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Metagenomic Next-Generation Sequencing in Clinical Microbiology: A Review of Diagnostic Utility, Comparative Performance, and Practical Implications Across Organ Systems

S Ruksakulpiwa.
Independent Scholar

Abstract: The swift and accurate identification of pathogens is essential for the effective management of infectious diseases. Traditional microbiological methods, though fundamental, often suffer from long turnaround times, limited sensitivity for fastidious or new organisms, and are impacted by prior antibiotic use. Metagenomic next-generation sequencing (mNGS) marks a significant shift by offering a culture-independent, hypothesis-free method for pathogen detection. This review compiles findings from recent studies, focusing on a 2024 retrospective analysis by Zhang et al., to critically assess mNGS's clinical use. The study shows that mNGS provides much higher sensitivity (78.05% vs. 24.39%) compared to culture, though it compromises on specificity (66.67% vs. 100.0%). Its greatest benefit is in diagnosing difficult infections, such as those from rare, new, or unculturable pathogens, and in directing treatment for immunocompromised patients. However, issues remain like distinguishing colonization from infection, interpreting background microbial signals (e.g., Epstein-Barr virus, Cytomegalovirus), and dealing with false negatives due to low pathogen presence. The review concludes that mNGS is an essential supplementary tool in clinical microbiology, especially for complex cases, although its findings should be carefully considered within the complete clinical scenario. Future efforts should aim at standardizing procedures, enhancing bioinformatic filtering, and performing large-scale prospective research to confirm its place in routine diagnostics.

Keywords: *metagenomic next-generation sequencing, mNGS, clinical diagnostics, infectious diseases, pathogen detection, antimicrobial stewardship, sensitivity and specificity.*

1. Introduction

Infectious diseases continue to rank among the top causes of illness and death worldwide, presenting an ongoing challenge to global public health systems [1]. The foundation of effective management lies in the swift and precise identification of the pathogen responsible, which allows for tailored antimicrobial treatment, enhances patient outcomes, and helps to combat the growing issue of antimicrobial resistance [2]. For many years, clinical microbiology laboratories have depended on a range of conventional techniques, such as culture, microscopy, serology, and pathogen-specific polymerase chain reaction (PCR) tests. Though these methods are extremely valuable, they come with inherent limitations. Culture, regarded as the traditional gold standard, is time-intensive (requiring between 24 to 72 hours or more), has a low success rate for difficult-to-grow or slow-growing organisms (e.g., Mycobacterium tuberculosis, fungi), and is adversely affected by prior antibiotic use [3]. Targeted PCR and serological tests offer faster results but necessitate prior suspicion of a specific pathogen, which makes them ineffective for identifying unexpected or new infectious agents [4].

The emergence of high-throughput sequencing technologies has marked the beginning of a new age in pathogen discovery and diagnostics. Metagenomic next-generation sequencing (mNGS) entails the extensive sequencing of all nucleic acids (DNA and/or RNA) in a clinical sample, which is then subjected to advanced computational analysis to pinpoint microbial sequences within the host material [5]. This non-culture-based, unbiased technique theoretically allows for the detection of any bacterium, virus, fungus, or parasite present in the sample, without requiring any prior assumptions.

An increasing amount of research has shown the effectiveness of mNGS in identifying infections in the central nervous system [6], respiratory system [7], and in cases of unexplained febrile illness [8]. However, as this technology moves from research settings to regular clinical use, essential questions about its overall diagnostic performance, cost-effectiveness, and best use across various organ systems and patient groups need to be answered. The recent retrospective observational study by Zhang et al. (2024) titled "Clinical application of metagenomic next-generation sequencing in patients with different organ system infection" offers a valuable real-world dataset to examine these issues [9].

1) *This review paper intends to thoroughly examine the present state of mNGS in clinical diagnostics. By focusing on the findings of Zhang et al. (2024) as a primary case study, we will critically assess how mNGS compares to traditional methods, highlighting its advantages and*

limitations across various pathogen types and sample matrices, its influence on clinical decision-making, and the practical challenges in interpreting results. Lastly, we will explore potential future developments for this transformative technology.

2. The mNGS Workflow: From Sample to Report

The clinical mNGS process involves a complex, multi-step workflow that can be summarized in three main stages: wet-lab processing, sequencing, and bioinformatic analysis (Figure 1).

Figure 1: Schematic of the Clinical mNGS Workflow.
(A simplistic flow chart would be included here showing: Sample Collection -> Nucleic Acid Extraction -> Library Preparation -> Sequencing -> Bioinformatic Analysis (Quality Control -> Human Read Filtering -> Alignment to Microbial Databases -> Report Generation) -> Clinical Interpretation.)

2.1. Wet-Lab Processing: The process begins with the extraction of total nucleic acid from a clinical sample (e.g., blood, BALF, CSF). This extract contains a overwhelming majority of host DNA/RNA. For RNA viruses, a reverse transcription step is incorporated. The extracted nucleic acids are then fragmented, and adapters are ligated to create a sequencing library. Notably, the library preparation protocol described by Zhang et al. is consistent with standard practices for illumina platforms [9, 10].

2.2. Sequencing: The prepared libraries are loaded onto a next-generation sequencer (e.g., Illumina, Oxford Nanopore), which generates millions to billions of short DNA sequences, or "reads."

2.3. Bioinformatic Analysis: This is the most critical and challenging phase. The raw sequencing data undergoes a rigorous computational pipeline:

- **Quality Control & Pre-processing:** Low-quality reads and sequencing adapters are removed.
- **Host Depletion:** Computational subtraction of human sequences is performed to enrich for microbial reads, significantly improving sensitivity [11].
- **Taxonomic Classification:** The remaining non-host reads are aligned against comprehensive reference databases containing genomic sequences from thousands of microbes.
- **Interpretation and Reporting:** The abundance of each microorganism is calculated. The final report lists detected microbes, often with semi-quantitative metrics like Reads Per Million (RPM). Crucially, as highlighted by Zhang et al., distinguishing clinically relevant pathogens from background noise, contamination, or colonization is a non-automated step requiring expert microbiological and clinical correlation [9].

3. Comparative Diagnostic Performance: mNGS vs. Conventional Methods

The study by Zhang et al. provides a clear, head-to-head comparison of mNGS and conventional methods (culture, smear, PCR, serology) in a cohort of 94 patients. Their findings are instrumental in quantifying the trade-offs inherent in this new technology.

3.1. Sensitivity and Specificity: The most striking result is the dramatic difference in sensitivity. Zhang et al. report a sensitivity of 78.05% for mNGS versus 24.39% for culture in confirmed infectious disease cases ($\chi^2 = 47.248$, $P < .001$). This ~53% increase in sensitivity is consistent with numerous other studies across various infection types [7, 12, 13]. This enhanced detection capability is primarily due to mNGS's ability to detect unculturable organisms, viruses, and pathogens affected by prior antibiotics.

Nevertheless, this heightened sensitivity comes with a trade-off in specificity. In their study, the specificity of mNGS stood at 66.67%, compared to 100% for cultures ($\chi^2 = 4.8$, $P = .028$). This decreased specificity highlights a critical issue: mNGS identifies microbial nucleic acid but cannot inherently differentiate between active infection, colonization, environmental contamination, or latent viral reactivation (such as EBV or CMV) [9, 14]. The significant number of "false positives" (22 patients in the ID group had pathogens detected by mNGS that were deemed not clinically significant) underscores that the result alone does not constitute a diagnosis but rather a piece of evidence that requires interpretation.

3.2. Predictive Values: The positive predictive value (PPV) of mNGS was high at 94.12%, meaning that if mNGS detected a pathogen, it was very likely to be a true infection. Conversely, the negative predictive value (NPV) was low (30.77%), indicating that a negative mNGS result does not reliably rule out infection [9]. This low NPV can be attributed to factors like low pathogen biomass, the presence of pathogens with tough cell walls (e.g., fungi) that are hard to lyse, or intracellular organisms.

Table 1: Comparative Diagnostic Metrics of mNGS vs. Culture (Adapted from Zhang et al., 2024)

Metric	mNGS	Conventional Culture	Statistical Significance
Sensitivity	78.05%	24.39%	$\chi^2 = 47.248$, $P < .001$
Specificity	66.67%	100.0%	$\chi^2 = 4.8$, $P = .028$
Positive Predictive Value (PPV)	94.12%	100%	N/A
Negative Predictive Value (NPV)	30.77%	16.22%	N/A
Concordance (Both Positive)	12.77% (12/94)		
Concordance (Both Negative)	23.4% (22/94)		
mNGS Positive / Culture Negative	59.57% (56/94)		
Culture Positive / mNGS Negative	4.26% (4/94)		

4. Performance Across Pathogen Types

The utility of mNGS is not uniform across all microbial kingdoms. Zhang et al.'s data allows for a breakdown of its performance by pathogen type.

4.1. Bacteria: mNGS showed a clear superior advantage in detecting bacterial pathogens ($\chi^2 = 32.614$, $P = .000$). It was particularly valuable for identifying fastidious bacteria like non-tuberculous mycobacteria (NTM), all 4 cases of which were diagnosed solely by mNGS in their study [9]. This aligns with studies on prosthetic joint infections and pneumonia, where mNGS identified causative bacteria missed by culture [15, 16].

4.2. Mycobacteria Tuberculosis (MTB): The performance for MTB was comparable between mNGS (8/14 detected) and conventional methods (7/14 detected) in the Zhang et al. study. This suggests mNGS is a useful, rapid adjunct for MTB diagnosis, especially given the slow growth culture, but may not yet surpass the sensitivity of advanced molecular tests like Xpert MTB/RIF in all scenarios [17].

4.3. Fungi: A critical finding was that mNGS showed **no statistically significant advantage** over conventional methods for fungal detection (42.1% vs. 15.79%, $\chi^2 = 3.199$, $P = .074$) [9]. This can be explained by the robust fungal cell wall that makes nucleic acid extraction difficult, and the low fungal burden in many infections. However, it excelled at identifying specific, rare fungi like *Pythium insidosum* and *Talaromyces marneffei*, which are difficult to culture [9, 18].

4.4. Viruses: Surprisingly, mNGS did not demonstrate a prominent advantage for virus detection in this study. This contrasts with other research highlighting its utility in diagnosing viral encephalitis and respiratory infections [6, 19]. The discrepancy may be due to sample type differences or the high background of latent herpesviruses (EBV, CMV), which comprised most of the viral reads and were often classified as "overinterpretation" in non-infectious contexts [9, 14].

2) *5. Impact on Clinical Management and Stewardship*

The true value of a diagnostic test is measured by its ability to improve patient outcomes. Zhang et al. provide concrete examples where mNGS directly guided successful therapeutic changes. In 6 patients, treatment was adjusted based on mNGS findings that identified rare pathogens (*Aspergillus terreus*, NTM, *Entamoeba histolytica*, *T. marneffei*, *P. insidiosum*) that were not covered by the initial empirical regimens [9]. This aligns with the core promise of mNGS: enabling precision medicine in infectious diseases by moving away from broad-spectrum empiric therapy towards targeted treatment, a key principle of antimicrobial stewardship [20].

Furthermore, the high negative predictive value in the non-infectious disease (NID) group (66.67% of NID patients had negative mNGS results) suggests a potential role for mNGS in **ruling out infection** and supporting alternative diagnoses (e.g., autoimmune, malignant), thus preventing unnecessary antibiotic courses [9, 21].

6. Challenges and Limitations

Despite its promise, the implementation of mNGS is fraught with challenges, many of which are evident in the Zhang et al. study.

6.1. Interpretation and "Overinterpretation": The foremost challenge is clinical interpretation. Distinguishing signal from noise is difficult. The detection of common environmental contaminants or latent viruses can lead to misdiagnosis and unnecessary treatment if not carefully correlated with clinical symptoms and other biomarkers. Zhang et al. list several cases of "overinterpretation," such as detecting Corynebacterium in CSF in a patient with meningismus or Anellovirus in ascites, which were likely not causative [9].

6.2. Standardization and Validation: There is a lack of standardized protocols for sample processing, sequencing depth, and bioinformatic pipelines. This makes it difficult to compare results across different laboratories and establish universal diagnostic thresholds [22].

6.3. Cost and Turnaround Time: While the cost of sequencing has plummeted, the total expense of mNGS (including library prep, sequencing, and bioinformatic expertise) remains higher than most conventional tests. Furthermore, the turnaround time, though faster than culture, is typically 24-48 hours, which is slower than rapid PCR panels [23].

6.4. Incomplete Pathogen Detection: mNGS is not infallible. False negatives occur due to low pathogen load, inefficient cell lysis (especially for fungi and parasites), the presence of host nucleases, or pathogens with extreme GC-content genomes [24]. Zhang et al. noted several cases where culture was positive but mNGS was negative, sometimes due to the analysis of an inconsistent specimen type (e.g., sputum culture positive for *K. pneumoniae* but BALF tested by mNGS was negative) [9].

3) *7. Future Directions*

To fully integrate mNGS into routine diagnostics, future efforts should focus on:

1. **Prospective Studies:** Large, multi-center prospective trials are needed to definitively establish its clinical utility and cost-effectiveness for specific syndromes.
2. **Standardization:** Developing and validating standardized protocols and quality control measures across laboratories.
3. **Improved Bioinformatics:** Enhancing algorithms to better filter out host and contaminant sequences and to predict antibiotic resistance genes from metagenomic data.
4. **Point-of-Care Sequencing:** Developing faster, simpler sequencing technologies that could provide results within a single working day.
5. **Integration with Host Response:** Combining mNGS data with host gene expression profiles (e.g., transcriptomics) to better differentiate infection from colonization and to assess the severity of the immune response [25].

Conclusion

The research conducted by Zhang et al. (2024) provides strong, real-world evidence of the revolutionary potential of metagenomic next-generation sequencing (mNGS) within clinical microbiology. This study clearly shows that mNGS significantly enhances diagnostic sensitivity over traditional culture-based methods, especially for identifying bacteria and rare pathogens, thereby directly impacting and refining treatment decisions in complex cases. However, it also underscores the complexity tied to this powerful technology. Due to its lower specificity and tendency to detect background microbes, careful, nuanced interpretation is essential. Rather than being seen as an independent "gold standard," mNGS should be utilized as a potent tool for generating hypotheses, to be integrated by clinicians and microbiologists with the broader context of clinical presentation, imaging results, and standard tests. As standardization progresses and costs decrease, mNGS is set to become a vital part of the diagnostic process for severe, mysterious, and hospital-acquired infections, ultimately ushering in a new era of precision in infectious disease medicine.

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