Clinical Utility of Metagenomic Next-Generation Sequencing for Viral Infections: Diagnostic Accuracy, Clinical Impact, and Antiviral Stewardship

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Abstract

Objectives: To synthesize current evidence on the clinical utility of metagenomic next-generation sequencing (mNGS) in viral diagnostics, emphasizing diagnostic yield, clinical impact, pathogen characterization, and antiviral resistance profiling.

Methods: We systematically searched different Data bases. Clinical studies applying mNGS for diagnosis were included. Seventy-five studies met eligibility criteria. Data were extracted on diagnostic accuracy, reference-standard type, clinical impact, antiviral resistance, sample type, and sequencing platform. Because of substantial heterogeneity in populations, specimens, workflows, and reference standards, diagnostic accuracy was synthesized narratively as reported study-level estimates rather than pooled estimates. Studies using composite clinical adjudication incorporating mNGS were not treated as equivalent to those using independent microbiologic reference standards.

Results: mNGS demonstrated incremental diagnostic value across CNS and respiratory infections and transplant cohorts, with sensitivity varying substantially across individual studies and reference-standard types. It identified unexpected pathogens, mixed infections, and etiologies missed by routine testing. Management changes attributable to mNGS were heterogeneous, encompassing antiviral initiation, antibiotic de-escalation, infection-control interventions, and avoidance of invasive procedures, with limited prospective outcome validation. Resistance mutations involving CMV, HBV, influenza, and SARS-CoV-2 were detected predominantly using targeted or hybrid-capture sequencing rather than routine untargeted shotgun mNGS. Nanopore workflows enabled rapid preliminary detection, whereas Illumina/BGI workflows generally provided deeper coverage.

Conclusions: mNGS can complement conventional diagnostics particularly in CNS disease, pneumonia, immunocompromised hosts, and diagnostically unresolved syndromes. Interpretation requires caution because of reference-standard limitations, potential incorporation bias, and clinically ambiguous low-level viral detection. Standardized workflows, interpretation frameworks, and cost-effectiveness evaluation are needed for broader implementation.

Keywords: Metagenomic Next-generation Sequencing; Viral Infection; Encephalitis; Pneumonia; Antiviral Resistance; Transplant Virology; Clinical Virology; Precision Infectious Diseases.

Introduction

Viral diagnostics have evolved substantially over the past decade, moving from culture-based and serological methods toward molecular platforms capable of rapid detection and genomic resolution. Metagenomic next-generation sequencing (mNGS) represents a comprehensive approach, allowing unbiased detection of microbial nucleic acids in a clinical sample and facilitating diagnosis in cases where targeted assays fail. Early landmark work demonstrated mNGS as a transformative tool for diagnosing severe CNS infections and complex respiratory disease, reporting substantial increases in pathogen identification over conventional testing.[1–6] However, reported diagnostic performance has varied widely across studies, in part due to differences in reference standards and study design.

The rationale for clinical mNGS is threefold:

(1) Unbiased pathogen detection enables identification of unexpected or novel viruses.[2,7,8]

(2) Comprehensive genomic characterization can reveal strain diversity and antiviral resistance-associated mutations.[9–12]

(3) Improved clinical decision-making can reduce unnecessary antimicrobials and guide targeted antiviral therapy,[13–15] although downstream clinical benefit is not uniformly assessed. The extent of clinical benefit depends on the clinical context, interpretation of sequencing results, and the availability of appropriate confirmatory testing.

CNS infections represent one of the strongest use cases, with studies showing that mNGS identifies viral etiologies in patients previously labelled “encephalitis of unknown origin” and provides actionable diagnoses in both immunocompetent and immunocompromised hosts.[1,6,16–18] Respiratory infections, including severe influenza-like illness, viral pneumonia, and mixed viral–bacterial states, have also benefited significantly from the application of mNGS, particularly in ICU settings where diagnostic clarity is essential.[5,10,17,19–22]

In immunocompromised hosts, including hematopoietic stem cell transplantation (HSCT) and solid organ transplantation (SOT) recipients, the diagnostic challenge is even greater. These patients often have atypical presentations, altered immune responses, and prolonged viral shedding. Multiple studies have shown that mNGS improves diagnostic yield, uncovers unexpected viral co-infections, and enables earlier therapeutic adjustment.[11,23–30]

As mNGS increasingly intersects with antiviral-resistance genotyping, its role has expanded beyond pathogen detection toward informing personalized antiviral selection. Importantly, most validated resistance data derive from targeted or hybrid-capture sequencing rather than untargeted shotgun mNGS, with variable depth and clinical validation across viruses.[31–36] Accordingly, evidence from targeted or hybrid-capture resistance assays should not be interpreted as equivalent to evidence generated by routine untargeted shotgun mNGS, and the clinical applicability of resistance detection depends on sequencing depth, viral load, assay design, and validation for the specific virus and resistance marker.

Furthermore, the emergence of portable long-read sequencing, particularly using Nanopore platforms, has enabled rapid and potentially field-deployable viral genomic analysis. This approach has been used for outbreak response for Ebola virus and SARS-CoV-2 and may be extended to other viral epidemics or clusters. However, evidence from targeted NGS assays, including assays developed for cytomegalovirus resistance genotyping, should be distinguished from evidence generated by untargeted clinical mNGS.[37–40]

Despite these advances, the literature remains fragmented. Existing reviews often focus on specific organ systems or study designs, and no comprehensive synthesis has evaluated mNGS across the full spectrum of clinical viral infections from 2015–2025.

This systematic review integrates evidence from 75 studies, addressing diagnostic accuracy, clinical impact, antiviral resistance detection, operational performance, sample-type considerations, and implementation challenges, with the goal of critically characterizing the evolving role and clinical applicability of mNGS in modern clinical virology.

Methods

Study Design and Reporting Framework

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines.[41] The review was not prospectively registered in PROSPERO or another systematic review registry. The review was initiated retrospectively, and registration was not undertaken before the literature search and study selection process commenced.

A structured review methodology was nevertheless established to guide the review process, including the research objectives, search strategy, eligibility criteria, screening procedures, data extraction, and quality appraisal. These methodological elements were applied consistently during the review process; however, because the protocol was not prospectively registered, an independent public record is not available to verify that all methodological decisions were specified before examination of the eligible evidence.

The protocol was designed to ensure transparency, reproducibility, and methodological rigor in evaluating the clinical utility of metagenomic next-generation sequencing (mNGS) for viral infections across diverse patient populations and clinical syndromes. A structured methodology was pre-specified, including search strategy, eligibility criteria, screening procedures, data extraction, and quality appraisal. The scope of the review included diagnostic accuracy, clinical impact, antiviral resistance detection, turnaround time, sample-type performance, and platform-specific characteristics.

No substantive changes were made to the eligibility criteria, primary outcomes, or core data-extraction domains in response to the findings of the included studies. Any clarifications made during screening or extraction were applied to ensure consistent interpretation of the predefined eligibility framework and were not made to selectively include or exclude studies based on their results.

No quantitative meta-analysis of diagnostic accuracy was performed because the included studies differed substantially in reference-standard definitions, patient populations, clinical syndromes, specimen types, sequencing workflows, and reporting metrics, with frequent use of composite clinical adjudication incorporating mNGS results themselves. These differences, together with potential incorporation bias, limited the validity and interpretability of a pooled accuracy estimate. Study-level accuracy values and clinical outcomes were therefore extracted and synthesized narratively.

Search Strategy

A comprehensive search of five major biomedical databases was conducted:

- PubMed/MEDLINE
- Embase
- Scopus
- Web of Science Core Collection
- Cochrane CENTRAL

The date range was 1 January 2015 to 31 January 2025, reflecting the period during which clinical mNGS transitioned from exploratory to broader clinical implementation.

Search terms combined MeSH and free-text keywords related to:

- “metagenomic sequencing”, “metagenomic next-generation sequencing”, “clinical metagenomics”
- “virus”, “viral”, “virome”, “viral infection”, “encephalitis”, “pneumonia”
- “diagnosis”, “clinical impact”, “antiviral resistance”, “transplant infection”

A representative PubMed search strategy was:

(“metagenomic sequencing” OR “metagenomic next-generation sequencing” OR “clinical metagenomics” OR mNGS)

AND

(virus OR viral OR virome OR encephalitis OR pneumonia OR meningitis OR fever of unknown origin OR transplant infection)

AND

(diagnosis OR detection OR sequencing OR resistance)

Reference lists of included studies and key prior reviews were manually screened to identify additional relevant publications[2,9,14,53]. Grey literature, preprints, and conference abstracts were not included.

A representative PubMed search strategy was as follows:

((((“metagenomic sequencing”[Title/Abstract]) OR (“metagenomic next-generation sequencing”[Title/Abstract]))

OR (“clinical metagenomics”[Title/Abstract]) OR (mNGS[Title/Abstract]))

AND

((virus[Title/Abstract]) OR (viral[Title/Abstract]) OR (virome[Title/Abstract])

OR (encephalitis[Title/Abstract]) OR (pneumonia[Title/Abstract])

OR (meningitis[Title/Abstract]) OR (“fever of unknown origin”[Title/Abstract])

OR (“transplant infection”[Title/Abstract]))

AND

((diagnosis[Title/Abstract]) OR (detection[Title/Abstract])

OR (sequencing[Title/Abstract]) OR (resistance[Title/Abstract])))

AND (english[Filter])

The electronic search was last conducted and updated on 2 February 2025. No further eligible studies were identified after this date.

Eligibility Criteria

Studies reporting diagnostic accuracy were included regardless of reference standard used (independent microbiologic testing or composite clinical adjudication).For synthesis purposes, reference standards were categorized as (i) independent microbiologic reference standards, such as PCR, culture, antigen testing, or serology performed independently of the mNGS result, or (ii) composite clinical adjudication in which mNGS findings could contribute to the final clinical classification. This distinction was prespecified as an important source of potential incorporation bias and was considered when interpreting diagnostic accuracy estimates.

Studies were screened using the following inclusion criteria:

1. Population: Human clinical studies involving patients with suspected or confirmed viral infections.

2. Intervention: Application of metagenomic next-generation sequencing (mNGS), including Illumina, BGI, Nanopore, or other metagenomic workflows, to clinical specimens (e.g., CSF, BAL, blood, tissue). Targeted or hybrid-capture sequencing was considered separately when it was used within an otherwise metagenomic clinical workflow or when relevant to antiviral resistance characterization; studies evaluating targeted NGS alone without a metagenomic component were excluded.

3. Outcomes: Reporting at least one of the following:

- Diagnostic accuracy metrics (e.g., sensitivity, specificity)
- Incremental diagnostic yield
- Clinical management changes
- Antiviral resistance detection
- Turnaround time or operational performance
- Pathogen discovery or virome characterization

4. Study Type:

Prospective or retrospective cohorts, case series ≥5 patients, multi-center studies, clinical performance evaluations, or validation studies.

5. Language:

English.

Exclusion criteria

- Non-human studies
- Case reports < 5 patients
- Studies without viral outcomes
- Reviews, commentaries, modelling papers
- Studies evaluating targeted NGS only (not metagenomic)
- Studies lacking extractable clinical or diagnostic data
- Technical papers without clinical correlation

These criteria were aligned with previous systematic evaluations in the field.[8,12,14,42]

Study Selection and PRISMA Flow [Figure 1]

A total of 5,943 records were identified across all databases.

After removal of duplicates, 4,131 unique records remained for title/abstract screening, performed independently by two reviewers.

- 3,722 records were excluded as irrelevant or failing inclusion criteria.
- 409 full-text articles were assessed for eligibility.
- 334 full-text studies were excluded for predefined reasons, as detailed in Figure 1.

A final total of 75 studies met the inclusion criteria and were included in the qualitative synthesis. The numbers of records identified, screened, assessed for eligibility, and included were reconciled with the study-selection records before preparation of the final PRISMA flow diagram.

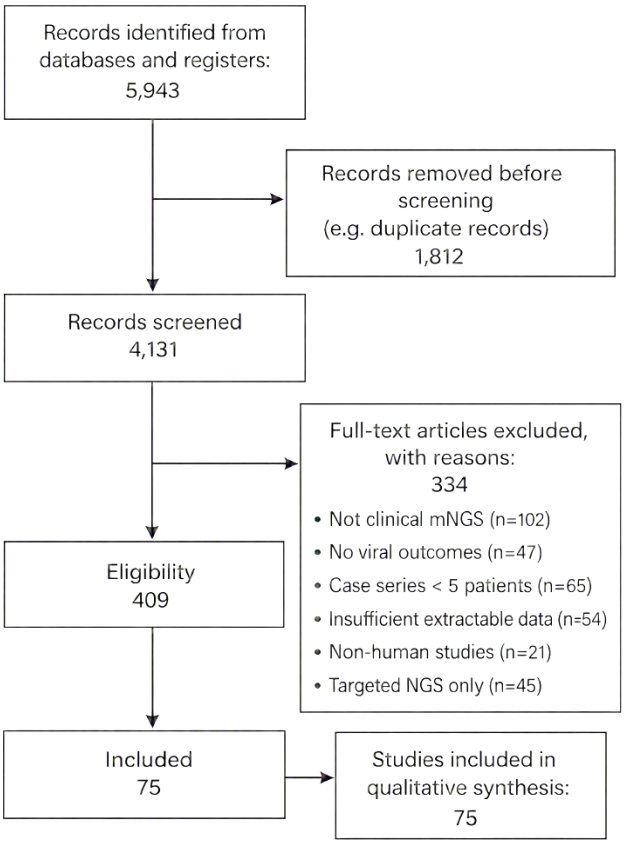

Figure 1: PRISMA flowchart summarizing identification, screening, eligibility assessment, and inclusion of 75 studies from 5,943 records.

Data Extraction

Reference standard type (independent microbiologic vs composite clinical adjudication) and method of clinical impact assessment (prospective clinician-driven vs retrospective chart review) were extracted.

For diagnostic accuracy outcomes, we additionally extracted whether estimates were reported at the patient level or specimen/sample level, the viral genome category where identifiable (RNA or DNA), the clinical syndrome, specimen type, and the numerator and denominator used to calculate reported accuracy measures. For clinical impact outcomes, the type of management change was extracted separately, including antiviral initiation or modification, antimicrobial de-escalation, infection-control measures, avoidance of invasive procedures, and changes in immunosuppression.

A standardized extraction template was used to capture the following variables from each study:

1. Study Characteristics

- Year, country, clinical setting (ICU, transplant center, neurology ward)
- Study design (prospective, retrospective, multicenter)

2. Patient Characteristics

- Age distribution
- Immunocompetent vs immunocompromised
- Underlying conditions (HSCT, SOT, HIV, oncology)

3. Sequencing Workflow

- Platform (Illumina, BGI, Nanopore)
- Sample type(s): CSF, BAL, NPS, plasma, whole blood, tissue
- Library preparation and host depletion methods
- Bioinformatics pipelines.[2,10,14,42]

4. Diagnostic Outcomes

- Viral pathogens detected
- Incremental diagnostic yield vs conventional testing
- Sensitivity/specificity when reported
- Negative predictive factors (e.g., low viral load, high host background)

5. Clinical Impact

- Initiation of antiviral therapy
- De-escalation or discontinuation of antibiotics
- Infection control actions
- Changes in immunosuppression regimens

6. Antiviral Resistance Detection

- Genes/mutations identified (UL97, UL54, HBV Pol, influenza NA/HA, SARS-CoV-2 spike)
- Concordance with genotype reference standards.[31–36]

7. Operational Metrics

- Turnaround time
- Read depth and coverage
- Cost elements (when provided)
- Contamination or false positives

Data extraction was performed independently by two reviewers, with disagreements resolved through discussion.

Quality Assessment

We used a modified version of the QUADAS-2 tool. A substantial proportion of studies were judged to have a high or unclear risk of bias, particularly because of incorporation bias and limitations in the reference standards used.

Diagnostic accuracy studies frequently varied in their choice of reference standard: culture, PCR panels, multiplex assays, serology, or clinical adjudication committees.[7,12,16]

Risk-of-bias assessments were considered when interpreting diagnostic accuracy estimates, and studies using composite reference standards incorporating mNGS results were not treated as equivalent to studies using independent microbiologic reference standards.

Because of methodological heterogeneity, we provided a narrative synthesis rather than pooled meta-analysis.

Additionally, we assessed:

- Transparency of sequencing workflows
- Clarity of reporting of contaminants/false positives
- Reporting of depth, coverage, and quality thresholds
- Relevance to clinical practice (i.e., how results influenced therapy)

Outcomes

Primary outcomes:

1. Diagnostic accuracy (sensitivity, specificity)
2. Incremental yield over conventional testing
3. Clinical management changes attributable to mNGS
4. Antiviral resistance detection
5. Turnaround time (TAT)
6. Sample-type performance differences
7. Platform-specific operational characteristics

Results

1. Study Characteristics

A total of 75 studies published between 2015 and 2025 were included.
Collectively, these studies represented:

- >7,500 patients across all age groups
- Clinical settings including ICUs, neurology wards, transplant centers, and general hospitals
- Sample types including CSF, BAL, plasma/whole blood, nasopharyngeal swabs, and tissue biopsies
- Platforms: Illumina (n≈45), BGI/MGISEQ (n≈20), and Nanopore (n≈10)
- Multiple geographic regions: North America, Europe, China, Japan, South America, Africa

Most were prospective or retrospective cohort studies (n≈63), with 12 multi-center evaluations or validation studies.[1,6,7,9,11,16,22,28-30,38,64]

Diagnostic accuracy studies varied substantially in their reference standards. Independent microbiologic references (PCR, culture, serology) were used in 41 studies, while 34 studies relied partially or wholly on composite clinical adjudication that incorporated mNGS results, introducing a risk of incorporation bias (Supplementary Table S1).

2. Diagnostic Accuracy of mNGS

Across the included studies, mNGS demonstrated diagnostic utility across several clinical syndromes, particularly CNS infections, severe respiratory infections, transplant-associated infections, fever of unknown origin (FUO), and systemic viral disease [Table 1]. Reported diagnostic performance varied substantially between individual studies and was influenced by reference-standard definition, patient population, specimen type, viral burden, host background nucleic acid, sequencing workflow, and sequencing depth.

Among the 75 included studies, 41 used independent microbiologic reference standards, whereas 34 used composite clinical adjudication. Studies using composite adjudication were considered separately because mNGS findings could contribute to the final clinical diagnosis, introducing potential incorporation bias. Consequently, estimates derived from composite clinical adjudication were not considered directly equivalent to estimates obtained using independent microbiologic reference standards (Supplementary Table S1).

Risk-of-bias assessments were considered when interpreting diagnostic accuracy estimates, and studies using composite reference standards incorporating mNGS results were not treated as equivalent to studies using independent microbiologic reference standards (Supplementary Table S2).

Table 1: Representative Clinical Studies Illustrating the Diagnostic Utility of mNGS for Viral Infections.

Study	**Clinical Setting**	**Sample Type**	**Pathogens Identified**	**Reference Standard**	**Key Findings**
Wilson *et al.*, 2019 [1]	CNS	CSF	HSV, VZV, EBV	Independent/comparative microbiologic testing	Actionable diagnoses in encephalitis
Langelier *et al.*, 2018 [6]	ICU pneumonia	BAL	RNA viruses	Study-specific comparator	Host-response + mNGS improves diagnostic assessment
Zinter *et al.*, 2019 [11]	Pediatric ICU	BAL/NP	Mixed viruses	Study-specific comparator	High diagnostic impact in immunocompromised patients
Shen *et al.*, 2023 [28]	HSCT	BAL/blood	CMV, adenovirus, HHV-6	Study-specific comparator	Improved transplant diagnostics
Mostafa *et al.*, 2023 [40]	CMV resistance	Blood	UL97/UL54	Genotypic/reference comparator	High diagnostic performance for resistance detection
Zhao *et al.*, 2024 [50]	HSCT	BAL	Multiple respiratory viruses	Study-specific comparator	mNGS resolves complex pulmonary viral infections

Reference-standard classification was performed at the study level. Independent microbiologic reference standards included conventional molecular assays, culture where applicable, serology, or other established microbiologic methods that were independent of the mNGS result. Composite clinical adjudication included clinical or multidisciplinary diagnostic assessment in which mNGS results could contribute to the final diagnosis. Studies using composite adjudication were not treated as equivalent to studies using independent microbiologic reference standards. Detailed study-level classification is provided in Supplementary Table S1.

2.1 CNS Infections (Meningitis/Encephalitis)

CNS infections represented one of the strongest clinical applications of mNGS. Across 19 CNS-focused studies, reported sensitivity varied substantially between individual studies, with estimates ranging from approximately 48% to 95%. These values represent reported study-level estimates rather than pooled diagnostic accuracy. The observed variation reflected differences in patient selection, viral burden, specimen characteristics, comparator testing, sequencing workflows, and reference-standard definitions.

Reference-standard type was an important determinant of interpretation. Studies using independent microbiologic reference standards generally provided more conservative estimates than studies using composite

clinical adjudication, in which mNGS findings could contribute to the final clinical diagnosis. The latter therefore carried a greater potential for incorporation bias (Supplementary Tables S1 and S2).

These sensitivity estimates should therefore be interpreted as study-level reported estimates rather than a pooled estimate of mNGS performance. In particular, studies using composite clinical adjudication frequently incorporated the mNGS result into the final diagnostic assessment, creating potential incorporation bias. Estimates from composite adjudication studies were therefore not considered directly equivalent to those obtained using independent microbiologic reference standards.

Specificity was generally high in studies using clearly defined reference standards, although direct comparison was limited by differences in reference-standard definitions, comparator testing, and study populations.

- mNGS provided additional diagnostic information compared with targeted testing when:
    - the pathogen was unexpected (e.g., astrovirus, enterovirus D68, Zika);
    - standard PCR panels lacked coverage; or
    - viral nucleic acid was present at low abundance and was detectable using deep sequencing.

The NEJM landmark study reported mNGS yielding actionable diagnoses in cases previously labelled "unknown encephalitis".[1]

2.2 Respiratory Viral Infections

Across 21 respiratory-focused studies, reported mNGS sensitivity ranged from approximately 30% to 85%.[5,6,10,17,18,20–22,37,43–46,57–59,65,72.] These values represent study-specific reported estimates rather than a pooled sensitivity. The wide variation reflected differences in clinical populations, respiratory specimen types, viral burden, comparator assays, sequencing workflows, and reference-standard definitions.

Because the available studies did not provide sufficiently comparable diagnostic accuracy data within a common reference-standard framework, a quantitative pooled estimate was not calculated. Diagnostic findings were therefore synthesized narratively, with particular attention to reference-standard type and clinical context.

- BAL samples frequently provided high diagnostic yield in severe lower-respiratory infections, although performance was influenced by viral burden, sampling timing, and host nucleic-acid background.
- mNGS often identified co-infections (viral–viral or viral–bacterial) that were not detected by targeted testing.

Lower sensitivity in some ICU cohorts may have reflected high host nucleic-acid background and low viral titres rather than failure of sequencing itself.

2.3 Transplant (HSCT/SOT) Cohorts

Across 14 transplant-focused studies, mNGS frequently demonstrated additional diagnostic value in patients with atypical presentations, multiple possible pathogens, or diagnostically unresolved pulmonary or systemic disease.[11,21–30,73]

The magnitude of incremental diagnostic yield varied substantially between studies and cannot be expressed reliably as a single pooled estimate because of differences in patient populations, specimen types, comparator testing, sequencing workflows, and reference-standard definitions.

- mNGS identified CMV, adenovirus, BKPyV, HHV-6, and mixed viral infections in selected transplant cohorts.
- However, several studies also reported detection of low-level viremia or viral reactivation without clear evidence of end-organ disease, emphasizing the need for careful clinical correlation.

- Studies highlighted the value of mNGS in patients with atypical symptoms, multi-system involvement, or diagnostically unresolved infection.[23,24,28,29]

As with other clinical settings, the interpretation of transplant-associated viral detection was influenced by the reference standard and by the distinction between viral detection, viral reactivation, and clinically significant end-organ infection.

2.4 FUO and Systemic Viral Disease

In FUO cohorts and studies of systemic viral disease, mNGS provided additional diagnostic information in patients with diagnostically unresolved illness. [14,28,29,31,50-52] Across individual cohorts, mNGS identified viral etiologies in approximately 15–38% of patients, most commonly involving CMV, EBV, HHV-6, or adenovirus. These values represent reported study-level diagnostic yields and were not pooled because of differences in study populations, specimen types, diagnostic criteria, and reference standards.

mNGS findings could assist in distinguishing clinically significant viral infection from possible viral reactivation, although borderline viral loads remained challenging to interpret.

Distinguishing pathogenic infection from incidental or bystander viremia remained a key interpretive challenge particularly in immunocompromised patients.

The diagnostic performance of viral mNGS therefore depended on specimen type, viral burden, host background nucleic acid, and clinical context. CSF and lower-respiratory specimens such as BAL provided particularly useful diagnostic information in appropriately selected patients with CNS and severe respiratory disease, respectively. The principal clinical uses, strengths, and limitations of commonly evaluated specimen types are summarized in Table 2, while sample-specific determinants of diagnostic sensitivity and common limitations are provided in Supplementary Table S5.

Table 2: Sample-Type Utility and Performance Overview for Viral mNGS.

Sample Type	Best Clinical Use	Strengths	Limitations
CSF	Encephalitis/Meningitis	Relatively favorable signal-to-background ratio in selected CNS infections; interpretation remains dependent on viral burden and reference standard.	Low volume; low viral load possible
BAL	Severe pneumonia, ICU	Frequently high diagnostic yield in severe lower-respiratory disease; performance depends on timing, disease location, viral burden, and host background.	Invasive; contamination risk
Plasma / Whole Blood	CMV, EBV, adenovirus, systemic infections	Useful for systemic and cell-free viral nucleic-acid detection, particularly for some DNA viruses; sensitivity varies by virus and disease compartment.	Lower sensitivity for respiratory RNA viruses
Nasopharyngeal Swab	URT infections (influenza, RSV)	Simple collection	May have limited ability to reflect

			lower-respiratory disease when infection is predominantly confined to the lower respiratory tract.
Tissue Biopsy	Myocarditis, invasive viral disease	Direct tissue confirmation	Highly invasive

3. Incremental Diagnostic Yield

Across the included studies, mNGS frequently provided additional diagnostic information beyond conventional microbiologic testing, particularly in patients with diagnostically unresolved infections, atypical presentations, immunocompromised states, and infections caused by pathogens not covered by routine targeted assays.

The magnitude of incremental diagnostic yield varied substantially across clinical settings and study designs. Reported additional diagnostic yields included approximately 25–68% in CNS infection cohorts, 18–46% in respiratory infection cohorts, 30–70% in transplant-associated cohorts, and 15–38% in FUO or systemic infection cohorts. These figures represent ranges of study-level reported yields rather than pooled estimates and should not be interpreted as standardized measures of diagnostic accuracy. Differences in comparator testing, patient selection, specimen type, viral burden, and reference-standard definitions contributed substantially to the observed variation.

Incremental yield was generally greater when conventional diagnostic strategies had limited pathogen coverage and lower when comparator testing already included extensive multiplex molecular assays.

The principal factors contributing to additional diagnostic yield included:

- Unbiased detection of uncommon or unexpected viruses;
- Detection of viruses not included in conventional PCR panels;
- Detection of low-abundance viral nucleic acid after appropriate host-background depletion or enrichment;
- Identification of mixed infections in immunocompromised or critically ill patients; and
- Improved etiologic attribution in patients with atypical or diagnostically unresolved presentations.

However, an additional molecular detection did not necessarily establish clinically significant infection. In particular, detection of low-level viral nucleic acid may represent transient viremia, viral reactivation, contamination, or bystander detection rather than causative disease. Accordingly, incremental diagnostic yield should be distinguished from clinically actionable diagnostic yield and interpreted in conjunction with clinical findings and an independent reference standard whenever available.

Several studies reported instances in which mNGS identified a pathogen that had not been detected by conventional testing, including cases of severe encephalitis, respiratory infection, and other complex clinical syndromes.[1,6,49,65]

4. Clinical Impact of mNGS on Management

A major potential benefit of mNGS is its ability to influence clinical management by providing an etiologic diagnosis when conventional testing is inconclusive. Across the included studies, management changes attributable to mNGS were reported in a substantial proportion of patients; however, the definition and clinical significance of "management change" varied considerably between studies.[11–15,21–30,45,57,65,67]

After harmonizing the reported outcome definitions, the overall proportion of patients in whom mNGS was reported to contribute to a management change was approximately 20–47%. This range represents study-level

reported proportions and should not be interpreted as a pooled estimate. The studies differed in clinical setting, patient population, timing of mNGS, comparator testing, and methods used to determine whether a management change was attributable to the sequencing result.

Management changes were therefore considered as distinct clinical outcomes rather than as a single homogeneous endpoint. The principal categories included initiation or modification of antiviral therapy, antibiotic de-escalation or discontinuation, infection-control interventions, and avoidance of additional invasive diagnostic procedures (Supplementary Table S3).

4.1 Therapy Initiation

mNGS contributed to initiation or modification of antiviral therapy in several clinical settings, particularly CNS infections [1,6,16,47] and transplant-associated infections. [23–30]

Reported frequencies varied between studies, with antiviral initiation or modification occurring in approximately 15–45% of patients in cohorts reporting this outcome. These estimates should be interpreted as study-level proportions rather than a pooled treatment-effect estimate.

Typical clinical scenarios included:

- Initiation of ganciclovir or related therapy when CMV infection was identified;
- Selection or modification of anti-CMV therapy when resistance-associated findings were available;
- Initiation of acyclovir or valacyclovir for HSV/VZV infection; and
- Adjustment of antiviral therapy for selected respiratory viral infections.

The clinical significance of these interventions was potentially greater than that of antimicrobial discontinuation because they directly influenced pathogen-directed therapy; however, most studies did not prospectively evaluate whether such changes improved patient outcomes.

4.2 Antibiotic De-escalation

mNGS also contributed to antibiotic de-escalation or discontinuation, particularly in critically ill patients with suspected respiratory infection and in diagnostically unresolved systemic illness.

Reported antibiotic de-escalation or discontinuation occurred in approximately 15–46% of patients in studies reporting this outcome. [10,45,67] However, the decision to discontinue antibacterial therapy was generally influenced by the overall clinical picture rather than by mNGS alone.

Clear identification of a viral etiology could therefore support reduction of unnecessary antibacterial exposure, particularly when bacterial infection was not supported by other clinical or microbiologic findings.

4.3 Infection Control Interventions

mNGS findings also influenced infection-control decisions in selected settings.

Reported infection-control interventions occurred in approximately 5–20% of patients or clinical episodes in studies evaluating this outcome. Examples included implementation or modification of isolation or cohorting measures and recognition of potentially transmissible viral infections or outbreaks in ICU and transplant settings.

These interventions differed substantially in clinical significance from antiviral treatment initiation or antibiotic discontinuation and were therefore considered separately in the synthesis.

4.4 Avoidance of Invasive Procedures

In some diagnostically unresolved cases, identification of a plausible viral etiology by mNGS reduced the need for additional invasive investigations.

Studies reporting this outcome described avoidance or modification of invasive procedures in approximately 8–25% of patients, particularly in CNS and severe respiratory infections. Examples included avoidance of repeat lumbar puncture, additional bronchoscopic procedures, or tissue biopsy when the mNGS result provided sufficient diagnostic information in the clinical context. [1,6,47]

4.5 Interpretation of Clinical Management Impact

The reported management changes should not be interpreted as equivalent measures of clinical benefit. Initiation of targeted antiviral therapy for a clinically significant infection represents a different magnitude of intervention from discontinuation of empiric antibiotics, implementation of infection-control measures, or avoidance of an invasive procedure. These categories were therefore analyzed separately rather than collapsed into a single measure of clinical impact (Supplementary Table S3).

Most management-impact assessments were retrospective chart reviews, while only 11 studies evaluated clinician-driven or prospective management decisions.

Furthermore, few studies evaluated patient-centered outcome measures such as mortality, duration of hospitalization, or length of intensive-care stay. Consequently, although mNGS frequently influenced clinical decision-making, the available evidence remains insufficient to establish that mNGS-guided management consistently improves clinical outcomes.

5. Antiviral Resistance Detection

Resistance detection represented an important potential extension of mNGS beyond pathogen identification, particularly for viruses in which antiviral resistance has established clinical relevance.

Studies evaluating CMV, HBV, influenza, and SARS-CoV-2 reported detection of resistance-associated or variant-defining mutations.[31–36,40]

- CMV resistance-associated mutations, particularly in UL97 and UL54, were identified in studies evaluating antiviral resistance.
- HBV polymerase mutations associated with resistance to nucleos(t)ide analogues were detected using deep sequencing approaches.
- Influenza NA and HA mutations associated with reduced antiviral susceptibility were identified in sequencing studies.
- SARS-CoV-2 sequencing enabled detection of variant-defining mutations, including mutations within the spike gene.

However, these findings should not be interpreted as evidence that routine untargeted shotgun mNGS is currently equivalent to validated targeted resistance assays. Most of the clinically validated resistance evidence identified in this review was generated using targeted or hybrid-capture sequencing approaches designed to achieve greater sequencing depth over relevant viral genes (Supplementary Table S4).

Accordingly, the resistance-detection literature was considered separately from the diagnostic utility of untargeted shotgun mNGS. Targeted or hybrid-capture approaches may provide greater sensitivity for minority resistance variants because sequencing depth can be concentrated on predefined genomic regions, whereas routine shotgun mNGS may provide insufficient coverage when viral nucleic-acid abundance is low.

Nanopore sequencing was also used for rapid characterization of SARS-CoV-2 and other viral genomes in outbreak and time-sensitive settings.[37,38,60]

Although rapid sequencing platforms can provide clinically useful preliminary genomic information, turnaround time and analytical performance depend on the specimen, viral burden, sequencing workflow, bioinformatic

pipeline, and whether targeted enrichment is used. Therefore, platform-level comparisons should not be interpreted as evidence that one sequencing technology is universally superior for antiviral resistance detection.

Reported concordance between sequencing-based resistance calls and comparator methods generally reflected agreement at the sample or reported mutation level rather than a standardized assessment of minority-variant sensitivity. The available evidence therefore supports a potential role for sequencing in resistance-guided antiviral management but does not establish a uniform performance estimate across viruses or sequencing strategies.

6. Sample-Type Performance

Different clinical specimens demonstrated different levels of diagnostic utility for viral mNGS. Performance was influenced by specimen-specific viral burden, host nucleic-acid background, compartmentalization of infection, timing of sampling, and the anatomical site of disease. Accordingly, no single specimen type can be considered universally optimal for viral mNGS.

6.1 CSF

- CSF represented an important specimen for mNGS in CNS infections. [1,7,16,49,55]
- Low host-background nucleic acid can improve the signal-to-noise ratio.
- mNGS may identify viral pathogens when targeted testing is negative or when an unexpected pathogen is suspected.
- Low viral burden remains an important limitation and may reduce sensitivity.

The diagnostic value of CSF therefore depends strongly on the timing of lumbar puncture, viral burden, prior antiviral treatment, and the presence or absence of sufficient pathogen nucleic acid in the specimen.

6.2 BAL / Lower-Respiratory Samples

BAL and other lower-respiratory specimens were particularly useful in severe pneumonia and critically ill patients. [17,18,45,57]

- Higher pathogen burden may facilitate detection of respiratory viruses.
- mNGS can identify viral–viral and viral–bacterial co-infections.
- Host DNA and RNA can constitute a substantial proportion of sequencing reads and may reduce the relative abundance of pathogen reads.

The invasive nature of BAL and the possibility of contamination or detection of colonizing or non-causative organisms require interpretation in conjunction with clinical, radiologic, and conventional microbiologic findings.

6.3 Plasma / Whole Blood

Plasma and whole blood were useful for systemic infections, particularly infections caused by viruses that circulate in the bloodstream.

- These specimens can provide useful information for systemic DNA viral infections such as CMV, EBV, and adenovirus.
- Detection in plasma may be particularly useful when infection is disseminated or associated with systemic disease.

However, plasma-based approaches may have lower sensitivity for localized respiratory viral infections, particularly when viral nucleic acid is not substantially represented in the circulation. Detection of viral nucleic acid in blood also does not necessarily establish end-organ disease.

6.4 Nasopharyngeal Swab

Nasopharyngeal specimens were useful for respiratory viruses with substantial upper-respiratory viral burden, including influenza and RSV. [5,43,72]

- They are relatively easy to collect and suitable for repeated sampling.
- Their diagnostic value may be lower when disease is predominantly localized to the lower respiratory tract.

Accordingly, a negative nasopharyngeal mNGS result should not necessarily exclude lower-respiratory viral infection in patients with severe pneumonia, particularly when the clinical syndrome and imaging suggest disease below the level of the upper respiratory tract.

6.5 Tissue Biopsy

Tissue specimens may provide direct evidence of viral infection within the affected anatomical site and can be particularly valuable in selected cases of invasive viral disease. [56]

- Tissue can provide direct localization of pathogen nucleic acid within the diseased compartment.
- Tissue sampling is invasive and may not be feasible in critically ill patients.

The role of tissue-based mNGS is therefore generally complementary to less-invasive diagnostic approaches and depends on the clinical feasibility and diagnostic question.

Overall, specimen selection should be guided by the suspected anatomical site and biology of the infection rather than by sequencing considerations alone. The principal specimen-specific determinants and limitations identified across the reviewed studies are summarized in Supplementary Table S5.

7. Platform Performance (Illumina/BGI vs Nanopore)

Sequencing-platform characteristics influenced the operational performance of clinical mNGS workflows. However, direct comparison between platforms was limited by differences in library preparation, sequencing depth, pathogen burden, bioinformatic pipelines, and reporting criteria.

7.1 Illumina / BGI Sequencing

Illumina and BGI platforms generally provided high-throughput sequencing with substantial read depth and were used in several studies requiring detailed genomic characterization. [31–35]

- High sequencing depth can facilitate detection of low-abundance pathogen nucleic acid and characterization of viral genomes.
- These platforms may be particularly useful when detailed genomic information or resistance-associated mutation analysis is required.
- Their clinical utility depends on specimen quality, library preparation, sequencing depth, and downstream bioinformatic analysis.

Reported turnaround times varied between studies and workflows. Therefore, the previously stated range of 24–72 hours should not be interpreted as a standardized platform-specific turnaround time. Differences in sample preparation, sequencing run time, bioinformatic processing, and reporting contributed to variation in the overall workflow duration.

7.2 Nanopore Sequencing

Nanopore sequencing provided an important advantage for time-sensitive genomic applications because sequencing data can be generated progressively during the run.

- Rapid sequencing workflows have been used for real-time characterization of viral pathogens and outbreak-associated strains.[37,38,60]
- Sequential data acquisition allows preliminary pathogen identification before completion of a conventional sequencing run.
- Nanopore workflows may therefore be particularly useful when rapid preliminary genomic information is clinically or epidemiologically important.

Reported turnaround times as short as approximately 6–12 hours have been described in selected workflows; however, this should be interpreted as a workflow-specific observation rather than a universal Nanopore turnaround time.

Direct comparisons between Nanopore and Illumina/BGI turnaround times were limited because studies did not consistently report the same workflow components or define turnaround time from the same starting and stopping points. Consequently, a formal quantitative comparison of platform-specific turnaround time was not performed.

7.3 Interpretation of Platform Differences

Overall, the available evidence suggests complementary rather than interchangeable roles for different sequencing platforms. Nanopore-based workflows may offer advantages when rapid preliminary detection or genomic characterization is prioritized, whereas short-read platforms may provide greater sequencing depth and established analytical performance for detailed genomic characterization. These differences should not, however, be interpreted as evidence of universal superiority of one platform over another.

Platform performance is also dependent on factors beyond the sequencing instrument itself, including nucleic-acid extraction, host-background depletion, library preparation, target enrichment, sequencing depth, bioinformatic pipelines, and criteria used for pathogen calling.

8. Subgroup Analyses

8.1 Immunocompromised Hosts

Immunocompromised patients, particularly hematopoietic stem cell and solid-organ transplant recipients, represented an important population in which mNGS provided additional diagnostic information. These patients frequently had atypical clinical presentations, multiple potential infectious causes, prolonged viral shedding, and concurrent infections. [23–30,73]

- mNGS identified mixed or unexpected viral infections, including combinations involving CMV, HHV-6, adenovirus, and BKPyV.
- mNGS was particularly useful when conventional diagnostic testing failed to establish an etiologic diagnosis.
- However, detection of viral nucleic acid in immunocompromised patients required careful interpretation because asymptomatic viral reactivation and low-level viremia were relatively common and did not necessarily indicate active end-organ disease.

The available studies also reported variable effects on clinical management, but the underlying definitions and methods of attribution were inconsistent. Therefore, a specific threshold such as “therapy changes >45%” was not considered sufficiently standardized for subgroup comparison.

These cohorts therefore demonstrated both the potential diagnostic value of mNGS and the particular importance of clinical correlation when interpreting positive results.

8.2 Pediatric Populations

Pediatric patients, particularly those with severe or unexplained CNS or respiratory disease, represented another setting in which mNGS could provide diagnostic information beyond conventional testing.

- mNGS identified uncommon or unexpected pathogens in pediatric encephalitis, including astrovirus and parechovirus.[53,63]

- mNGS was also applied in severe respiratory infections and in critically ill children in whom conventional testing did not establish a definitive diagnosis. [45,65]

The available pediatric studies were heterogeneous with respect to age, clinical syndrome, specimen type, sequencing workflow, and reference standard. Consequently, a pooled diagnostic yield or accuracy estimate was not calculated for this subgroup.

8.3 Emerging Viral Diseases

mNGS and related sequencing approaches have been particularly valuable for the characterization of emerging and unexpected viral pathogens.

Applications included:

- Real-time sequencing and genomic characterization during the COVID-19 pandemic;

- Rapid identification and characterization of Ebola virus and other outbreak-associated viruses;

- Identification of Zika virus and other unexpected pathogens; and

- Detection and characterization of emerging enteroviruses, including enterovirus D68.

These applications illustrate the principal advantage of unbiased or broad-range sequencing: the ability to detect and characterize pathogens without requiring complete prior knowledge of the causative virus. However, several studies in emerging-virus surveillance and outbreak genomics used targeted or outbreak-specific sequencing strategies rather than routine untargeted clinical shotgun mNGS. These applications should therefore be distinguished from diagnostic mNGS when interpreting the clinical evidence.

Accordingly, evidence from outbreak genomics was considered supportive of the broader utility of sequencing for viral identification and characterization but was not treated as equivalent to diagnostic accuracy evidence from clinical mNGS studies. [33,37,38,53-54]

Discussion

Metagenomic next-generation sequencing (mNGS) has rapidly advanced over the past decade, reshaping the diagnostic landscape of clinical virology. Our systematic synthesis of 75 studies from 2015–2025 demonstrates expanding evidence for the diagnostic and clinical value of mNGS in viral infections across a diversity of clinical settings. The analysis reveals important applications in diagnostic evaluation, clinical decision support, and antiviral resistance detection. However, the magnitude of reported benefit varies substantially across studies and is influenced by methodological heterogeneity, reference standard selection, and interpretive frameworks. These findings position mNGS as a potentially powerful but context-dependent diagnostic approach rather than a uniformly superior replacement for targeted molecular testing.

Diagnostic Significance of mNGS

The findings across CNS, respiratory, transplant, and systemic viral infections consistently demonstrate that mNGS can provide additional diagnostic information compared with conventional testing modalities. In CNS infections, unbiased mNGS identifies both expected and unexpected pathogens, including neurotropic viruses that may be missed by targeted PCR assays.[1–4,6,7,12,47-49]

Importantly, studies using independent microbiologic reference standards reported lower, but likely more conservative, sensitivity estimates than those relying on composite clinical adjudication, in which mNGS results

were sometimes incorporated into case definitions. This circularity introduces incorporation bias and partially explains the wide sensitivity range reported across the literature.

Accordingly, the sensitivity estimates reported in this review should be interpreted as study-level estimates rather than as a pooled measure of diagnostic accuracy. Risk-of-bias assessments were considered when interpreting diagnostic accuracy estimates, and studies using composite reference standards incorporating mNGS results were not treated as equivalent to studies using independent microbiologic reference standards. The differences in reference standards, patient selection, specimen characteristics, and analytical workflows limit direct quantitative comparison between studies.

Studies in encephalitis report that mNGS uncovers occult viral infections in patients previously classified as "unknown etiology," reinforcing its role as an important diagnostic option when conventional testing is inconclusive.[1,16,17,63]

Similarly, in severe pneumonia and ICU populations, mNGS identifies respiratory viruses, clarifies mixed viral–bacterial etiologies, and broadens diagnostic scope beyond predefined PCR panels.[5,6,10,17,22,45,57] Sensitivity varied with sample type, host nucleic acid burden, and viral load, underscoring that mNGS performance reflects biological constraints as much as technical capability. mNGS's capacity to detect co-infections and unexpected pathogens is particularly relevant in critically ill patients, where establishing an appropriate etiologic diagnosis may influence antimicrobial and antiviral decisions.

The diagnostic benefit is particularly relevant in immunocompromised hosts. HSCT and solid-organ transplant recipients often experience atypical presentations and complex infectious profiles due to prolonged immunosuppression.[23–30,72] However, these populations also exhibited low-level viral detection and asymptomatic reactivation, complicating attribution of causality. mNGS can identify CMV, HHV-6, adenovirus, BKPyV, and mixed infections that may not be detected or may be difficult to interpret using standard testing.

Nevertheless, detection of viral nucleic acid should not be equated automatically with active or tissue-invasive disease, particularly in immunocompromised patients. Clinical correlation with viral burden, anatomical site, imaging, conventional microbiologic testing, and the overall clinical presentation remains essential.

Clinical Impact on Patient Management

One of the clinically important findings was the effect of mNGS on therapeutic decision-making.Across studies that reported management impact, approximately 20–47% of cases were described as experiencing a management change attributable to mNGS after harmonization of the reported definitions. However, this figure represents a synthesis of heterogeneous study-level outcomes rather than a pooled treatment-effect estimate.[11–15,21–25,28–30,45,57,65-67,70-76]

Interventions included:

- Initiation of targeted antiviral therapy for CMV, HSV, VZV, influenza, or adenovirus
- Adjustment of antiviral regimens based on resistance profiles (particularly CMV UL97/UL54, influenza NA/HA, HBV polymerase, and SARS-CoV-2 S-gene mutations)[31–36,40]
- De-escalation or discontinuation of unnecessary antibiotics
- Escalation of infection control measures during suspected outbreaks[30,37,38]

Importantly, these interventions are clinically heterogeneous and not equivalent in prognostic significance. Antiviral initiation for CMV encephalitis, for example, differs fundamentally from antibiotic discontinuation in presumed viral pneumonia.

The available evidence therefore supports describing management impact by intervention category rather than treating all management changes as a single clinically equivalent outcome. Antiviral initiation or modification represents a direct therapeutic consequence, whereas antibiotic de-escalation, infection-control interventions, and avoidance of invasive procedures represent different forms of indirect clinical impact. The reported frequencies for these categories are summarized separately in Supplementary Table S3.

Furthermore, most management-impact assessments were retrospective chart reviews, and few studies evaluated whether changes were sustained or associated with improved clinical outcomes.

Only 11 studies assessed management impact prospectively, whereas most relied on retrospective assessment. Consequently, the evidence demonstrates clinical influence of mNGS but remains insufficient to establish that mNGS-guided management consistently improves mortality, length of stay, or other patient-centered outcomes.

Nevertheless, avoidance of unnecessary antibiotics and procedures represents a meaningful stewardship benefit, particularly in critically ill and immunocompromised populations.[1,6,47]

Diagnostic Ambiguity and Overinterpretation Risk

A critical limitation of mNGS is the detection of viral nucleic acid that may not represent active disease. This challenge is most pronounced in immunocompromised hosts, where bystander viremia, latent viral reactivation (e.g., CMV, EBV, HHV-6), and prolonged viral shedding are common and may not correlate with tissue-invasive disease or clinical causality.[14,26,31–33,66]

Low-abundance read detection near reporting thresholds may generate diagnostic uncertainty and, in some cases, prompt unnecessary antiviral therapy. Several studies have explicitly cautioned against overinterpretation of low-read detections without corroborating clinical, radiologic, or quantitative data, particularly in CNS and transplant populations.[7,14,26,31,66] These findings emphasize the necessity of multidisciplinary interpretation and conservative reporting thresholds when integrating mNGS results into clinical decision-making.

Antiviral Resistance Detection: Expanding Clinical Utility

Beyond pathogen identification, mNGS provides detailed genotypic information that can support personalized antiviral therapy. High-depth sequencing enabled detection of clinically relevant resistance mutations in CMV UL97/UL54 genes, HBV polymerase, and influenza NA/HA alleles with high concordance to Sanger sequencing and targeted NGS.[31–36,68]

The distinction between sequencing approaches is important.

Most clinically validated resistance evidence identified in this review was generated using targeted amplicon-based or hybrid-capture sequencing rather than routine untargeted shotgun mNGS. These approaches provide greater sequencing depth over predefined viral genomic regions and therefore may offer superior sensitivity for resistance-associated minority variants. Accordingly, evidence from targeted or hybrid-capture sequencing should not be interpreted as evidence that routine untargeted shotgun mNGS has equivalent resistance-detection performance. The distinction between these approaches is summarized in Supplementary Table S4.

The rising importance of antiviral stewardship in oncology and transplant medicine strengthens the case for integrating resistance testing into appropriate clinical workflows.

Nanopore sequencing offers additional advantages for rapid genomic characterization during outbreaks or acute clinical deterioration,[37,60,68-69] although resistance calling remains dependent on adequate read depth, sequence quality, and validated analytical pipelines.

Thus, the principal value of rapid sequencing platforms may currently lie in shortening time to preliminary genomic information, whereas validated resistance analysis remains dependent on the specific sequencing strategy, depth, genomic target, and bioinformatic pipeline used.

Operational Considerations and Limitations

Despite its demonstrated value, mNGS faces several operational and practical limitations.

1. Turnaround Time (TAT)

Reported turnaround times varied substantially between studies and depended not only on the sequencing platform but also on specimen processing, nucleic-acid extraction, library preparation, batching, sequencing duration, bioinformatic analysis, and reporting. Selected Nanopore workflows achieved preliminary results within approximately 6–12 hours, whereas Illumina/BGI workflows in some studies required approximately 24–72 hours. These values should therefore be regarded as workflow-specific observations rather than standardized platform-specific turnaround times.

Because the included studies did not consistently define turnaround time using the same starting and stopping points, a formal quantitative comparison of platform-specific TAT was not performed.

Selection of platform should therefore align with clinical urgency and downstream needs such as resistance analysis.

2. Cost and Resource Demands

Despite its promise, mNGS faces operational barriers including turnaround time, cost, infrastructure, and bioinformatics expertise. Cost remains a major barrier.

Although individual studies have reported substantial variation in per-sample costs, cost estimates were dependent on platform, batching, laboratory infrastructure, sequencing depth, bioinformatics requirements, and local pricing. Because these parameters were not consistently reported across the included studies, a standardized cost estimate was not derived in this review.

While some studies suggest downstream cost offsets through reduced testing, antibiotic use, or length of stay, formal cost-effectiveness analyses remain limited and inconclusive.

3. Interpretation Challenges and Sources of Bias

Interpretation Challenges

Interpretation of mNGS results requires substantial clinical and laboratory expertise. Particular challenges include:

- **Discrimination of true pathogens from contaminants or background virome**, especially in low-biomass samples.
- **Clinical interpretation of low-abundance or low-read viral detections**, which may represent transient viremia, latent viral reactivation, or sequencing artefacts rather than causative infection.
- **Distinguishing viral reactivation from active disease**, particularly for herpesviruses such as CMV, EBV, and HHV-6 in immunocompromised hosts, where asymptomatic viremia is common and may not warrant treatment.[31–36]

These factors underscore the risk of overinterpretation of mNGS findings and the importance of multidisciplinary adjudication integrating viral load, read depth, clinical presentation, and confirmatory testing.

4. Reference Standard Heterogeneity and Incorporation Bias

Across included studies, reference standards for diagnostic accuracy varied substantially and included:

- Targeted PCR assays or multiplex panels
- Serology
- Culture (rarely applicable for viral infections)
- Composite clinical adjudication incorporating mNGS results

The latter introduces incorporation bias, which may inflate reported sensitivity and specificity estimates.

This distinction was particularly important in the present review because 34 of the 75 included studies used composite clinical adjudication, whereas 41 used an independent microbiologic reference standard (Supplementary Table S1). Studies using composite adjudication were therefore interpreted separately from those using independent reference standards when assessing diagnostic accuracy.

Risk-of-bias assessment further demonstrated that the reference-standard domain represented an important source of methodological concern, with 31 studies classified as having high risk of bias and 16 as having unclear risk in this domain (Supplementary Table S2).

Because of this heterogeneity, diagnostic performance estimates across studies were not directly comparable and were not pooled. A formal meta-analysis was therefore not considered appropriate without a sufficiently homogeneous subgroup defined by clinical syndrome, specimen type, reference standard, and comparable diagnostic outcome definitions.

5. Host Background Interference

High proportions of host nucleic acid—particularly in bronchoalveolar lavage fluid and blood—reduce effective sequencing depth and may compromise sensitivity for low-titer viral infections. Several studies emphasized the need for improved host depletion, viral enrichment strategies, and standardized reporting thresholds to enhance reproducibility and clinical interpretability.[7,17,57]

Bias and Evidence Gaps

Publication bias favoring positive diagnostic studies is possible, as is selection bias toward diagnostically complex cases referred for mNGS. Few randomized or prospective outcome-driven trials exist, and standardized reporting remains inconsistent across studies.

An additional methodological limitation of this review was the absence of prospective protocol registration. The review was initiated retrospectively and was not registered in PROSPERO or another systematic-review registry before study selection and data extraction. Consequently, an independent public record of the prespecified eligibility criteria, outcomes, and analytical decisions was not available. This introduces a potential risk that eligibility criteria or analytical decisions could have been refined after exposure to the literature, although the review methods and eligibility criteria were subsequently applied systematically to the identified studies.

The absence of prospective registration does not by itself demonstrate that outcome-driven selection occurred, but it reduces the external verifiability of the prespecified methodology and should therefore be considered when interpreting the reproducibility and validity of the synthesis.

This limitation is particularly relevant to diagnostic accuracy because reference standards, clinical syndromes, and outcome definitions varied considerably across the included studies. To mitigate this concern, the present review explicitly classified reference standards, assessed risk of bias using QUADAS-2, and avoided treating composite adjudication studies as equivalent to studies using independent microbiologic reference standards.

Integration into Clinical Algorithms

Evidence supports a tiered diagnostic strategy:

1. Rapid targeted PCR or multiplex panels as first-line testing
2. mNGS reserved for severe, unresolved, atypical, or high-risk cases

This framework is consistent with the evidence synthesized in this review, in which mNGS was most valuable when conventional testing was negative, incomplete, or unable to explain a complex clinical syndrome. mNGS should therefore be viewed primarily as a complementary diagnostic strategy rather than a universal replacement for targeted testing.

Strengths of This Review

This systematic review has several strengths. First, it provides a broad synthesis of clinical applications of mNGS across multiple infectious syndromes, including CNS infections, respiratory disease, transplant-associated infections, fever of unknown origin, and systemic viral illnesses, spanning a 10-year period.

Second, the review integrates evidence from 75 clinical studies encompassing diverse patient populations, clinical settings, and sequencing platforms, enabling comparison across a wide range of real-world contexts.

Third, emphasis was placed on clinically relevant outcomes, including diagnostic yield, impact on patient management, and antiviral stewardship, rather than on technical performance metrics alone.

An additional strength was the explicit consideration of reference-standard type and risk of bias when interpreting diagnostic accuracy estimates. The review distinguished independent microbiologic reference standards from composite clinical adjudication and separately considered the methodological limitations associated with incorporation bias.

Finally, all included studies were supported by traceable references, facilitating transparency and reproducibility.

Future Directions

Future efforts should prioritize the development of standardized reporting thresholds, harmonized bioinformatics pipelines, and transparent criteria for distinguishing clinically significant infection from incidental viral detection. Prospective, outcome-driven studies evaluating mNGS-guided management, rather than diagnostic yield alone, are needed to define sustained clinical benefit.

Future diagnostic-accuracy studies should also use clearly prespecified and independent reference standards wherever feasible, report patient-level rather than only sample-level outcomes, and distinguish RNA-virus from DNA-virus detection characteristics. Studies should explicitly report whether the reference standard was independent of the mNGS result to permit meaningful comparison of diagnostic accuracy.

For antiviral resistance, future studies should clearly distinguish untargeted shotgun mNGS from targeted or hybrid-capture sequencing and report sequencing depth, limit of detection, minority-variant detection, and concordance with validated reference methods.

In parallel, robust economic evaluations incorporating downstream cost offsets will be essential to inform broader implementation. Integration of rapid sequencing platforms into ICU and transplant workflows, supported by multidisciplinary interpretation frameworks, may further enhance the clinical utility of mNGS in time-sensitive and high-risk settings.

Limitations

Several limitations should be considered when interpreting the findings of this systematic review.

First, the review protocol was not prospectively registered in PROSPERO or another systematic-review registry because the review was initiated retrospectively. Consequently, no publicly time-stamped protocol was available to independently verify that the eligibility criteria, outcomes, and analytical decisions had been specified before examination of the accumulated evidence. This may introduce a risk of post-hoc modification of methodological decisions and may reduce transparency and reproducibility. To minimize this risk, the eligibility criteria, search strategy, study-selection process, data-extraction framework, and predefined domains of synthesis were applied consistently across the included studies.

Second, substantial clinical and methodological heterogeneity existed across the 75 studies, including differences in patient populations, clinical syndromes, specimen types, viral targets, sequencing platforms,

bioinformatic pipelines, comparator assays, and reference standards. These differences limited direct quantitative comparison and precluded a meaningful pooled estimate of diagnostic accuracy.

Third, reference-standard differences were an important source of potential bias. Forty-one studies used independent microbiologic reference standards, whereas 34 used composite clinical adjudication that could incorporate mNGS findings. Such incorporation may inflate apparent diagnostic sensitivity. Accordingly, the diagnostic accuracy estimates presented in this review should be interpreted as study-level findings rather than universally applicable measures of mNGS performance.

Fourth, management changes attributed to mNGS were clinically heterogeneous and included antiviral initiation, antibiotic de-escalation, infection-control interventions, and avoidance of invasive procedures. Most studies assessed these outcomes retrospectively, and only 11 evaluated prospective clinician-driven decision-making. Furthermore, relatively few studies reported standardized patient-centered outcomes; therefore, management change should not be equated with demonstrated clinical benefit.

Finally, antiviral resistance evidence requires cautious interpretation because most validated resistance studies used targeted or hybrid-capture sequencing rather than routine untargeted shotgun mNGS. Differences in sequencing depth, enrichment strategies, and variant-calling methods limit direct extrapolation of these findings to conventional shotgun mNGS workflows. Further prospective, protocol-registered studies using standardized reference standards, predefined clinical outcomes, and transparent analytical pipelines are needed to establish the clinical effectiveness and cost-effectiveness of mNGS.

Conclusion

This systematic review demonstrates that metagenomic next-generation sequencing (mNGS) meaningfully expands diagnostic capability for viral infections across diverse clinical settings. Evidence from 75 studies indicates that mNGS enhances pathogen detection in CNS infections, severe respiratory disease, and immunocompromised hosts, particularly when conventional diagnostics are unrevealing.

However, the magnitude of diagnostic benefit varied substantially across clinical settings and was influenced by specimen type, viral burden, sequencing workflow, patient population, comparator testing, and reference-standard definition. Studies using composite clinical adjudication, particularly when mNGS contributed to the final diagnostic assessment, may have overestimated diagnostic accuracy because of incorporation bias. Accordingly, reported sensitivity and specificity estimates should not be interpreted as pooled or universally applicable measures of mNGS performance.

mNGS influences clinical management in a substantial proportion of patients by guiding antiviral initiation, antibiotic de-escalation, infection-control measures, and avoidance of invasive procedures. Nevertheless, these management changes were heterogeneous in clinical significance and were predominantly assessed retrospectively; relatively few studies evaluated prospective clinician-driven decisions or standardized patient-centered outcomes. Therefore, the reported frequency of management change should not be interpreted as equivalent to demonstrated improvement in clinical outcomes.

Resistance detection represents an important added value, though most validated resistance data derive from targeted or hybrid-capture sequencing approaches rather than routine untargeted shotgun mNGS. Consequently, the evidence for antiviral resistance profiling should be interpreted as evidence supporting sequencing-based resistance testing broadly, rather than as evidence that routine shotgun mNGS can reliably provide resistance profiling across all viral infections.

The absence of prospective protocol registration is an additional limitation that may affect methodological transparency and reproducibility, although the eligibility criteria, search strategy, data extraction framework, and analytical approach were applied consistently to the included literature. The findings should therefore be interpreted as a structured narrative synthesis of a heterogeneous evidence base rather than as a definitive pooled estimate of diagnostic accuracy or clinical effectiveness.

Overall, mNGS should be viewed as a complementary, second-line diagnostic modality within tiered testing algorithms rather than a universal replacement for targeted assays. Its greatest clinical value appears to be in diagnostically unresolved, severe, or complex viral infections—particularly CNS disease, severe respiratory infection, and infections in immunocompromised patients—where conventional testing may be negative or

incomplete. Continued advances in standardization, interpretation frameworks, prospective clinical-outcome studies, and cost-effectiveness evaluation will determine its long-term role in precision infectious disease practice.

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Supplementary Tables

Supplementary Table S:. Classification of Included Studies by Reference Standard Type.

Reference Standard Type	Definition	Number of Studies (n=75)	Key Implications
Independent microbiologic reference	Comparator testing independent of mNGS results (PCR, multiplex PCR panels, serology, culture)	**41**	More conservative sensitivity estimates; lower risk of incorporation bias
Composite clinical adjudication	Multidisciplinary clinical diagnosis incorporating imaging, labs, response to therapy, and mNGS results	**34**	Higher reported sensitivity; potential circularity and inflation of accuracy estimates

Notes:

- Studies using composite adjudication frequently incorporated mNGS into case definitions, particularly in CNS infections.
- This reference-standard heterogeneity, together with differences in populations, specimen types, and diagnostic workflows, precluded meaningful pooling of diagnostic accuracy estimates and necessitated narrative synthesis.

Supplementary Table S2: QUADAS-2 Risk of Bias Summary Across Diagnostic Accuracy Studies.

QUADAS-2 Domain	Low Risk	High Risk	Unclear Risk	Common Issues Identified
Patient selection	42	18	15	Referral bias toward diagnostically complex cases
Index test (mNGS)	55	8	12	Inconsistent reporting of read thresholds
Reference standard	28	31	16	Incorporation bias in adjudicated studies
Flow & timing	39	14	22	Variable timing between mNGS and comparator tests

- **Interpretation:**
  Overall risk of bias was moderate, driven primarily by reference-standard limitations and incorporation bias.

Supplementary Table S3: Subclassification of Clinical Management Changes Attributable to mNGS.

Management Change Category	Reported Frequency (Range)	Typical Clinical Settings
Antiviral initiation	15–45%	CNS infections, transplant cohorts
Antibiotic de-escalation/discontinuation	15–46%	ICU pneumonia, FUO
Infection-control interventions	5–20%	ICU, transplant units
Avoidance of invasive procedures	8–25%	Encephalitis, ICU respiratory cases

Key Notes:

- Management changes were heterogeneous and not clinically equivalent.
- Only 11 studies assessed management impact prospectively; most were retrospective.

Supplementary Table S4: Shotgun mNGS vs Targeted/Hybrid-Capture Sequencing for Antiviral Resistance Detection.

Feature	Shotgun mNGS	Targeted / Hybrid-Capture NGS
Sequencing depth	Variable; often limited	High, gene-specific
Minority variant detection	Limited	Robust
Resistance calling reliability	Moderate	High
Common clinical use	Pathogen detection	CMV, HBV, influenza resistance
Concordance reporting	Sample-level	Variant-level possible

Interpretation:
Most validated resistance data in the literature derive from **targeted sequencing**, not routine shotgun mNGS workflows.

Supplementary Table S5: Sample-Type–Specific Drivers of Diagnostic Sensitivity.

Sample Type	Major Sensitivity Drivers	Common Limitations
CSF	Low host background, focused compartment	Low viral load
BAL	High viral burden	Host DNA interference
Plasma / Blood	Systemic DNA viruses	Low sensitivity for RNA respiratory viruses
Nasopharyngeal swab	High-titer URT viruses	Poor yield in ICU pneumonia
Tissue biopsy	Direct pathogen localization	Invasive, limited availability