sequencing (mNGS) has become a revolutionary tool in clinical infectious disease diagnostics, offering remarkable abilities for unbiased pathogen detection. This thorough review explores the development of mNGS from research settings to clinical use, evaluating its applications across various infectious diseases such as central nervous system infections, respiratory tract issues, bloodstream infections, and detection of emerging pathogens. We assess the main sequencing platforms, including Illumina, Oxford Nanopore Technologies, and MGI sequencing systems, discussing their benefits and drawbacks in clinical environments. The review critically examines the current obstacles in implementing mNGS clinically, such as complexities in sample processing, bottlenecks in bioinformatics analysis, lack of standardization, and cost factors. By analyzing clinical performance data in depth, we compare the diagnostic accuracy of mNGS with traditional methods across different infection types, emphasizing its improved detection rates for hard-to-culture organisms and its limitations in samples with high host background. The review also investigates emerging solutions to these technical challenges, like enhanced sample preparation techniques, automated bioinformatics pipelines, and standardized quality control practices. We address the economic impact of mNGS adoption, regulatory issues, and the technology's potential role in antimicrobial stewardship and outbreak investigations. Finally, we offer insights into the future of clinical metagenomics, including its integration with artificial intelligence, the creation of rapid sequencing protocols, and its expansion into personalized medicine. This review provides a comprehensive resource for clinicians, lab professionals, and researchers interested in the current status and future possibilities of clinical metagenomics in infectious disease diagnostics.

Keywords: *Metagenomics, advanced sequencing techniques, infectious illnesses, pathogen identification, medical diagnostics, computational biology, drug resistance, CSF, respiratory diseases*

1. Introduction

The field of infectious disease diagnostics has seen a significant evolution over the last twenty years, primarily due to breakthroughs in molecular technologies and an enriched comprehension of microbial diversity. Although traditional diagnostic methods are essential to clinical microbiology, they often lack the ability to tackle the intricacies of contemporary infectious disease issues. While culture-based techniques are specific and quantitative, they are time-intensive and unable to identify fastidious or non-culturable organisms. On the other hand, molecular methods, such as polymerase chain reaction (PCR), are quick and sensitive but restricted by their focus and inability to identify unforeseen or new pathogens.

In this context, metagenomics next-generation sequencing (mNGS) has emerged as a revolutionary diagnostic approach that addresses many limitations of conventional methods. By sequencing all nucleic acids present in clinical samples without prior amplification bias, mNGS provides an unbiased view of the microbial landscape, enabling detection of bacteria, viruses, fungi, and parasites in a single assay (Liu & Ma, 2024). This capability has proven particularly valuable in cases where conventional diagnostics fail, with studies indicating that 40-50% of serious infections remain undiagnosed using traditional methods.

The journey of mNGS from research curiosity to clinical reality has been accelerated by several convergent factors. The dramatic reduction in sequencing costs, from over $100 million for the first human genome to less than $1,000 today, has made routine clinical sequencing economically feasible. Simultaneously, improvements in sequencing throughput and turnaround times have brought mNGS within the timeframe requirements of acute clinical care. The development of sophisticated bioinformatics tools has begun to address the complex challenge of extracting meaningful clinical information from vast datasets.

The COVID-19 pandemic marked a pivotal moment for clinical metagenomics, highlighting the critical necessity for swift pathogen identification and the transformative capability of genomic technologies in public health responses. The swift detection of SARS-CoV-2 using metagenomic methods, alongside real-time tracking of viral evolution and transmission trends, illustrated the technology's promise for individual patient care and population-wide surveillance.

However, the translation of mNGS from research to routine clinical practice faces significant challenges. The complexity of sample processing, the need for sophisticated bioinformatics expertise, and the absence of standardized workflows have created barriers to widespread adoption. Questions regarding result interpretation, clinical significance of detected organisms,

and integration with existing diagnostic algorithms remain areas of active investigation and debate.

This review offers an in-depth assessment of the present landscape of clinical metagenomics, highlighting its notable achievements as well as its persistent challenges. We investigate the technological foundations underpinning clinical mNGS, evaluate performance data across various clinical applications, and consider the practical factors that affect implementation decisions. By critically analyzing published studies and emerging trends, we seek to equip clinicians and researchers with a detailed understanding of how mNGS can enhance current practices and identify areas requiring further advancement.

2. Evolution of Sequencing Technologies and Clinical Applications

2.1 Historical Development of DNA Sequencing

The advancements in DNA sequencing technologies have markedly enhanced speed, precision, and affordability. This progress began in 1977 with Sanger sequencing, which held its status as the benchmark for more than thirty years. Frederick Sanger's chain termination method offered exceptionally accurate sequencing but was constrained by its limited throughput and the high cost per base, rendering extensive sequencing projects financially challenging.

The debut of automated DNA sequencers in the 1980s, particularly the ABI 373 system, signified the start of commercial DNA sequencing. These devices facilitated the Human Genome Project and laid the groundwork for contemporary molecular diagnostics. Nonetheless, the limited throughput and significant expenses of first-generation sequencing confined its clinical uses to specialized genetic testing and research purposes.

The groundbreaking advancement occurred in 2005 with the advent of massively parallel sequencing technologies, spearheaded by 454 Life Sciences and their pyrosequencing platform. This innovation was soon succeeded by Illumina's Solexa system in 2006 and Applied Biosystems' SOLiD platform in 2007. These second-generation technologies boosted throughput exponentially and significantly lowered the cost per base, rendering comprehensive genomic analysis feasible in clinical settings.

The advent of third-generation sequencing technologies, spearheaded by Pacific Biosciences and Oxford Nanopore Technologies, has brought forth new capabilities such as real-time sequencing and exceptionally long read lengths. These innovations are especially significant for clinical metagenomics, as the capacity to sequence entire genomes or identify structural variations can offer essential diagnostic insights.

2.2 Current Sequencing Platforms in Clinical Practice

Illumina Platforms: Illumina sequencing systems dominate the clinical metagenomics landscape, representing over 50% of published studies in infectious disease applications. The technology's strength lies in its high accuracy (>99%) and established workflow protocols. Current clinical platforms range from benchtop systems like the MiSeq and NextSeq series to high-throughput systems like the NovaSeq platform.

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Oxford Nanopore Technologies (ONT): The advent of third-generation sequencing technologies, spearheaded by Pacific Biosciences and Oxford Nanopore Technologies, has brought forth new capabilities such as real-time sequencing and exceptionally long read lengths. These innovations are especially significant for clinical metagenomics, as the capacity to sequence entire genomes or identify structural variations can offer essential diagnostic insights.

The capacity to produce real-time data renders ONT platforms especially appealing for urgent clinical scenarios where swift results are crucial. Research has shown that pathogen identification can be accomplished in as little as 6 hours with ONT platforms, in contrast to the 24-48 hours required by traditional mNGS methods. The ultra-long reads generated by nanopore sequencing can cover entire bacterial genomes, allowing for a more thorough examination of antimicrobial resistance mechanisms and virulence factors.

Nevertheless, the elevated error rates linked to nanopore sequencing (ranging from 5-15%, in contrast to less than 1% for Illumina) necessitate the use of specialized bioinformatics techniques and may hinder the detection sensitivity for organisms present in low abundance. Recent advancements in basecalling algorithms and consensus methods have substantially lowered error rates, thereby enhancing the feasibility of ONT platforms for clinical use.

MGI Sequencing Platforms: The sequencing platforms provided by MGI (previously known as BGI) serve as a significant alternative to Illumina systems, especially in markets where minimizing expenses is crucial. The MGISEQ lineup utilizes DNA nanobead (DNB) technology in conjunction with combinatorial probe-anchor synthesis (cPAS) chemistry to deliver high throughput while keeping costs low.

The recent authorization of MGISEQ platforms for clinical application in multiple countries has broadened the choices available to clinical laboratories aiming to adopt mNGS. Comparative research has demonstrated that, for most uses, the performance of these platforms is on par with Illumina's, although some variations in GC bias and the uniformity of coverage have been noted.

2.3 Technical Considerations for Platform Selection

Selecting a sequencing platform for clinical metagenomic next-generation sequencing (mNGS) involves several considerations, such as the need for rapid results, the volume of samples to be processed, budgetary constraints, and targeted

clinical uses. Each platform has unique strengths and weaknesses that should be thoughtfully assessed based on the planned application.

Table 1. Comparison of Major Sequencing Platforms for Clinical mNGS

Platform	Throughput	Read Length	Accuracy	Turnaround Time	Cost per Sample	Key Advantages
Illumina MiSeq	25M reads	2×300 bp	99.9%	24-55 hours	$50-150	High accuracy, established workflows
Illumina NextSeq	400M reads	2×150 bp	99.9%	12-30 hours	$30-100	High throughput, moderate cost
ONT MinION	50 Gb	Variable	95-98%	6-24 hours	$100-300	Rapid results, portable
ONT PromethION	>10 Tb	Variable	95-98%	12-48 hours	$50-200	Ultra-high throughput, long reads
MGISEQ-2000	300M reads	2×150 bp	99.8%	24-48 hours	$40-120	Cost-effective, comparable accuracy

For critical clinical needs like meningitis or sepsis, platforms that provide swift results might be favored even if the cost per sample is higher. On the other hand, for regular monitoring or research purposes, high-throughput platforms designed for cost efficiency may be more suitable.

3. Clinical mNGS Workflow and Technical Implementation

3.1 Sample Collection and Processing

The success of clinical mNGS begins with appropriate sample collection and processing, which differs significantly from conventional microbiology practices. Unlike culture-based methods that can amplify small numbers of organisms, mNGS requires adequate quantities of nucleic acids for reliable detection. This fundamental difference necessitates careful attention to pre-analytical factors that can significantly impact diagnostic yield.

The conditions under which samples are transported and stored are especially important for metagenomic next-generation sequencing (mNGS) applications. RNA viruses are notably prone to breaking down during transport. Additionally, nucleic acids may fragment, and detection sensitivity can decrease due to repeated freeze-thaw cycles. Research indicates that samples handled within six hours of being collected perform significantly better than those subjected to prolonged storage.

The performance of mNGS is also affected by the volume and type of clinical specimen. For adult blood samples, usually collected in amounts of 8-10 mL, centrifugation and plasma separation are necessary to concentrate the cell-free DNA. When dealing with cerebrospinal fluid, which often comes in limited quantities, concentration steps might be needed to obtain sufficient nucleic acid yields. Respiratory samples such as bronchoalveolar lavage fluid and sputum may need to be pretreated with mucolytic agents to enhance the efficiency of nucleic acid extraction.

Sample-Specific Considerations:

Cerebrospinal Fluid (CSF): CSF offers both the largest opportunity and the biggest obstacle for clinical mNGS. Due to the typically sterile condition of CSF, any organism found is likely to be clinically important. Nonetheless, the low biological material in these samples makes them especially prone to contamination and difficulties in extraction. Research indicates that CSF samples containing a high amount of host DNA (more than 90%) exhibit considerably lower sensitivity in detecting pathogens.

Blood and Plasma: Bloodstream infections pose specific difficulties for mNGS because of the substantial presence of host DNA and the usually low levels of circulating pathogen nucleic acids. Extracting cell-free DNA from plasma has become the favored method as it minimizes the host background and enriches the pathogen-derived nucleic acids that are released from infected tissues.

Respiratory Samples: The intricate microbiome within the respiratory tract necessitates a cautious analysis of mNGS results to differentiate between organisms that are simply colonizing and those that are actual pathogens. Bronchoalveolar lavage fluid is typically more effective at distinguishing colonization from infection than samples from the upper respiratory tract.

3.2 Nucleic Acid Extraction and Library Preparation

Extracting high-quality nucleic acids is an essential challenge in clinical mNGS workflows. Whereas PCR-based assays can endure some level of nucleic acid degradation, mNGS demands intact, high-molecular-weight DNA and RNA to ensure optimal library construction and sequencing performance.

The selection of an extraction technique greatly affects the range of organisms identified and the assay's overall sensitivity.

For organisms with sturdy cell walls, such as mycobacteria and fungi, mechanical lysis using bead beating is typically required. Nonetheless, these rigorous extraction conditions can potentially degrade viral RNA or harm other sensitive targets.

Recent advancements in extraction techniques have been aimed at tackling the issue of depleting host DNA. Differential lysis, probe capture, and enzymatic depletion are among the methods that have shown potential for minimizing host background. However, each of these approaches has certain limitations and may unintentionally eliminate pathogen nucleic acids.

Quality Control and Contamination Prevention:

The exceptional sensitivity of mNGS makes it highly prone to contamination from environmental sources, reagents, and cross-contamination between different samples. Thorough quality control measures are essential to tackle contamination at various stages, including the screening of reagents, monitoring of environmental factors, and validation from batch to batch.

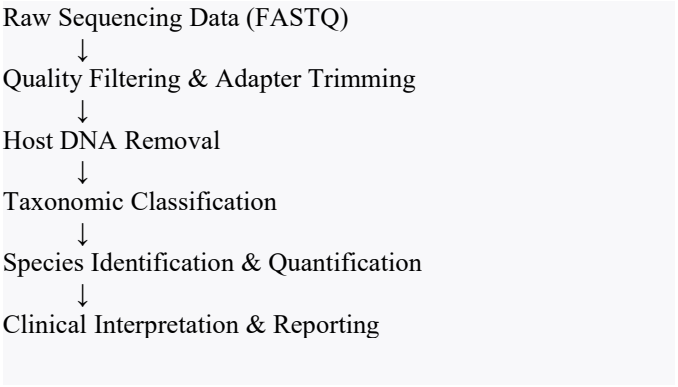
Incorporating negative controls into each sequencing run has become a routine procedure, yet interpreting background contaminants continues to pose difficulties. Frequently detected contaminants such as Propionibacterium acnes and various environmental bacteria might also serve as genuine pathogens in specific clinical situations, necessitating thorough clinical correlation.

Positive controls serve multiple functions including validation of extraction efficiency, library preparation success, and bioinformatics pipeline performance. The choice of positive control organisms should reflect the types of pathogens expected in clinical samples and include representatives of bacteria, viruses, fungi, and parasites as appropriate.

3.3 Bioinformatics Analysis Pipeline

The analysis of mNGS data through bioinformatics is arguably the most intricate part of the whole process, as it demands specialized knowledge and computational resources not always accessible in all clinical labs. The initial sequencing data, usually produced in FASTQ format, must go through several processing stages before yielding results that can be used in a clinical context.

Figure 1. Clinical mNGS Bioinformatics Workflow



Quality Control and Data Filtering: Initial data processing focuses on removing low-quality sequences, adapter contamination, and extremely short reads that may interfere with downstream analysis. Quality thresholds typically include minimum Q30 base quality scores and minimum read lengths of 50-75 base pairs.

Host DNA Subtraction: Eliminating human-derived sequences is a crucial part of mNGS analysis since human DNA usually makes up 90-99% of the sequences found in clinical samples. This involves aligning the sequences to human reference genomes and then subtracting the reads that match. It is important to exercise caution in this process to ensure that pathogen sequences, which might resemble host sequences, are not mistakenly removed, especially in the case of organisms with integrated or episomal DNA.

Taxonomic Classification: The non-host sequences that remain are subject to taxonomic classification by aligning them with extensive pathogen databases. To enhance detection sensitivity, a combination of databases is used, such as NCBI RefSeq, GenBank, and other specialized pathogen databases. The selection of alignment parameters and similarity thresholds plays a crucial role in determining the sensitivity and specificity of organism detection.

Clinical Interpretation: To convert bioinformatics findings into reports that are significant in a clinical setting requires advanced algorithms that take into account various factors, such as the abundance of organisms, the clinical context, and established patterns of contamination. Creating standardized criteria for interpretation continues to be a field of ongoing research and development.

4. Clinical Applications Across Infectious Disease Specialties

4.1 Central Nervous System Infections

Infections of the central nervous system (CNS) stand out as a particularly important area for the application of clinical metagenomic next-generation sequencing (mNGS). This is due to the urgent need for quick diagnosis and the common shortcomings of standard methods. Research consistently reveals that conventional techniques fail to diagnose 40-60% of suspected CNS infections, forcing healthcare providers to depend on empirical antimicrobial treatment.

The first landmark clinical application of mNGS was reported in 2014 when Wilson and colleagues used metagenomic sequencing to diagnose neuroleptospirosis in a 14-year-old patient who had undergone extensive conventional testing without success. This case highlighted the transformative potential of unbiased sequencing for detecting unexpected pathogens in critical clinical situations.

Later, more extensive studies have offered detailed insights into the clinical usefulness of mNGS in diagnosing CNS infections. Miller and his team carried out a thorough validation study involving 95 CSF specimens, revealing a 73% sensitivity and 99% specificity when measured against composite reference standards. Remarkably, sensitivity rose to 89% when samples with significant host DNA interference were removed, emphasizing the crucial role of sample quality in the effectiveness of mNGS.

Table 2. Clinical Performance of mNGS for CNS Infections by Pathogen Type

Pathogen Category	Number of Studies	Sensitivity Range	Specificity Range	Key Advantages	Limitations
Bacterial Meningitis	8	65-90%	95-99%	Detects fastidious organisms	Lower sensitivity than culture for common pathogens
Viral Encephalitis	12	70-95%	98-100%	Broad viral detection, including novel viruses	May miss low-viral load cases
Fungal CNS Infections	6	76-100%	95-98%	Superior for Cryptococcus and Aspergillus	Variable performance for endemic fungi
Tuberculous Meningitis	10	27-85%	98-100%	Faster than culture, detects drug resistance	Sensitivity varies with bacterial load
Multiple Pathogens	4	85-95%	92-98%	Simultaneous detection capability	Complex interpretation

The effectiveness of mNGS differs considerably depending on the type of pathogen and the clinical context. One of its most effective uses is in cases of viral encephalitis, where mNGS shows higher detection rates than targeted PCR panels, especially for uncommon or new viruses. This technology has proven to be instrumental in identifying new pathogens, including viruses that were not previously recognized and unusual manifestations of known organisms.

Cryptococcal meningitis has become an especially effective application for mNGS, as numerous studies have shown sensitivity rates of over 90%. This impressive effectiveness is probably due to the relatively high number of organisms in cryptococcal infections and the durable nature of fungal DNA found in clinical samples.

Tuberculous meningitis continues to present difficulties for mNGS applications, with studies indicating a sensitivity range of 27% to 85%. This variability in performance is probably due to factors such as the generally low bacterial load in cases of tuberculous meningitis, the complex cell wall structure of mycobacteria that may withstand typical extraction procedures, and the significant presence of host DNA in numerous CSF samples.

4.2 Respiratory Tract Infections

Respiratory tract infections are the leading reason for mNGS testing in numerous clinical laboratories. This is due to the intricate nature of respiratory microbiomes and the frequent shortcomings of traditional diagnostics in identifying the pathogens involved. The difficulty in differentiating between colonization and infection in respiratory samples further complicates result interpretation, necessitating meticulous clinical correlation.

Lower Respiratory Tract Infections: Research on the effectiveness of mNGS for diagnosing lower respiratory tract infections has repeatedly shown that it offers better detection rates than traditional techniques. Huang and his team found that mNGS had a positive detection rate of 89%, significantly outpacing the 25.7% rate achieved by conventional methods in cases of peripheral pulmonary infections. Likewise, Chen and his colleagues discovered that mNGS had a detection rate of 65%, compared to a mere 20% for culture methods in patients with lower respiratory tract infections.

The exceptional efficacy of mNGS in diagnosing respiratory infections seems to be influenced by a number of elements. The impartial approach of mNGS permits the identification of elusive organisms that are challenging or unfeasible to culture, such as Pneumocystis jirovecii, Legionella species, and atypical bacteria. Furthermore, mNGS has the capability to identify multiple co-infecting organisms at the same time, a situation often seen in patients with compromised immune systems and those with intricate underlying health issues.

Mycobacterial Detection: The effectiveness of mNGS in identifying Mycobacterium tuberculosis in respiratory specimens has yielded varied outcomes in various studies. Certain findings indicate that its sensitivity surpasses that of traditional techniques, whereas others reveal similar or lesser performance. This discrepancy is probably due to variations in sample processing methods, especially the crucial role of sufficient mechanical lysis to break down the mycobacterial cell wall.

Liu and his team proposed that integrating mNGS with traditional detection techniques might enhance the overall diagnostic effectiveness for tuberculosis. This blended strategy takes advantage of the prompt results and drug resistance data offered by mNGS, while preserving the quantitative details supplied by culture methods.

Fungal Respiratory Infections: mNGS has demonstrated significant potential for identifying invasive fungal infections,

which are infamously challenging to diagnose with traditional techniques. Research by Yang and his team revealed a notably higher sensitivity for pulmonary fungal infections using mNGS (80%) as opposed to standard tests (44.4%). This technology seems especially proficient at detecting Aspergillus species and other molds that may not consistently grow in culture.

Viral Respiratory Infections: The effectiveness of mNGS in detecting respiratory viruses has been inconsistent, with various studies indicating that its sensitivity is often inferior to that of targeted PCR techniques. This diminished performance is probably due to the lower analytical sensitivity of mNGS in contrast to optimized PCR tests, as well as the difficulties in identifying RNA viruses within complex clinical samples. Nonetheless, mNGS provides considerable benefits in identifying new or emerging respiratory viruses that might not be covered by standard PCR panels.

4.3 Bloodstream Infections and Sepsis

Rapid diagnostic methods are crucial for addressing bloodstream infections, as postponing the correct antimicrobial treatment is linked to higher mortality rates and extended hospital stays. Although conventional blood culture techniques are still considered the gold standard for diagnosing sepsis, they have notable drawbacks such as low sensitivity (5-50%) and lengthy turnaround times.

The use of mNGS for bloodstream infections has mainly centered on examining cell-free DNA (cfDNA) obtained from plasma samples. This method provides several theoretical benefits, such as non-invasive sampling, increased sensitivity because of lower host cell interference, and the capacity to identify pathogens that might not be alive in blood cultures.

Clinical Performance Data: Blauwkamp and his team conducted one of the most extensive validation studies on mNGS for detecting bloodstream infections, involving 350 sepsis alert patients. Their research revealed a 93.7% concordance between mNGS and blood culture findings, with over 85% of mNGS results available within 24 hours, as opposed to the 3-5 day timeframe for blood culture results. Notably, 53.7% of the mNGS reports detected one or more microorganisms, indicating a possible enhancement in diagnostic yield over traditional culture methods alone.

Jing and their team carried out a detailed comparison between mNGS and traditional techniques for diagnosing bloodstream infections. They found that mNGS had a sensitivity of 87.1% and a specificity of 80.2%, based on a composite reference standard. The reduced specificity is probably due to identifying organisms that might not grow in culture because they are either not abundant enough or possibly indicative of actual, though temporary, bacteremia.

Challenges and Limitations: Various elements make it challenging to interpret mNGS results from blood samples accurately. The presence of bacterial DNA in healthy people has been well established, leading to uncertainties about the clinical importance of low-level positive findings. Furthermore, pathogen DNA can remain in the plasma even after effective antimicrobial treatment, resulting in extended positive results that may not indicate an active infection.

The cost-effectiveness of mNGS for detecting bloodstream infections is still being actively explored. Although the expense per test is notably greater than that of traditional blood cultures, the potential advantages—such as shorter hospital stays, enhanced antimicrobial management, and improved patient outcomes—may justify the costs in carefully chosen groups of patients.

4.4 Other Clinical Applications

Ocular Infections: Infectious keratitis and endophthalmitis represent challenging diagnostic scenarios where conventional methods frequently fail to identify causative organisms. The limited sample volumes available from ocular specimens and the presence of fastidious organisms make these conditions particularly suitable for mNGS approaches.

Parekh and collaborators illustrated the usefulness of shotgun sequencing in diagnosing corneal infections, whereas Lalitha and her team assessed the application of metagenomic deep sequencing for infectious keratitis. Despite their limited scale, these studies indicate potential advantages in identifying rare or difficult-to-culture organisms that might be missed by standard techniques.

Prosthetic Joint Infections: The diagnosis of prosthetic joint infections remains challenging due to the frequent presence of biofilm-forming organisms and the need to distinguish infection from aseptic loosening. mNGS has shown promise for detecting organisms in sonication fluid from removed prostheses, potentially offering improved diagnostic yield compared to conventional culture methods.

Immunocompromised Patients: Patients with primary or acquired immunodeficiency present unique diagnostic challenges due to their susceptibility to opportunistic infections and atypical presentations of common pathogens. mNGS has shown particular utility in these populations, with several studies demonstrating improved diagnostic yield compared to conventional methods.

5. Technical Challenges and Limitations

5.1 Sample Processing and Host Background Issues

One of the most significant technical challenges facing clinical mNGS is the overwhelming presence of host nucleic acids in clinical samples. In typical infectious disease scenarios, pathogen-derived DNA or RNA represents less than 1% of the total nucleic acid content, with the remainder consisting of human-derived material. This extreme imbalance creates multiple downstream challenges including reduced sequencing efficiency, increased computational requirements, and decreased sensitivity for pathogen detection.

The problem is particularly acute in certain sample types. Cerebrospinal fluid samples from patients with viral encephalitis may contain 97-99% human sequences, making pathogen detection extremely challenging. Similarly, tissue biopsies and normally sterile body fluids often contain high proportions of host DNA that can mask pathogen signals.

Host Depletion Strategies: Several approaches have been developed to address host DNA contamination, each with specific advantages and limitations. Differential lysis methods attempt to selectively lyse host cells while preserving pathogen cells, but this approach may also remove certain pathogens, particularly viruses that reside within host cells. Chemical depletion using compounds such as saponin has shown promise for reducing host DNA background, but optimization is required for different sample types and pathogen categories.

Enzymatic approaches using DNase treatment can selectively degrade free host DNA while preserving encapsulated pathogen nucleic acids. However, this method is primarily effective for detecting intact pathogens and may miss cell-free pathogen DNA that could provide diagnostic information. Probe-based capture methods can selectively enrich pathogen sequences, but require prior knowledge of potential targets and may miss novel or unexpected organisms.

Computational Solutions: Bioinformatics approaches to host depletion have become increasingly sophisticated, utilizing multiple reference genomes and advanced alignment algorithms to identify and remove host sequences. However, the computational burden of processing millions of sequences against large reference databases can be substantial, requiring specialized infrastructure and expertise.

Recent developments in cloud-based computing and optimized alignment algorithms have helped address some computational challenges, but the fundamental issue of host background remains a significant limitation for mNGS sensitivity, particularly in low-biomass samples.

5.2 Standardization and Quality Assurance

The complexity of mNGS workflows and the lack of standardized protocols represent significant barriers to widespread clinical adoption. Unlike conventional microbiology methods that have decades of standardization and quality assurance development, clinical mNGS lacks universally accepted standards for sample processing, bioinformatics analysis, and result interpretation.

Laboratory Standardization Challenges: Each step of the mNGS workflow, from sample collection to final reporting, requires careful optimization and validation. Differences in extraction methods, library preparation protocols, sequencing platforms, and bioinformatics pipelines can all influence final results, making comparison between laboratories and studies challenging.

The absence of certified reference materials specifically designed for mNGS validation compounds these challenges. While some commercial standards are available, they may not adequately represent the complexity and diversity of clinical samples encountered in routine practice. The development of comprehensive quality control materials that include appropriate organism mixtures, concentration ranges, and sample matrices remains an active area of development.

Proficiency Testing: External quality assessment programs for clinical mNGS are still in early stages of development. The complexity of generating appropriate proficiency testing samples that reflect real-world clinical scenarios while maintaining stability during shipping and storage presents unique challenges not encountered with conventional microbiology testing.

Several professional organizations including the American Society for Microbiology and European Society of Clinical Microbiology and Infectious Diseases have begun developing guidelines for clinical mNGS implementation, but comprehensive standards remain under development.

5.3 Bioinformatics Challenges and Data Interpretation

The bioinformatics analysis of mNGS data represents one of the most complex aspects of clinical implementation, requiring sophisticated algorithms and extensive computational resources. The volume of data generated by modern sequencing platforms can overwhelm traditional laboratory information systems and require specialized storage and processing infrastructure.

Database Challenges: The accuracy of pathogen identification depends critically on the quality and comprehensiveness of reference databases used for sequence comparison. Public databases such as GenBank contain sequences of variable quality and annotation accuracy, while specialized pathogen databases may lack comprehensive coverage of organism diversity.

The rapid evolution of pathogen genomes, particularly RNA viruses, means that reference databases require continuous updating to maintain accuracy. Additionally, the presence of closely related organisms in databases can lead to ambiguous taxonomic assignments that complicate clinical interpretation.

Quantification and Clinical Significance: Unlike culture-based methods that provide inherent quantification through colony counting, mNGS results are typically reported as sequence read counts or relative abundance measures. The translation of these metrics into clinically meaningful information remains challenging, particularly for distinguishing colonization from infection.

The development of standardized metrics such as stringently mapped read numbers (SMRN) has helped provide more consistent quantification approaches, but optimal thresholds for clinical decision-making remain uncertain and likely vary by sample type and pathogen category.

Table 3. Major Technical Challenges in Clinical mNGS Implementation

Challenge Category	Specific Issues	Current Solutions	Limitations of Solutions
Host Background	90-99% human DNA in samples	Depletion methods, computational removal	May remove pathogen sequences, increased complexity
Standardization	Variable protocols across labs	Emerging guidelines, validation studies	Limited consensus, ongoing development
Bioinformatics	Complex data analysis pipelines	Commercial software, cloud platforms	Requires specialized expertise, high costs
Quality Control	Lack of reference materials	Internal controls, spike-in standards	Limited availability, validation challenges
Data Interpretation	Converting reads to clinical decisions	Standardized metrics, expert review	Threshold uncertainty, clinical correlation needed
Cost	High per-sample costs	Batch processing, platform optimization	Still expensive compared to conventional methods

5.4 Result Interpretation and Clinical Correlation

The interpretation of mNGS results requires sophisticated clinical judgment that goes well beyond simple positive/negative determinations typical of conventional diagnostic tests. The unbiased nature of mNGS means that results may include organisms representing true pathogens, colonizing flora, laboratory contaminants, or environmental organisms with unclear clinical significance.

Pathogen Versus Commensal Distinction: The challenge of distinguishing clinically significant organisms from background flora is particularly complex in mNGS due to the technology's ability to detect organisms at very low levels. Organisms that might be dismissed as contaminants in culture-based methods may actually represent true but low-level infections detectable only through mNGS.

The clinical context becomes critical for result interpretation, requiring close collaboration between laboratory personnel, infectious disease specialists, and treating physicians. Factors including patient immune status, clinical presentation, anatomic site of infection, and concurrent antimicrobial therapy all influence the significance of detected organisms.

Novel and Unexpected Findings: One of the strengths of mNGS is its ability to detect novel or unexpected pathogens that might be missed by conventional methods. However, this capability also creates challenges for result interpretation when organisms are detected that have limited or no previous clinical documentation.

The detection of novel organisms requires careful validation including confirmation through alternative methods when possible, literature review to assess potential pathogenicity, and consideration of epidemiological factors that might support or refute clinical significance.

6. Regulatory and Quality Management Considerations

6.1 Regulatory Landscape for Clinical mNGS

The regulatory route for clinical mNGS differs greatly across various regions and continues to progress as the technology advances. Unlike traditional diagnostic tests that usually focus on specific analytes with clearly defined performance traits, mNGS poses a more intricate regulatory issue because of its impartial nature and its capability to identify thousands of different organisms.

In the United States, the Food and Drug Administration (FDA) regulates clinical mNGS via several avenues. Certain mNGS assays have been granted FDA clearance as particular diagnostic tools, while others are provided as laboratory-developed tests (LDTs) under the provisions of the Clinical Laboratory Improvement Amendments (CLIA). The regulatory scene is continually changing as the FDA formulates frameworks for diagnostics based on next-generation sequencing.

International Regulatory Approaches: European regulatory bodies have adopted similar approaches to mNGS oversight, with emphasis on clinical validation and quality management systems. The In Vitro Diagnostic Medical Devices Regulation (IVDR) has established requirements for NGS-based diagnostics that include comprehensive analytical and clinical validation studies.

Other countries have developed their own regulatory frameworks, with varying requirements for validation, quality control, and clinical oversight. The harmonization of international standards remains an ongoing challenge that may impact global implementation of clinical mNGS technologies.

6.2 Quality Management Systems

The implementation of clinical mNGS requires comprehensive quality management systems that address the unique challenges associated with high-complexity molecular testing. Unlike conventional microbiology methods with decades of quality assurance development, mNGS requires novel approaches to quality control, proficiency testing, and result validation.

Pre-analytical Quality Control: Sample collection, transport, and processing represent critical control points for clinical mNGS. Quality management systems must address factors including sample stability, storage conditions, contamination

prevention, and processing timelines. The development of standard operating procedures that account for the unique requirements of mNGS is essential for consistent performance.

Analytical Quality Control: The analytical phase of mNGS includes multiple steps that require independent quality control measures. Nucleic acid extraction efficiency, library preparation quality, sequencing performance, and bioinformatics pipeline functionality all require monitoring and validation. The development of appropriate positive and negative controls that adequately represent the complexity of clinical samples remains challenging.

Post-analytical Quality Control: Result interpretation and reporting require quality oversight that ensures appropriate clinical correlation and meaningful communication of findings. The complexity of mNGS results necessitates review by personnel with specialized expertise in both microbiology and bioinformatics.

6.3 Laboratory Accreditation and Competency

Clinical laboratories offering mNGS testing must demonstrate competency through appropriate accreditation processes and personnel qualifications. The multidisciplinary nature of mNGS requires expertise spanning microbiology, molecular biology, bioinformatics, and clinical interpretation.

Personnel Requirements: The successful implementation of clinical mNGS requires personnel with diverse skill sets that may not be available in traditional microbiology laboratories. Bioinformatics expertise is particularly critical, as the analysis of mNGS data requires sophisticated computational skills and understanding of genomic databases.

Training programs for mNGS implementation have been developed by professional organizations and commercial vendors, but comprehensive educational resources remain limited. The development of standardized competency assessments and certification programs represents an important need for the field.

Laboratory Infrastructure: Clinical mNGS implementation requires specialized infrastructure including high-performance computing systems, data storage capabilities, and network security measures appropriate for handling patient genomic data. The costs and complexity of this infrastructure may limit implementation to larger clinical laboratories or regional reference centers.

7. Economic Considerations and Cost-Effectiveness

7.1 Direct Costs of mNGS Implementation

The economic implications of clinical mNGS implementation extend beyond simple per-test costs to encompass infrastructure investment, personnel training, quality assurance programs, and ongoing operational expenses. Understanding these comprehensive costs is essential for healthcare institutions considering mNGS adoption.

Capital Equipment Costs: Modern sequencing platforms require substantial initial investments ranging from $100,000 for benchtop systems to over $1 million for high-throughput platforms. Additional infrastructure costs include computing hardware, data storage systems, and laboratory modifications required for NGS operations. The total capital investment for mNGS implementation typically ranges from $500,000 to $2 million depending on platform selection and laboratory size.

Operational Costs: Per-sample costs for mNGS vary significantly depending on platform selection, sample throughput, and specific protocols used. Current estimates range from $100 to $500 per sample, with costs generally decreasing as sample volumes increase. These costs include reagents, consumables, labor, and allocated infrastructure expenses.

When compared to conventional diagnostic approaches, mNGS costs appear high on a per-test basis. However, direct cost comparisons may not capture the full economic value proposition, particularly when considering the comprehensive nature of mNGS testing and potential cost savings from reduced need for multiple targeted tests.

Table 4. Economic Analysis of mNGS Implementation

Cost Category	Initial Investment	Annual Operating Cost	Cost per Sample
Platform (Mid-range)	$300,000	$50,000	$25-50
Infrastructure	$200,000	$75,000	$15-30
Personnel	$150,000	$200,000	$40-80
Reagents/Consumables	-	$100,000	$50-150
Quality Control	$25,000	$25,000	$5-15
Total	**$675,000**	**$450,000**	**$135-325**

7.2 Cost-Effectiveness Analysis

Several studies have attempted to evaluate the cost-effectiveness of mNGS compared to conventional diagnostic approaches, with mixed results depending on the clinical application and analytical framework used. The comprehensive nature of mNGS testing complicates traditional cost-effectiveness analysis, as the technology may replace multiple conventional tests while providing additional diagnostic information.

Hospital Length of Stay Impact: Several studies have suggested that mNGS may reduce hospital length of stay through faster diagnosis and more targeted antimicrobial therapy. Bajaj and colleagues reported cost-effectiveness for mNGS in preventing hospitalizations in cirrhosis patients through improved management of infectious complications. However, these studies have generally been small-scale and may not be representative of broader clinical populations.

Antimicrobial Stewardship Benefits: The ability of mNGS to provide rapid identification of pathogens and antimicrobial resistance markers may support improved antimicrobial stewardship programs. Reduced use of broad-spectrum antibiotics and shortened treatment courses could generate significant cost savings while improving patient outcomes. However, quantifying these benefits requires longer-term studies that account for complex clinical factors.

Value-Based Care Considerations: In healthcare systems moving toward value-based payment models, the comprehensive diagnostic information provided by mNGS may align well with quality metrics and outcome-based reimbursement. The technology's potential to improve diagnostic accuracy and reduce unnecessary treatments could generate value under these payment structures.

7.3 Reimbursement and Market Access

The reimbursement landscape for clinical mNGS remains complex and varies significantly across different healthcare systems and geographic regions. In the United States, reimbursement policies are still evolving, with different approaches taken by Medicare, Medicaid, and private insurance providers.

Current Reimbursement Status: Some mNGS applications have achieved favorable reimbursement status, particularly for infectious disease applications in critical care settings. However, coverage policies often include specific requirements for clinical indication, sample type, and laboratory qualifications that may limit broader adoption.

The development of appropriate Current Procedural Terminology (CPT) codes for mNGS has been an ongoing process, with new codes introduced to better reflect the complexity and clinical value of these assays. However, reimbursement rates may not fully cover the costs of testing, particularly during the early phases of implementation.

Global Market Considerations: International markets for clinical mNGS vary significantly in terms of healthcare infrastructure, regulatory requirements, and economic resources. Developed markets with sophisticated healthcare systems may be early adopters, while resource-limited settings may require different implementation strategies focused on cost reduction and simplified workflows.

The potential for mNGS to address diagnostic gaps in resource-limited settings has generated interest in innovative funding mechanisms including public-private partnerships and international development programs. However, the technical complexity and infrastructure requirements of current mNGS workflows may limit implementation in these settings without significant technological simplification.

8. Future Directions and Emerging Technologies

8.1 Technological Advances in Sequencing Platforms

The rapid evolution of DNA sequencing technologies continues to drive improvements in clinical mNGS capabilities, with new platforms and methodologies emerging regularly. These advances focus on addressing current limitations including cost, turnaround time, ease of use, and analytical performance.

Ultra-Rapid Sequencing Protocols: Recent developments have focused on dramatically reducing the time-to-result for clinical mNGS. Oxford Nanopore Technologies has demonstrated pathogen identification in as little as 6 hours using optimized protocols, while Illumina has developed rapid sequencing chemistries that can provide results within 24 hours. These improvements bring mNGS turnaround times closer to those required for urgent clinical decision-making.

Portable Sequencing Systems: The development of portable sequencing platforms has significant implications for point-of-care diagnostics and resource-limited settings. The Oxford Nanopore MinION device has demonstrated feasibility for field deployment, with applications in outbreak investigation and remote diagnostic settings. Further miniaturization and automation may enable broader deployment of sequencing capabilities outside traditional laboratory settings.

Single-Cell Sequencing Integration: Emerging single-cell sequencing technologies offer the potential to analyze host-pathogen interactions at unprecedented resolution. These approaches could provide insights into infection mechanisms, immune responses, and treatment resistance that may inform therapeutic decision-making.

8.2 Artificial Intelligence and Machine Learning Integration

The integration of artificial intelligence (AI) and machine learning (ML) approaches with clinical mNGS represents a promising avenue for addressing current limitations and expanding diagnostic capabilities. These technologies can potentially improve multiple aspects of the mNGS workflow from sample processing optimization to result interpretation.

Automated Bioinformatics Analysis: Machine learning algorithms can potentially automate many aspects of mNGS data analysis, reducing the need for specialized bioinformatics expertise and improving consistency of results. Automated quality control, contamination detection, and result interpretation could make mNGS more accessible to smaller laboratories and resource-limited settings.

Predictive Modeling: AI approaches could enable predictive modeling of antimicrobial resistance, treatment response, and clinical outcomes based on genomic data. These capabilities could transform mNGS from a purely diagnostic tool to a predictive platform that guides therapeutic decision-making and patient management.

Real-Time Analysis: The integration of AI with real-time sequencing platforms could enable immediate analysis and interpretation of sequencing data as it is generated. This capability could dramatically reduce turnaround times and enable dynamic clinical decision-making based on evolving genomic information.

8.3 Integration with Clinical Decision Support Systems

The successful clinical implementation of mNGS will likely require integration with sophisticated clinical decision support systems that can contextualize genomic information within broader clinical frameworks. These systems could help clinicians interpret complex mNGS results and translate them into actionable clinical decisions.

Electronic Health Record Integration: Seamless integration of mNGS results with electronic health record systems could improve clinical workflow and enable better correlation of genomic findings with clinical data. Advanced integration could include automated alerts for concerning findings, treatment recommendations based on resistance profiles, and longitudinal tracking of pathogen evolution.

Clinical Guidelines Integration: The development of evidence-based clinical guidelines for mNGS utilization could improve appropriate test ordering and result interpretation. Integration of these guidelines into clinical decision support systems could help standardize clinical practice and improve outcomes.

8.4 Expanded Clinical Applications

Future developments in clinical mNGS are likely to expand beyond infectious disease diagnostics to encompass broader applications in precision medicine and population health.

Microbiome Analysis: The extension of mNGS to comprehensive microbiome analysis could provide insights into the relationship between microbial communities and human health. These applications could inform treatment decisions in conditions ranging from inflammatory bowel disease to cancer immunotherapy response.

Pharmacogenomics Integration: The combination of mNGS with pharmacogenomic analysis could enable personalized antimicrobial therapy selection based on both pathogen characteristics and host genetic factors affecting drug metabolism and response.

Surveillance and Outbreak Investigation: Enhanced mNGS capabilities could transform infectious disease surveillance and outbreak investigation through real-time pathogen monitoring, transmission tracking, and emergence detection. These applications could significantly improve public health responses to infectious disease threats.

9. Global Health Impact and Implementation Strategies

9.1 Applications in Resource-Limited Settings

The potential impact of clinical mNGS on global health is significant, particularly in resource-limited settings where diagnostic capabilities are often inadequate and infectious disease burdens are high. However, the successful implementation of mNGS in these settings requires addressing unique challenges related to infrastructure, expertise, and cost.

Infrastructure Challenges: Many resource-limited settings lack the reliable electricity, internet connectivity, and climate control systems required for sophisticated sequencing platforms. The development of portable, battery-powered sequencing systems with reduced infrastructure requirements could enable deployment in remote locations and field settings.

Cost Considerations: While mNGS costs have decreased significantly, they remain prohibitive for many resource-limited settings. Alternative funding mechanisms including international development programs, public-private partnerships, and tiered pricing strategies may be necessary to enable global access to mNGS technologies.

Capacity Building: The successful implementation of mNGS requires substantial technical expertise in molecular biology, bioinformatics, and clinical interpretation. Training programs and knowledge transfer initiatives will be critical for building local capacity and ensuring sustainable implementation.

9.2 Telemedicine and Remote Diagnostics

The integration of mNGS with telemedicine platforms could enable remote diagnostic services that extend sophisticated testing capabilities to underserved populations. Cloud-based analysis platforms combined with portable sequencing systems could enable sample collection and processing in remote locations with expert interpretation provided remotely.

Quality Assurance for Remote Testing: Ensuring appropriate quality control and result validation for remote mNGS testing presents unique challenges. The development of standardized protocols, remote monitoring capabilities, and quality assurance programs specifically designed for distributed testing networks will be essential.

Data Security and Privacy: Remote and telemedicine applications of mNGS must address data security and patient privacy concerns, particularly when genomic information crosses international borders. Appropriate encryption, data governance policies, and regulatory compliance measures are essential for protecting patient information.

9.3 Pandemic Preparedness and Response

The COVID-19 pandemic demonstrated both the potential and limitations of genomic technologies for infectious disease response. Future pandemic preparedness strategies will likely incorporate mNGS as a critical component of surveillance, outbreak investigation, and response coordination.

Early Detection Systems: Global surveillance networks incorporating mNGS could enable early detection of emerging pathogens and rapid characterization of their transmission potential and clinical significance. These systems could provide critical early warning capabilities for public health authorities.

Rapid Response Protocols: The development of standardized protocols for rapid deployment of mNGS capabilities during infectious disease emergencies could improve response times

and coordination. These protocols should address sample collection, transport, analysis, and result dissemination in emergency settings.

International Coordination: Effective pandemic response using mNGS requires international coordination and data sharing agreements that enable rapid sharing of genomic information while protecting patient privacy and national interests. The development of appropriate governance frameworks for global genomic surveillance represents an important priority.

10. Conclusions

Clinical metagenomics next-generation sequencing has emerged as a transformative technology that addresses many longstanding limitations of conventional infectious disease diagnostics. The ability to detect all pathogens present in clinical samples without prior knowledge or cultivation requirements represents a fundamental advance that has already demonstrated clinical value across diverse applications.

The evidence reviewed in this comprehensive analysis demonstrates that mNGS consistently provides superior diagnostic yield compared to conventional methods, particularly for difficult-to-culture organisms, rare pathogens, and complex infections involving multiple organisms. The technology has proven especially valuable in critical clinical scenarios such as central nervous system infections, where rapid and accurate diagnosis is essential for optimal patient outcomes.

However, the path from laboratory innovation to routine clinical implementation remains complex and challenging. Technical hurdles including host DNA background, bioinformatics complexity, and result interpretation require ongoing attention and development. The absence of standardized protocols and quality assurance frameworks represents a significant barrier to widespread adoption, while cost considerations continue to limit accessibility in many healthcare settings.

The evolution of sequencing technologies continues to address many current limitations, with improvements in speed, cost, and ease of use making mNGS increasingly practical for clinical applications. The integration of artificial intelligence and machine learning approaches holds particular promise for automating complex bioinformatics workflows and improving result interpretation. These advances, combined with growing clinical evidence and improving reimbursement policies, suggest that mNGS will become increasingly integrated into routine clinical practice.

The global health implications of clinical mNGS are profound, with the potential to address diagnostic gaps in resource-limited settings and improve infectious disease surveillance and outbreak response capabilities. However, realizing this potential will require sustained efforts to address infrastructure limitations, build local capacity, and develop sustainable funding mechanisms.

Looking toward the future, clinical mNGS is likely to expand beyond its current applications to encompass broader aspects of precision medicine including microbiome analysis, pharmacogenomics, and personalized therapeutic selection. The technology's ability to provide comprehensive genomic information from clinical samples positions it as a cornerstone of future precision medicine approaches.

The successful integration of mNGS into clinical practice will require continued collaboration between technologists, clinicians, regulators, and healthcare systems. The development of appropriate clinical guidelines, quality standards, and reimbursement policies will be essential for ensuring that the benefits of this powerful technology are realized while maintaining appropriate safeguards for patient safety and data security.

As we stand at the intersection of technological capability and clinical need, clinical metagenomics represents both a remarkable achievement and a promising foundation for future advances in infectious disease diagnostics and precision medicine. The journey from research curiosity to clinical standard of care is well underway, with the potential to fundamentally transform how we detect, understand, and treat infectious diseases in the decades ahead.

The evidence presented in this review supports the conclusion that mNGS has matured from an experimental research tool to a clinically valuable diagnostic modality that addresses critical gaps in conventional infectious disease diagnostics. While challenges remain, the trajectory of technological development, clinical validation, and practical implementation suggests that mNGS will play an increasingly important role in clinical microbiology and infectious disease management.

The transformation of infectious disease diagnostics through metagenomic approaches represents more than just technological advancement; it embodies a fundamental shift toward more comprehensive, unbiased, and informative diagnostic approaches that better serve both individual patients and public health objectives. As we continue to refine these technologies and address remaining challenges, the full potential of clinical metagenomics to improve human health remains an exciting prospect for the future of medicine.

Acknowledgments

The authors acknowledge the significant contributions of researchers and clinicians worldwide who have advanced the field of clinical metagenomics through their dedication to improving infectious disease diagnostics. We recognize the pioneering work of investigators who conducted early clinical validation studies and the ongoing efforts of those working to address the technical and practical challenges that remain.

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