

Research progress of metagenomic next-generation sequencing in infectious diseases of the spine: a systematic review

Ziyan Zhu^{ID} and Xinxin Miao^{ID}

Ther Adv Infect Dis

2026, Vol. 13: 1–19

DOI: 10.1177/
20499361251412789

© The Author(s), 2026.
Article reuse guidelines:
sagepub.com/journals-
permissions

Abstract

Background: Infectious diseases of the spine (IDS) cause structural destruction and abscess formation, requiring precise early diagnosis. While conventional culture methods show limited sensitivity and slow turnaround, metagenomic next-generation sequencing (mNGS) offers a promising alternative with its broader pathogen spectrum, rapid turnaround time, high detection rate, and sensitivity, showing significant advantages in the diagnosis of IDS.

Objectives: This systematic review aims to synthesize the current evidence on the advantages and clinical utility of mNGS in diagnosing and managing IDS, focusing on pyogenic and granulomatous spinal infections.

Design: The systematic review conducted in accordance with PRISMA guidelines.

Data sources and methods: A comprehensive literature search was performed across nine electronic databases (including PubMed, Web of Science, and Embase) from 2010 to April 2025. Studies reporting on mNGS for pathogen detection in patients with suspected or confirmed spinal infections were included. The quality of included observational studies was assessed using the STROBE checklist. Data on detection spectrum, rate, sensitivity, turnaround time, and clinical impact were extracted and synthesized narratively due to high heterogeneity.

Results: Twenty-nine studies (25 retrospective studies and 4 case reports) from China were included. mNGS demonstrated a significantly broader detection spectrum, identifying common pathogens (e.g., *Staphylococcus aureus*, *Mycobacterium tuberculosis*) as well as rare and fastidious organisms that were missed by conventional methods. The pooled detection rate of mNGS (36.8%–95.5%) was consistently and significantly higher than that of culture (5.9%–59.2%). mNGS also showed superior sensitivity (39%–94.7%) compared to culture. The average turnaround time for mNGS (29–53 h) was substantially faster than for culture (2–10 days). mNGS-guided therapy was associated with improved clinical outcomes, including significant reductions in inflammatory markers.

Conclusion: mNGS represents a powerful diagnostic tool for IDS, offering broader detection spectrum, higher detection rate, faster turnaround time, and greater sensitivity compared to conventional methods. This enables more targeted antimicrobial therapy and improves clinical management. Challenges including high costs and difficulty in distinguishing colonization from infection remain. Future efforts should focus on technical optimization, workflow automation, protocol standardization, and outcome validation in larger prospective studies.

Trial registration: CRD420251170912.

Keywords: detection, infections, metagenomic next-generation sequencing (mNGS), pathogen, spine

Correspondence to:

Xinxin Miao
Department of
Orthopedics, The Second
Affiliated Hospital,
Jiangxi Medical College,
Nanchang University,
Nanchang, Jiangxi,
330006, China

Jiangxi Provincial Key
Laboratory of Spine and
Spinal Cord Disease,
Nanchang, Jiangxi,
330006, China

Institute of Minimally
Invasive Orthopedics,
Nanchang University,
Jiangxi, 330006, China
Ndefy22076@ncu.edu.cn

Ziyan Zhu
Department of Clinical
Medicine, School of
Queen Mary, Nanchang
University, Nanchang,
Jiangxi, 330006, China

Received: 14 April 2025; revised manuscript accepted: 16 December 2025.

Introduction

Infectious diseases of the spine (IDS) refer to infectious disorders caused by pathogenic microorganisms that lead to structural destruction of various spinal components (vertebral bodies, intervertebral discs, spinal appendages, spinal canal, and adjacent paravertebral tissues), abscess formation, and localized or systemic manifestations of infection.^{1–3} These conditions are collectively termed spinal infections, encompassing spondylitis (vertebral osteomyelitis), pyogenic spondylitis, sepsis, and epidural abscess.⁴ The clinical incidence of spinal infections in the general population is approximately 2.4 cases per 100,000 individuals, while the incidence in the elderly population (particularly those aged 70 years and above) can reach 6.5 cases per 100,000 individuals.⁵ The spine, as the central axial skeleton of the human body, plays a critical role in connecting the upper and lower limbs. Spinal infections can cause pathological changes in the vertebrae, their appendages, and surrounding tissues, potentially leading to abscess formation that compresses neural structures.⁶ This may result in neurological dysfunction, severe paralysis, or even death. *Failure to promptly identify the causative pathogen and control disease progression may significantly increase both disability and mortality, while imposing substantial economic burdens on patients.*⁷ However, spinal infection patients often present with nonspecific clinical symptoms and atypical imaging manifestations, frequently leading to missed diagnoses, misdiagnoses, or even inappropriate treatment.^{8,9} Therefore, obtaining rapid and accurate pathogen detection results is crucial for the early diagnosis and treatment of IDS.

According to the type of pathogen and host immune response, IDS can be classified into two main categories: (1) pyogenic spinal infections, caused by pyogenic bacteria, are pathologically characterized by suppurative inflammation.^{1–3} These include pyogenic spondylitis (discitis, vertebral osteomyelitis, and infectious spondylitis), paraspinal abscesses, and epidural abscesses. Pathogens responsible for pyogenic spondylitis include Gram-positive bacteria, notably *Staphylococcus aureus* (SA), and Gram-negative bacteria such as *Escherichia coli* and enterococci, among others. (2) Granulomatous spinal infections, caused by specific pathogens, typically present with granulomatous inflammation as their pathological feature. These include spinal

tuberculosis (STB), brucellosis spondylitis (BS), fungal spondylitis, and parasitic spondylitis. The diagnosis of spinal infections is primarily based on patient signs, clinical symptoms, laboratory tests, and imaging studies. Although the conventional microbial culture is the gold standard for diagnosis, it has several limitations such as prolonged incubation time, difficulty in culturing certain microorganisms, and suboptimal sensitivity and detection rates. These drawbacks not only frequently lead to diagnostic delays and missed diagnoses but also significantly compromise treatment efficacy and prognosis of patients with IDS.

To overcome the limitations of traditional culture methods, nucleic acid-based sequencing technologies have rapidly developed. As a representative of first-generation DNA sequencing technology, Sanger sequencing utilizes dideoxynucleotides to randomly terminate DNA strand elongation during synthesis, enabling sequence reading through electrophoretic separation. Although this method offers long read lengths and high accuracy, its low throughput and high cost have limited its widespread clinical application. To address these issues, next-generation sequencing technologies with higher throughput and faster speeds emerged, gradually evolving into second and third-generation sequencing. Among these, metagenomic next-generation sequencing (mNGS) has recently demonstrated great potential in pathogen detection for spinal infections. mNGS is a high-throughput, unbiased sequencing technology that directly extracts all nucleic acids from clinical samples (e.g., blood, lesioned tissue, and pus), and rapidly and accurately identifies microorganisms in the samples by comparing the sequenced results with pathogen databases. The basic procedure of mNGS mainly consists of experimental manipulation (wet experiment) and bioinformatics analysis (dry experiment).¹⁰ The wet experiment includes sample pretreatment, nucleic acid extraction, library construction, and online sequencing. The dry experiment includes data quality control (assessing the quality of raw sequencing data, filtering out low-quality sequences, adapter sequences, and duplicate reads to ensure the accuracy and reliability of downstream analyses), human sequence removal (aligning sequencing data to the human reference genome to identify and remove host-derived nucleic acid sequences, thereby enriching trace pathogen sequences and reducing background interference), microbial species comparison and

identification (aligning and identifying high-quality non-human sequences after host removal against a comprehensive microbial genome database), and analysis of drug resistance genes and virulence genes (aligning sequencing data with known antimicrobial resistance (AMR) gene and virulence factor databases to identify relevant genes carried by pathogens). Depending on the experimental requirements and conditions, different sequencing platforms (e.g., Illumina, Ion Torrent, PacBio, BGI, Oxford Nanopore) and technical approaches (e.g., 16S rRNA bacterial metagenomics, viral metagenomics, fungal metagenomics, hybridization-based targeted NGS, and unbiased metagenomic NGS) are available, as shown in Tables 1 and 2.

In addition, the technical requirements of mNGS are very high: (1) avoidance of contamination and standardization of samples during collection, preservation, or transport; (2) difficulties in collection and extraction: mNGS is difficult to collect specimens from spinal infection sites, with the risk of neurovascular injuries,²² and Conventional cell lysis techniques remain challenging for DNA extraction from intracellular bacteria with structurally specialized cell walls (e.g., *Mycobacteria*)²³; (3) Databases: bacteria, viruses, fungi, parasites, and other pathogens; and (4) Background bacteria need to be monitored. In 2014, mNGS was first applied to the clinic by Wilson et al., when it successfully diagnosed a case of intracranial *leptospirosis* infection.²⁴ In 2017, Greninger et al.²⁵ used mNGS to detect positive for *enterovirus/rhinovirus* in 16 swabs via the FilmArray system. In 2019, Zhang et al.²⁶ confirmed the diagnosis of mNGS via osteomyelitis caused by *Cryptococcus neoformans*. In 2019, Wilson et al.²⁷ used mNGS to identify neurologic infections with clinical effect and guide treatment. With continuous technological advancements, mNGS has more and more applications in the field of medicine, including respiratory infections, bloodstream infections (BSI), central nervous system infections, osteoarticular infections, gastrointestinal or abdominal infections, skin and soft tissue infections, and urinary tract infections.

Given the urgent demands for the advancement of clinical technologies and the treatment in the spinal infection, this review examines the current research landscape of mNGS in IDS (focusing on pyogenic spondylitis and granulomatous

spinal infections) and summarizes the existing clinical evidence to provide a basis for in-depth research (an overview of mNGS is provided in Figure 1).

Method

This review adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA²⁸) guidelines. It aims to systematically retrieve, screen, and evaluate the existing literature on “Research Progress of Metagenomic Next-Generation Sequencing in Infectious Diseases of the Spine: A Systematic Review.” The complete PRISMA checklist is provided in Supplemental File 1. For the narrative synthesis, items pertaining to meta-analysis were marked as “not applicable” (N/A).

Literature search strategy

A systematic literature search was conducted across the following electronic databases: PubMed, Web of Science, Scopus, Science Direct, Embase, Google Scholar, China National Knowledge Infrastructure (CNKI), Wanfang Database, and Chinese Medical Journal Network (CMJN). The final search date for PubMed and Web of Science was April 20, 2025, while for Scopus, Science Direct, Embase, Google Scholar, CNKI, Wanfang, and CMJN, it was April 21, 2025. The search period is from 2010 to April 19, 2025. The search strategy involved combining medical subject headings (MeSH) terms and keywords such as “Spine (MeSH),” “Infections (MeSH),” “Spinal Diseases (MeSH),” “Osteomyelitis (MeSH),” “Spinal Cord Diseases (MeSH),” “Tuberculosis, Spinal (MeSH),” “Spondylitis (MeSH),” “High-Throughput Nucleotide Sequencing (MeSH),” “Metagenomics (MeSH),” “Sequence Analysis, DNA (MeSH),” “Genomics (MeSH),” “Molecular Diagnostic Techniques (MeSH),” “Diagnosis (MeSH),” “Spinal Infection,” “Pyogenic Spondylitis,” “Granulomatous Spinal Infections,” “Vertebral osteomyelitis,” “mNGS” or “Metagenomic next-generation sequencing,” and “Detection” using Boolean operators. Adjustments to the search terms were made based on the requirements of each database. This systematic review was retrospectively registered with PROSOERO (CRD420251170912) to ensure protocol transparency, with no changes made to the pre-specified methods.

Table 1. Different sequencing platforms (Illumina, Ion Torrent, PacBio, BGI, Oxford Nanopore).

Sequencing platform	Applications	Sample type	Strengths	Limitations	Ref.
Second-generation sequencing					
Illumina	Whole genome sequencing; whole exome sequencing; targeted sequencing; transcriptome sequencing; epigenome sequencing; exome sequencing; Small RNA sequencing; ChIP-Seq	DNA (genomic, long fragments, target-enriched, exome-captured, immunoprecipitated); RNA (total RNA, small RNA)	High sequencing depth; high accuracy	Slow; phasing difficulties	11
Ion Torrent	Transcriptome profiling; splice site identification	DNA (genomic, amplicons, target-enriched, ChIP-enriched); RNA (total RNA)	Long read length; fast; low substitution error rate	Difficulty sequencing through homopolymer regions	11, 12
BGI	3 M project; human genome re-sequencing; palaeogenomic ancient DNA sequencing; detection of small noncoding RNAs	DNA (genomic, metagenomic, ancient); RNA (small RNA, transcriptome)	High throughput; low cost; high sequencing depth	Short read length; resequencing only in applications	13, 14
Third-generation sequencing					
PacBio	Full length transcript sequencing; targeted/amplicon sequencing; metagenomics sequencing	DNA (amplicons, long fragments, metagenomic); RNA (full-length cDNA isoforms); tissue; thallus; interstitial fluid; environmental samples; water filter membrane	Long read length; fast	High cost; high error rate; large instrument footprint	11, 15
Oxford Nanopore	DNA sequencing; amplicons sequencing; cDNA sequencing; direct RNA sequencing	DNA (amplicons, target-enriched, native, crosslinked chromatin samples); RNA (direct RNA, cDNA)	Fast; small instrument footprint; portability; real-time data analysis	High error rate	11, 15
"Genome Coverage" (or "Breadth of Coverage") refers to the proportion of the reference genome covered by at least one sequencing read. "Sequencing Depth" (or "Depth of Coverage") refers to the average number of times a given base in the genome is sequenced. NGS, next-generation sequencing.					

Literature inclusion and exclusion criteria

Inclusion criteria. Literature inclusion was defined according to the PICOS framework:

(P) Population: Patients with clinically suspected or confirmed spinal infections, regardless of age, gender, or spinal infection type. Infection types include pyogenic spinal infections (pyogenic spondylitis (discitis, vertebral osteomyelitis, and

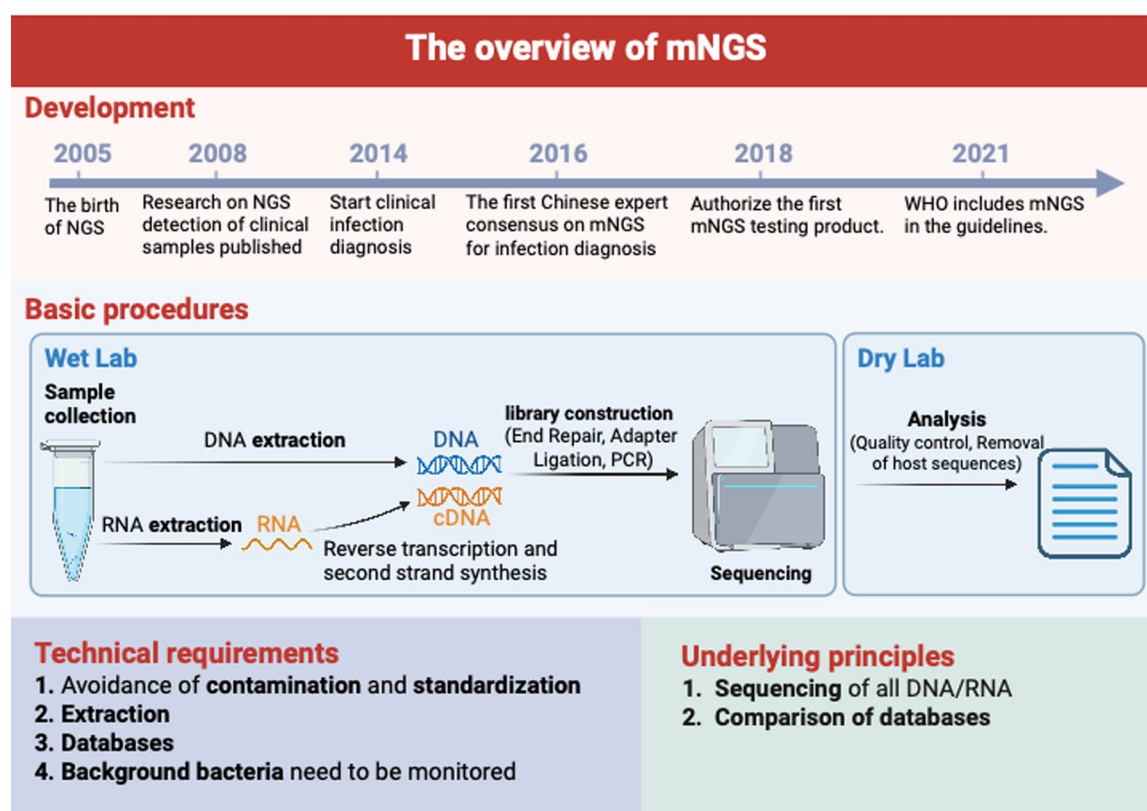
infectious spondylitis), paravertebral abscesses, and epidural abscesses) and granulomatous spinal infections (spinal tuberculosis/STB, brucellosis spondylitis/BS, fungal spondylitis, and parasitic spondylitis).

(I) Intervention: Pathogen detection using mNGS on clinical samples (e.g., blood, lesioned tissue, or pus).

Table 2. Different technical approaches (16S rRNA bacterial metagenomics, viral metagenomics, fungal metagenomics, hybridization-based targeted NGS, and unbiased metagenomic NGS).

Technical approaches	Applications	Strengths	Limitations	Ref.
Targeted (amplicon-based) metagenomics	16S sequencing (bacteria and archaea); 18S Sequencing (eukaryote); ITS sequencing (fungi); probe-based capture	High throughput; low cost; fast; high accuracy; high efficiency; high sensitivity	Limited genome coverage; limited resolution; high bias	15
Shotgun metagenomics	Microbial diversity profiling (virus, bacteria, fungi, and parasite); functional gene and pathway discovery; novel microbe and pathogen identification; antibiotic resistance surveillance	High throughput; fast; wide genome coverage; primer-free detection; high resolution; no culturing needed	Contamination; high cost; high host DNA burden; low sequencing depth; database limitations	15–17
Hybridization-based targeted NGS	Genotyping; exome sequencing; gene discovery; rare variant; indel; copy number variation	Low cost; high efficiency; the multiplexing capacity of hybridization capture	Limited genome coverage; probe design bias	18–21

NGS, next-generation sequencing.

**Figure 1.** The overview of mNGS, including development, underlying principles, basic procedures, and technical requirements.

Source: Created with BioRender.com.

mNGS, metagenomic next-generation sequencing.

(C) Comparator: The diagnostic performance of mNGS was compared against that of traditional microbiological methods (e.g., culture, targeted PCR, serology) and/or a comprehensive clinical diagnostic standard (based on clinical presentation, imaging, histopathology, etc.).

(O) Outcomes: The primary outcomes included the pathogen detection spectrum, detection rate, turnaround time, and sensitivity of mNGS. Secondary outcomes encompassed technical procedures, limitations, and clinical implication.

(S) Study Design: case reports, and retrospective studies.

Exclusion criteria. The exclusion criteria were as follows:

Relevance: Studies not pertinent to the research progress of mNGS in IDS.

Impact factor: Articles published in journals with an Impact Factor of less than 1.

Publication date: Publications outside the search period from January 1, 2010, to April 19, 2025.

Language: Articles published in languages other than Chinese or English.

Study type and quality: Non-original research publications such as commentaries, conference abstracts, and editorials; studies with unsatisfactory methodological quality; and articles for which the full text was inaccessible. Higher-impact literature was prioritized where possible.

Population: Animal studies.

Literature screening process

All records retrieved from the databases were screened by two independent reviewers. The initial screening utilized electronic database filters to exclude publications with an impact factor below 1 and those published outside the date range from 2010 to April 19, 2025. Subsequently, titles, keywords, and abstracts were reviewed to exclude clearly irrelevant records. With the exception of 14 articles for which the full text could not be retrieved, the remaining publications were downloaded for full-text assessment. Studies that were irrelevant due to study type, population, or low quality were excluded to finalize the literature

inclusion. Any discrepancies during the screening process were resolved through discussion between the reviewers or by consulting a third reviewer. The detailed literature selection process is illustrated in the PRISMA flow diagram (Figure 2).

Quality assessment of included studies

The reporting quality of the final included observational studies was assessed for adherence to the STROBE guideline. Two reviewers independently evaluated each included study using the official STROBE checklist, which comprises 22 items. Compliance with each item was rated as: “adequately reported” (1 point), “partially reported” (0.5 points), “not reported” (0 points), or “not applicable.” Any discrepancies arising during the assessment were resolved through discussion between the two reviewers or by consultation with a third reviewer. The summarized assessment results are presented in Supplemental File 2 (Quality Assessment of Included Studies).

Data synthesis and analysis methods

Supplemental File 3 (Data Summary Table of Included Literature) summarizes the fundamental characteristics of the 29 included studies. Data extraction was conducted independently by two reviewers using a pre-designed data extraction form, with any discrepancies resolved through discussion or consultation with a third reviewer. It is important to note that our data extraction relied exclusively on information available in the published articles and their Supplemental Materials. We did not contact the authors of the primary studies to obtain missing or unreported data, as the necessary information for our analysis was publicly accessible. The extracted data included the following (in cases where data were missing or ambiguously reported in a study, this was denoted as “N/A” or clarified based on information from its main text, tables, or figures):

- (1) Basic study information: title, first author, year of publication, country, impact factor, and study design.
- (2) Characteristics of the study population: sample size and type of spinal infection.
- (3) Research methods and interventions: gold standard, and mNGS technology platform.

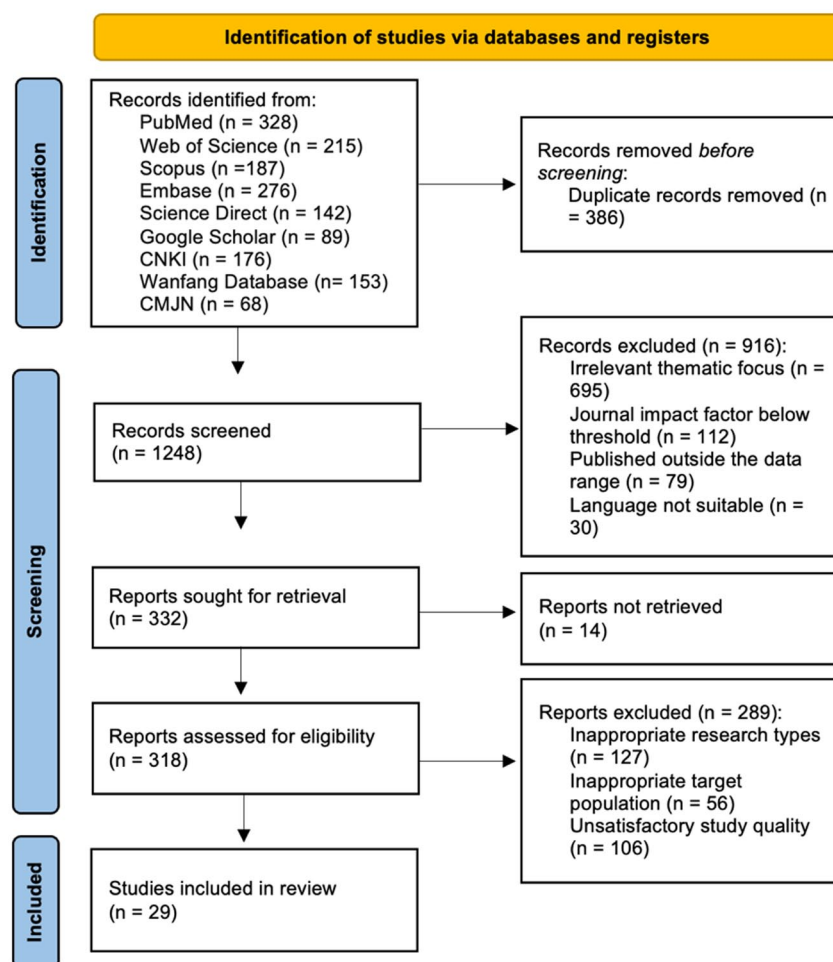


Figure 2. PRISMA flowchart.
PRISMA, preferred reporting items for systematic review and meta-analysis.

- (4) Outcome measures: The primary outcomes were the pathogen spectrum, detection rate, turnaround time, and sensitivity of mNGS. Additionally, data on targeted NGS for identifying AMR genes were extracted.

Data summary: All included studies were conducted in China, comprising 25 retrospective studies and 4 case reports. The journals' impact factors ranged from 1.188 to 11.9, with publication dates spanning from 2020 to 2025. The sample sizes varied widely, from 1 to 380 cases. The types of spinal infections covered were comprehensive, though pyogenic spondylitis and granulomatous spinal infections were predominant. The primary mNGS platforms utilized were Illumina, BGISEQ-50, and MGISEQ-2000.

Most studies used histopathological findings or culture results as the diagnostic reference standard and compared the diagnostic outcomes and efficacy of mNGS against traditional microbiological methods. According to the STROBE checklist assessment, the reporting quality scores of the included studies ranged from 52.27% to 96.15%.

Due to significant clinical and methodological heterogeneity among the included studies (e.g., differences in patient populations, mNGS platforms, and reference standards), a narrative synthesis was adopted. The outcomes were grouped thematically according to the objectives of the review (e.g., advantages of mNGS, applications in specific infections). Within each theme, results were presented in structured tables (Tables 3–6) and summarized narratively.

Table 3. A summary table by Shi et al.,²⁹ Zhang et al.,³⁰ and Huang et al.,³¹ including study design, CMT methods, main pathogens, diagnostic yield (CMT and mNGS), and specimen type.

Author (s) and Ref.	Study design	CMT methods	Main pathogens	Diagnostic yield (CMT and mNGS)	Specimen type
Shi et al. ²⁹	Retrospective study	Microbial culture	<i>S. aureus</i> , <i>Mycobacterium tuberculosis</i> , <i>Streptococcus</i> spp., <i>anaerobes</i> , <i>E. coli</i> , <i>S. epidermidis</i> , <i>Brucella melitensis</i> , <i>B. melitensis</i>	Microbial culture (27.16%) mNGS (77.78%)	Site-specific samples (tissue)
Zhang et al. ³⁰	Retrospective study	Microbial culture + histopathological analysis	<i>S. aureus</i> , <i>M. tuberculosis</i> , <i>E. coli</i> , <i>Streptococcus</i> spp.	Microbial culture (27.7%) Histopathological analysis (41%) mNGS (45%)	Site-specific samples (tissue and pus)
Huang et al. ³¹	Retrospective study	Microbial culture	<i>M. tuberculosis</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , <i>Brucella</i> , <i>E. coli</i>	Microbial culture (59.6%) mNGS (80.9%)	Site-specific samples (tissue)
mNGS, metagenomic next-generation sequencing.					

Table 4. A summary table by Qi et al.,³² Li et al.,³³ and Liu et al.,³⁴ including detection targets, gold-standard diagnostic methods, specimen type, detected pathogens, mNGS detection rates, and when available, kappa agreement statistics between mNGS and conventional detection methods.

Author (s) and Ref.	Detection target	Gold standard	Specimen type	Detected pathogens	mNGS detection rate	The kappa agreement
Qi et al. ³²	17 surgical specimens	Conventional cultures	mNGS: site-specific samples (pus and tissue) Culture: blood and site-specific samples (pus)	<i>S. aureus</i> , <i>Streptococcus</i> , <i>E. coli</i> , <i>Brucella melitensis</i> , and various <i>Bacillus</i> species etc.	82.4%	N/A
Li et al. ³³	301 patients	Microbial culture	Blood and site-specific samples (pus and tissue)	<i>S. aureus</i> , <i>Streptococcus</i> , <i>E. coli</i> , <i>S. epidermidis</i> , <i>Klebsiella pneumoniae</i> , <i>M. minuta</i> , <i>Pseudomonas aeruginosa</i> , <i>Corynebacterium</i> , <i>Prevotella</i> , <i>B. fragilis</i> , <i>C. burnetii</i> , <i>C. acnes</i> , and <i>C. perfringens</i> , etc.	77.9%	0.12 (<0.40)
Liu et al. ³⁴	54 samples	Isolating culture	Site-specific samples (pus and tissue lavage fluid)	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Streptococcus</i> , <i>Salmonella typhi</i> , and <i>Prevotella intermedia</i> , etc.	90.7%	N/A
mNGS, metagenomic next-generation sequencing.						

Table 5. A summary table by Jin et al.,²³ Guo et al.,³⁵ Jin et al.,³⁶ Ye et al.,³⁷ and Li et al.,³⁸ including detection targets, gold-standard diagnostic methods, specimen type, mNGS detection rates, and when available, kappa agreement statistics between mNGS and conventional detection methods.

Author (s) and Ref.	Detection target	Gold standard	Specimen type	mNGS detection rate	The kappa agreement
Jin et al. ²³	24 patients	Clinical diagnosis	Site-specific samples (tissue)	62.5%	N/A
Guo et al. ³⁵	112 patients	Clinical diagnosis (pathological results)	Blood and site-specific samples (tissue and pus)	80.4%	0.700
Jin et al. ³⁶	203 patients	Culture, CRS	Site-specific samples (tissue)	71.2%	N/A
Ye et al. ³⁷ (case report)	1 patient	<i>Mycobacterium tuberculosis</i> culture	Site-specific samples (tissue and bronchoalveolar lavage fluid)	N/A	N/A
Li et al. ³⁸	100 samples	Culture, CRS	Blood and site-specific samples (pus and tissue)	81%	N/A
mNGS, metagenomic next-generation sequencing.					

Advantages of mNGS in the diagnosis of spinal infections

Detection spectrum

Bacteria. Conventional bacterial culture methods are often limited by factors such as poor cultivability and stringent growth requirements. In contrast, mNGS directly detects nucleic acid sequences, bypassing the need for microbial cultivation. As a result, mNGS offers a broader pathogen detection spectrum, demonstrating significant advantages in diagnosing spinal infections.

In pyogenic spinal infections, common Gram-positive pathogens include *S. aureus* (very common) and *Streptococcus* spp. (common).⁴² Common Gram-negative pathogens include *E. coli* (common), while anaerobes are found occasionally. Shi et al.²⁹ detected a total of 44 pathogens, primarily *S. aureus*, *E. coli*, and *S. epidermidis*, by microbial culture in 162 patients with spinal infections; and one or more of these pathogens, by mNGS, in 86 patients with SI and negative culture results. These included 30 cases of *S. aureus* (22.22%), 12 cases of *Streptococcus* spp. (8.89%), 11 cases of anaerobes (8.15%), and 10 cases of *E. coli* (7.41%). Zhang et al.³⁰ detected 31 positive cases by culture, including *S. aureus* (8 cases, 26%) and *Streptococcus* spp.; 51 positive cases by mNGS, identifying 16 cases of *S. aureus* (31%), 4 cases of *E. coli* (8%), and several cases of *Streptococcus* spp. etc.; 27 cases of pyogenic infection were detected by histopathology. In the study

by Huang et al.,³¹ 13 cases of *S. epidermidis*, 11 cases of *S. aureus*, and 7 cases of *E. coli* were isolated by culture; 15 cases of *S. aureus* and 7 cases of *E. coli* were detected by mNGS; and 18 potentially pathogenic species, such as *Mycoplasma genitalium* and *Gardnerella vaginalis*, were successfully detected by mNGS alone from culture-negative samples. In addition, some rare pathogens were detected by mNGS, such as *Neisseria elongata*,³⁰ *Klebsiella variicola*,⁴³ *Acinetobacter baumannii*, *Peptostreptococcus stomatis*, *Klebsiella pneumoniae* and *Prevotella intermedia*, *Pseudomonas fluorescens*,⁴⁴ *Corynebacterium* spp., *Kocuria polaris*, *Acidovorax temperans*, and *Moraxella osloensis*, *Bacteroides fragilis*,³³ and *Treponema denticola*.

In granulomatous spinal infections, *M. tuberculosis* is a very common pathogen, while *Brucella* spp. is a common pathogen. Zhang et al.³⁰ found that culture tests identified 11 pathogens, with *M. tuberculosis* being the most prevalent (13 cases, 42%); mNGS detected 20 pathogens, with *M. tuberculosis* being present in 25 cases (49%); and histopathology identified 18 cases of tuberculous infection. In the research by Huang et al.,³¹ the most frequently isolated microorganisms in culture-based methods were *M. tuberculosis* ($n=21$), followed by *Brucella* ($n=7$), among others; the most common microorganisms detected by mNGS-based assays were *M. tuberculosis* complex (MBTC) (39 cases), followed by *Brucella* (9 cases), and even *Behcet's* *Kerchus* bodies were detected. In addition, there are some studies that

Table 6. A summary table by Du et al.,³⁹ Yin et al.,⁴⁰ and Lv et al.,⁴¹ including detection targets, gold-standard diagnostic methods, specimen type, mNGS detection rates, and when available, kappa agreement statistics between mNGS and conventional detection methods.

Author (s) and Ref.	Detection target	Gold standard	Specimen type	mNGS detection rate	The kappa agreement
Du et al. ³⁹ (case report)	1 patient	Isolation of <i>Brucella</i> spp.	mNGS: Site-specific samples (tissue) Culture: Blood and site-specific samples (pus and tissue)	N/A	N/A
Yin et al. ⁴⁰	95 patients	Bacterial culture	Blood and site-specific samples (pus and tissue)	88.42%	N/A
Lv et al. ⁴¹	76 patients	Clinical diagnosis	Site-specific samples (pus and tissue)	77.6%	N/A

mNGS, metagenomic next-generation sequencing.

have detected by mNGS in spinal infections the rare *Caustic-dependent bacilli*, *Fusobacterium fortuitum*, *Mycobacterium fortitum coagulans*,³⁵ *M. iranense*,⁴⁵ *Chryseobacterium* spp.,⁴⁴ *Nocardia* spp.,²³ *M. xenopi*,⁴⁶ *K. aerogenes*⁴⁷ (a summary of the main studies is presented in Table 3).

These studies suggest that mNGS can detect the most common and some rare bacterial pathogens, whether in pyogenic spondylitis or granulomatous spinal infections. Although mNGS has a broad spectrum of bacterial pathogens in spinal infections, it is crucial to determine their pathogenicity in clinical diagnosis, that is, to differentiate between true pathogens and contaminants or colonizers, especially when rare or low-biomass microorganisms are detected, such as *Bordetella bronchiseptica*²⁹ (considering the possibility of Q fever) and *Veillonella atypica*⁴⁴ (possibly environmental contamination), need to be diagnosed by combining traditional culture, histopathology.

Virus. Viruses are one of the common pathogens of spinal infections.⁴ Traditional viral detection methods, such as serological testing and PCR, are simple to operate and inexpensive, but can only identify known viruses. Viruses are highly genetically variable and prone to mutations, which continuously generate new variants during replication. Uncommon viruses and novel variants can only be detected using mNGS. By mNGS, human herpesviruses are among the most frequently (very common) detected viruses. Wang et al.⁴⁸ applied mNGS in 60 cases of clinically acute

spinal infections with negative culture and/or smear (CMT) results, identifying 46 pathogen-positive cases, including 8 viral infections. Zhang et al.⁴⁹ detected *human beta-herpesvirus 5*, *human herpesvirus 1*, and *human gamma-herpesvirus 4* in 17 cases of IDS using mNGS (requires joint clinical information for diagnosis). Wan et al.⁴⁵ analyzed samples from IDS patients with sepsis and identified eight different viruses, including *human parvovirus B19*, *human herpesvirus 5*, and *torque teno virus*. Similarly, Wang et al.⁴⁴ reported the presence of *human gamma-herpesvirus 4*, *human beta-herpesvirus 6A*, and *torque teno virus* in 41 diagnosed spinal infection cases via mNGS. These studies demonstrate that while the unique replication and survival mechanisms of viruses increase the difficulty of detection and diagnosis, mNGS overcomes these limitations, showing significant advantages. However, none of these studies has clarified whether their viral detection results were supported by histopathological evidence, such as the observation of typical cytopathic effects. Therefore, in clinical practice, the diagnosis of the virus requires a combination of virus detection in the tissues and histopathology evaluation, especially if the site of infection is unusual for the detected pathogen in the given host (e.g., high levels of *Human herpesvirus* in immunocompromised individuals or the potential symbiont *Torque teno virus*).

Fungi. In clinical practice, fungal spinal infections are relatively uncommon accounting for only 0.5%–1.6% of cases.⁵⁰ The clinical signs and

symptoms of fungal infections are not distinctly different from other types of spinal infections, as well as the imaging findings lack specificity, which make diagnosis more challenging.⁵¹ Furthermore, conventional fungal cultures suffer from low sensitivity and prolonged turnaround times, significantly hindering diagnostic accuracy and timeliness. Many studies have found that mNGS can accurately and rapidly identify pathogens and has an advantage over traditional methods in diagnosing fungal infections of the spine, which significantly reduce misdiagnosis and missed diagnosis rates. Via mNGS, the most frequently detected fungi are *Aspergillus* (common), followed by *Candida* (occasional). By mNGS, Gou et al.³⁵ detected eight cases of fungi, primarily *A. flavus*, *A. fumigatus*, and *A. sydowii*. Wan et al.⁴⁵ identified 51 fungal species in cervical spine injury-associated sepsis, including *S. lycopersici*, *Nakaseomyces delphensis*, *A. niger*, *Candida hubeiensis*, and *Trichophyton mentagrophytes*. Liu et al. diagnosed *A. fumigatus* infection in a retrospective analysis of 47 suspected cases. Li et al.²² identified five *Aspergillus* and two *Candida* infections among 380 IDS patients. Wang et al.⁴⁴ reported *Stachybotrys chartarum* in 41 spinal infection cases. Similarly, mNGS detected some rare fungi such as *Fusarium solani*,⁴⁰ *Aspergillus fumigatus*, and *Talaromyces marneffei*.²⁸ The above studies confirm that mNGS also demonstrates higher diagnostic yield for fungal pathogens, compared to conventional methods. Similarly, fungal sensations need to be co-diagnosed with the Key criterion being histopathology. As *Malassezia restricta* (skin commensal) and *Stachybotrys chartarum* (environmental mold) may reflect contamination.

Parasite. It is noteworthy that among the literature included in this review, no cases of parasitic spinal infections diagnosed by mNGS were identified. This finding underscores the extreme rarity of parasites as etiological agents of spinal infections. Several factors may contribute to this phenomenon: (1) parasitic infections exhibit distinct geographical predilections, being more prevalent in regions with developing economies, poor sanitary conditions, or well-developed livestock industries. (2) These infections often disseminate to the spine via hematogenous or intracranial routes.^{52,53} Their clinical presentations and imaging features can mimic those of tuberculous spondylitis or neoplastic lesions, potentially leading to diagnostic challenges or misclassification. Consequently, parasites are not typically considered a primary differential

diagnosis for spinal infections in non-endemic areas during routine clinical practice. Nevertheless, clinicians should maintain a high index of suspicion for atypical cases originating from endemic regions or with relevant exposure history.

Detection rate

In recent years, with the continuous improvement and development of mNGS technology, its diagnostic value in spinal infections has gained increasing recognition. Numerous studies have successively used mNGS to diagnose spinal infection. Some of these studies compared traditional diagnostic methods with mNGS and found that mNGS had a high detection rate. *The detection rate was defined as the percentage of samples with ≥ 1 pathogenic microbe identified by mNGS or other diagnostic methods.* Zhang et al.³⁰ counted a detection rate of 27.7% by culture in 112 patients, and 41% and 45% by histology and mNGS, respectively, in 111 patients. Li et al.³³ reported that the detection rate of mNGS (77.9%) was significantly higher than that of microbial culture (27.2%). Zhang et al.⁴ found mNGS exhibited higher detection rates (86.32%) for spinal infections than culture, procalcitonin/PCT (30.33%), white blood cell count/WBC (17.39%), and interferon-gamma release assays/IGRAs (38.57%). Additionally, Li et al.²² demonstrated in 44 cases of spinal *Brucella* infection, the positive rate of mNGS was 95.5%, which was higher than that of agglutination test (81.8%) and culture (20.5%); in 20 cases of tuberculosis, the positive rate of mNGS (85.0%) was also higher than that of culture (3/17), PCR test (12/17), *M. tuberculosis* nucleic acid test (6/19) and pathology tests (7/19).

The high detection rate of mNGS is due to its unique technological principle: (1) Unbiased amplification: unlike culture or PCR, mNGS detects all microbial nucleic acids without prior targeting, enabling identification of fastidious/unculturable pathogens (e.g., *Brucella*, anaerobes like *Prevotella*) and polymicrobial infections (e.g., co-detection of *S. aureus* and *E. coli* in pyogenic abscesses); (2) Enhanced sensitivity: It is because mNGS has a high detection rate that it is capable of comprehensive pathogen identification, complementation of pathogens that are undetected by traditional methods and differentiation between TB and NTM infections, thus providing patients with more accurate clinical diagnosis and more effective treatment, including early targeted

therapy and management of antibiotic use. However, it is important to note that the high detection rate of mNGS may include the detection of non-pathogenic microorganisms. Because mNGS can detect all microbial nucleic acids in samples but cannot differentiate between true pathogens and colonizing microorganisms, environmental contaminants, and commensal flora.

The high detection rates of mNGS for spinal infections also depend on optimized library preparation, which differs significantly between DNA-based organisms (e.g., bacteria, fungi, mycobacteria) and RNA viruses (e.g., enteroviruses, HIV in rare spinal infections).^{54,55} In the DNA Pathogen Workflow, the first step involves breaking the cell wall through mechanical/enzymatic lysis to extract microbial DNA. Next, human DNA is removed to enhance detection signals. Finally, library preparation is performed through fragmentation, adapter ligation, and PCR amplification. In contrast, the RNA Virus Workflow requires RNase-free conditions to prevent degradation and DNase treatment to remove contaminating DNA during RNA extraction. The RNA is then converted into cDNA via reverse transcription and double-stranded DNA synthesis. Finally, the library is prepared through fragmentation, adapter ligation, and PCR amplification.

Turnaround time

The mNGS process consists of four key steps: (1) sample collection and nucleic acid extraction; (2) DNA Library preparation and quality control: construction of metagenomic libraries, quantitatively detection and sequencing preparation; (3) mNGS Sequencing: high-throughput sequencing of DNA on sequencing platforms; (4) data processing and analysis: removal of low-quality reads and human host sequences, with remaining sequences aligned against pathogenic microorganism databases for pathogen identification.^{9,56} In contrast, conventional culture methods involve sample collection, prolonged incubation, and subsequent pathogen diagnosis. Compared to traditional culture methods, the mNGS method eliminates the need for culture, saving a lot of time. Comparative studies demonstrate: Wang et al.⁴³ first extracted DNA from 400 μ L of blood using the MagEN Biotech Microbial DNA Extraction Kit (D6318). They then constructed a ~350bp genomic library using the Illumina Nextera XT Library Prep Kit. Sequencing was performed on

the Illumina NextSeq 550Dx platform (SE75 strategy). Finally, after removing reads containing sequencing adapters, reads with >10% N bases, reads with >50% low-quality bases (Q -value ≤ 10), and human host sequences, the remaining data were analyzed. The entire mNGS process took 2.16 ± 0.69 days, which was substantially shorter than bacterial culture (4.74 ± 1.71 days) and histopathology (3.04 ± 1.06 days). In the study by Li et al.,⁹ DNA was first extracted from 300 μ L of infected tissue using the TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH, Beijing, China). The extracted DNA underwent blunt-end repair, barcode adapter ligation, and unbiased amplification via PCR using BGI reagents (BGI, Tianjin, China) to construct a 100–150 bp genomic library. Sequencing was performed on the MGISEQ-2000 platform (MGI, Shenzhen, China). For data analysis, low-quality and short reads (length < 35 bp) were filtered out, and human host sequences were removed using the Burrows–Wheeler Alignment tool (version 0.7.10-r789; Cambridge, MA, USA) by mapping to the human reference genome (hg19). The study demonstrated that mNGS could provide results in <2 days, significantly faster than standard cultures (2–10 days). In acute spinal infections, Wang et al.⁴⁸ employed a methodology similar to the aforementioned study, utilizing the BGISEQ-50/MGISEQ-2000 platform for mNGS detection. The results demonstrated that mNGS achieved pathogen identification within 29–53 h, significantly faster than conventional cultures (90.88 ± 8.33 h; $p < 0.05$). These data show that mNGS has a higher detection speed than traditional methods, which is conducive to early diagnosis and treatment planning as well as improving prognosis, reducing the complication rate, and slowing down the deterioration of the disease.

Detection sensitivity

A study by Zhou et al.⁵⁷ demonstrated that conventional microbial culture exhibits low sensitivity, failing to meet clinical diagnostic requirements for spinal infections. Furthermore, multiple studies have confirmed that mNGS demonstrates relatively higher sensitivity compared to traditional microbial culture methods. Key findings include: Zhang et al.⁵⁶ reported mNGS sensitivity (82.1%) was higher than tissue culture (17.9%) ($\chi^2 = 0.02$, $p < 0.001$). Shi et al.²⁹ documented 77.78% sensitivity for mNGS versus 27.16% for traditional culture. Cheng et al.⁵⁸ demonstrated that in

spinal infections, mNGS exhibited significantly higher sensitivity (approximately 84%) compared to conventional culture methods (32%) ($p < 0.001$). For spinal tuberculosis specifically, mNGS showed equivalent diagnostic sensitivity to T-SPOT (both 90.91%), while conventional culture methods demonstrated near-zero sensitivity. Additionally, there are several studies that provide more sophisticated statistics on the sensitivity, for instance. Wang et al.⁵¹ demonstrated mNGS maintained higher sensitivity in both TB (72.7%) and non-TB groups (80.0%), compared to bacterial culture (36.4% and 11.1%, respectively). Lin et al.,⁵⁹ in a retrospective analysis of clinical data from patients with suspected SI treated at their hospital between January 2010 and December 2011, reported that mNGS could detect 88.9% (8/9) of pathogens identified by conventional culture, with a genus-level sensitivity of 100% (8/8) and a species-level sensitivity of 87.5% (7/8). Moreover, the sensitivity of mNGS in detecting pathogens was 87.1%, which significantly exceeded that of conventional culture (25.8%). While mNGS can accurately identify pathogenic microorganisms and offers notable advantages over traditional culture-based methods in infection diagnosis, its sensitivity for low-biomass pathogens (e.g., early-stage or localized infections) may still lag targeted PCR assays. For instance, Xpert MTB/RIF has demonstrated higher sensitivity in diagnosing spinal tuberculosis.⁶⁰ Therefore, while mNGS is a powerful tool, positive histopathological or culture results remain the diagnostic gold standard for spinal infections in clinical practice.

Application of mNGS in diagnosing various types of spinal infections

Pyogenic spondylitis

Pyogenic spondylitis refers to spinal infection caused by pyogenic bacteria, which usually manifests as vertebral osteomyelitis and discitis. The annual incidence ranges from 0.4 to 2.0 cases per 100,000 population, with its occurrence being associated with chronic debilitating conditions, age, and gender (males are affected 1.5–3 times more frequently than females).^{61–63} While mortality rate remains low, untreated pyogenic spondylitis may form abscesses at the primary lesion. The abscess may not only compress the nerves, which can cause neurological symptoms including paralysis, but also destroy the vertebral body

and intervertebral discs leading to kyphosis deformity.⁶⁴ Therefore, early and accurate diagnosis and treatment are crucial for patients with septic spondylitis.

The gold standard for diagnosing pyogenic spondylitis remains pathogen isolation through culture. However, culture-based methods depend on pathogen cultivability, are time-consuming, and prone to missed detections.⁶⁵ mNGS technology has demonstrated significant advantages in diagnosing pyogenic spondylitis, showing promising clinical utility: Qi et al.³² detected, by performing mNGS on 17 surgical specimens, that the common pathogens of pyogenic spinal infections, including *S. aureus*, *Streptococcus*, *E. coli*, *Brucella*, and various *Bacillus* species. Notably, the sensitivity of mNGS (82.4%) was significantly higher than that of conventional microbial culture (5.9%) ($p < 0.001$). Li et al.³³ analyzed data from 301 patients with PS and discovered a broad pathogen spectrum (41 species in total) via mNGS. The most common Gram-positive bacterium was *S. aureus*, while the most prevalent Gram-negative bacterium was *E. coli*. Furthermore, mNGS detected several rare microorganisms, including *Prevotella* (four cases), *Bacteroides fragilis* (two cases), *Coxiella burnetii* (four cases), *Cutibacterium acnes* (two cases), and *Clostridium perfringens* (one case). In one case, mNGS simultaneously identified *Porphyromonas gingivalis*, *Treponema denticola*, and *S. constellatus*. Liu et al.³⁴ subjected 54 samples to both conventional microbial culture and mNGS. The mNGS assay detected 15 pathogenic microorganisms in total, with *S. aureus* accounting for 38.8%, *E. coli* for 16.3%, and *Streptococcus* for 8.2%, along with rare cases of *Salmonella typhi* and *Prevotella intermedia* (one case each). The detection rate in the mNGS group (90.7%, 49 cases) was significantly higher than that in the conventional microbial culture group (59.2%, 32 cases). Additionally, the average turnaround time for mNGS (1.4 ± 0.3 days) was shorter than that for conventional microbial culture (7.3 ± 2.5 days), with both differences being statistically significant (the summary of the above studies is shown in Table 4). The results of these studies demonstrate the promising potential of mNGS in the diagnosis of pyogenic spondylitis.

Granulomatous spinal infections

Granulomatous infections refer to spinal infections caused by specific pathogens, often presenting as

granulomatous lesions.^{1–3} These include spinal tuberculosis, brucellar spondylitis, fungal spondylitis, and parasitic spondylitis.

Tuberculosis (TB) is an ancient infectious disease caused by *M. tuberculosis*, threatening public health.⁶⁶ According to the 2023 Global TB Report, approximately 10.6 million people developed TB in 2022, with an incidence rate of 133 per 100,000 population.⁶⁷ Spinal tuberculosis, the most common form of osteoarticular TB, accounts for 50% of cases.⁶⁸ Common diagnostic methods include mNGS, microbial culture, Xpert MTB/RIF assay, and T-SPOT. Li et al.⁶⁹ reported that the sensitivities of T-SPOT, Xpert MTB/RIF, mNGS, mNGS + Xpert MTB/RIF, and mNGS + T-SPOT tests were 92.7%, 53.7%, 39.0%, 73.2%, and 97.6%, respectively. Thus, combining mNGS can enhance the sensitivity of both Xpert MTB/RIF and T-SPOT.TB tests. Current research applications of mNGS for spinal tuberculosis detection include: Ye et al.³⁷ reported a rare case of disseminated TB diagnosed via mNGS, which detected *M. tuberculosis* complex in both spinal surgical specimens and bronchoalveolar lavage fluid. Guo et al.³⁵ detected 34 cases of tuberculous infection using mNGS among 112 patients and demonstrated that mNGS outperformed microbial culture in detection rates of spinal infection (80.4% vs 19.6%), spinal TB (69.39% vs 34.69%), and non-tuberculous infections (88.89% vs 53.97%). Jin et al.²³ identified *M. tuberculosis* as the predominant pathogen detected by mNGS. The overall detection rate of mNGS reached 62.5%, significantly higher than that of conventional culture methods (30%). Jin et al.³⁶ analyzed 203 specimens, identifying 79 cases of positive *M. tuberculosis* via mNGS. Notably, mNGS could differentiate TB from non-tuberculous infections, which is a capability lacking in TB-specific assays like Xpert MTB/RIF. Additionally, when compared using a composite reference standard (CRS), mNGS and culture showed comparable sensitivities (71.2% vs 73.0%). Li et al.³⁸ detected 36 cases of spinal tuberculosis using mNGS in 100 samples from 114 patients. Their key findings revealed that for spinal tuberculosis: There is no statistically significant difference in sensitivity between mNGS and either Xpert or T-SPOT.TB ($p=1.000$ and $p=0.430$, respectively). The sensitivity of the mNGS assay was higher than that of MGIT 960 culture ($p<0.001$) and pathological examination ($p=0.006$). For non-tuberculous infections: The

sensitivity of the mNGS assay was higher than that of bacterial culture ($p<0.001$) and pathological examination ($p<0.001$). (The summary of the above studies is shown in Table 5).

Brucellosis, a zoonosis caused by *Brucella* spp., is endemic in northern China and frequently misdiagnosed. Du et al.³⁹ documented a non-endemic case of brucellar spondylitis confirmed by mNGS. Yin et al.⁴⁰ identified *Brucella* infection in 15.79% of cases (15/95) within their cohort of spinal infection patients. In a separate study, Lv et al.⁴¹ detected *B. melitensis* in 3 of 76 consecutive patients with suspected spinal infections (The summary of the above studies is shown in Table 6). Multiple studies have identified *Brucella* in clinical samples, highlighting mNGS as a powerful tool to enhance detection accuracy for specific pathogens and guide a variety of infectious diseases, particularly rare or diagnostically challenging cases.

The role of mNGS in the treatment of spinal infections

The high detection rate (90.7%³⁴) and sensitivity (85.7%²²) of mNGS enable precise pathogen identification, facilitating accurate diagnosis and targeted therapy while mitigating antibiotic misuse and resistance development. Additionally, its rapid turnaround time (36–48h²³) supports early diagnosis, thereby improving therapeutic effectiveness and reducing complications (e.g., spinal cord compression, systemic infection). Furthermore, its broad pathogen coverage enhances detection of rare microorganisms and culture-negative viruses, significantly decreasing misdiagnosis and missed diagnoses. Zhang et al.⁴ demonstrated that mNGS-guided therapy resulted in progressive declines in postoperative C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels, and by 30 days post-operation, there was a statistically significant difference in ESR between empiric and targeted treatment groups. Qi et al.³² administered targeted antibiotic therapy based on mNGS in 14 patients, resulting in marked reductions in postoperative visual analog score, WBC, PCT, and CRP ($p<0.01$). Jin et al.²³ provided anti-*Nocardia* treatment to the patient who was diagnosed with *Nocardia* spp. via mNGS, leading to significant radiographic improvement on follow-up MRI. In summary, with the assistance of mNGS technology, most of the patients who received

timely treatment recovered well, fully demonstrating the value of early diagnosis in guiding the precise treatment of IDS. However, the detection cost of mNGS remains high and variable, depending on the sequencing technology employed and other factors, which may substantially increase the economic burden on patients.⁵⁸ In addition, mNGS requires a high-throughput sequencing platform and a complex bioinformatics analysis system. The associated equipment and maintenance costs are high, placing high demands on healthcare organizations. Furthermore, mNGS testing has not yet been widely included in health insurance reimbursement, limiting its adoption in the clinic.

Impact of sample type on detection efficacy

In the diagnosis of infectious diseases such as spinal infections, the choice of sample type is a critical factor determining detection sensitivity. Although most spinal infections are transmitted via the bloodstream, suggesting the potential presence of pathogens in the blood, samples collected directly from the infection site (including tissue and pus) demonstrate superior diagnostic performance in practice. Multiple studies provide evidence supporting this: Qi et al.³² reported that for pyogenic spinal infections, the sensitivity of mNGS using site-specific samples (tissue or pus) was 82.4%, whereas the sensitivity of conventional culture using blood samples was only 5.9%. In the study by Wan et al.,⁴⁵ when blood culture was used as the gold standard, the sensitivity and specificity of mNGS were 66.7% and 12.5%, respectively. However, when the gold standard combined results from blood culture and other cultures (sputum, catheter drainage fluid, and urine), the sensitivity and specificity of mNGS increased to 86.4% and 20.0%. The above results indicate that blood culture has certain limitations as a gold standard; moreover, when the sample type expands from blood alone to include tissue or pus, the detection efficacy of mNGS shows an improvement.

When focusing on site-specific samples, mNGS technology demonstrates higher sensitivity compared to traditional methods. For instance, Zhang et al.⁴⁹ concurrently performed mNGS and microbial culture on 21 pus samples and found that the sensitivity of mNGS was 84.2%, compared to 42.1% for culture. Similarly, Li et al.³⁸ reported that for site-specific samples (tissue and

pus), the sensitivity of mNGS reached 89.0%, significantly higher than that of culture (including MGIT 960 and conventional culture, 28.1%) and histopathological examination (42.9%). These data not only highlight the importance of sample type for detection efficacy but also reveal the value of site-specific samples in the diagnosis of spinal infections, which are considered one of the preferred sample types for achieving accurate diagnosis.

Limitations and future perspectives

The clinical application of mNGS for detecting spinal infections faces several practical challenges. Although mNGS can identify a broad spectrum of pathogens, it cannot definitively discriminate between current infection, opportunistic colonization, or commensal organisms, nor can it confirm active infection. Therefore, the results of mNGS must always be interpreted in correlation with clinical manifestations, imaging findings, inflammatory markers (such as CRP and ESR), and histopathological evidence.

Furthermore, the widespread adoption of mNGS is confronted with significant technical limitations. These include the risk of sample contamination, amplicon contamination, interference from high levels of host nucleic acids, a lack of standardized procedures, and a dependency on skilled bioinformatic experts—especially in low-resource settings. To further improve the detection efficacy and clinical application value of mNGS, the following improvements could be implemented: (1) Comprehensive technical optimization: optimize sample processing and analytical methods to reduce background and host DNA interference. Enhance nucleic acid extraction protocols and expand microbial sequence databases to improve test specificity and sensitivity. (2) Implementation of fully automated mNGS workflows: for example, Luan et al.⁷⁰ developed an integrated system (NGSmaster) that automates wet laboratory workflows, including nucleic acid extraction, PCR-free library preparation, and purification. Since most experimental procedures are performed in closed chambers without requiring PCR amplification, this fully automated system can significantly reduce hands-on time while minimizing microbial contamination, thereby streamlining the mNGS process to reduce testing costs and healthcare burdens. (3) Standardization of mNGS testing protocols: establish unified procedures for consistent

and reproducible results across laboratories. (4) Expansion of clinical applications: broaden the use of mNGS to detect drug-resistant genes and dynamically monitor pathogen load and resistance gene profiles during treatment. This approach would provide more accurate, real-time clinical evidence to guide therapy, promote faster patient recovery, and prevent complications.

Limitations of this review

This study has several limitations. (1) Although we systematically searched both Chinese and English databases, the original data from all included studies were not available and were not supplemented by contacting the authors, which may have resulted in incomplete data extraction. (2) The inclusion criterion of “impact factor greater than 1,” intended to ensure quality, may have excluded valuable studies published in emerging or regional journals, thereby introducing selection bias. (3) The high heterogeneity among the included studies precluded a meta-analysis to obtain more precise effect estimates, thus necessitating a narrative synthesis.

Summary

mNGS is an advancing pathogen detection technology with broad pathogen spectrum, high detection rate and sensitivity, and rapid turnaround time, which plays a pivotal role in the diagnosis and treatment of both pyogenic spondylitis and granulomatous spinal infections. Compared with traditional diagnostic techniques, the results of mNGS can not only provide key evidence for the diagnosis of IDS, reducing the missed or misdiagnosed cases, but also guide the precise treatment and improve the prognosis of patients with spinal infections, which has broad clinical application prospects. However, it is noteworthy that the choice of sample type can significantly affect the diagnostic performance of mNGS. Additionally, mNGS still faces common challenges such as specificity, cost, and technical difficulty, which need to be systematically overcome by further in-depth research. In the future, with the development of interdisciplinary technology, the advancement of clinical multidisciplinary model, and the guidance of intelligent demand in complex clinical scenarios, mNGS will provide more comprehensive and efficient support for the precise diagnosis and treatment of IDS.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions

Ziyan Zhu: Writing – original draft.

Xinxin Miao: Writing – review & editing.

Acknowledgments

The authors gratefully acknowledge Queen Mary University of London for providing institutional access to critical academic databases and electronic library resources essential for this comprehensive review. The graphical abstract was created using BioRender.com. Special thanks are extended to Jianjian Deng for his assistance in refining the core literature search strategy, Rui Ding for supporting the mapping of research progress to visually present the development trajectory of the field and supplementing case studies, Huajun Pan for contributing to the writing of the core theoretical sections and analyzing research hotspots and controversies, and Dingwen He for reviewing the accuracy of literature citations, addressing logical inconsistencies, and optimizing the structure and language of the review.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Key Project of Jiangxi Province Key Research and Development Program [20243BB191009]; Youth Fund Project of Jiangxi Province Natural Science Foundation [20224BAB216032].

Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

This is a review article, and all data cited are available in the referenced publications.

ORCID iDs

Ziyan Zhu  <https://orcid.org/0009-0008-7661-5948>

Xinxin Miao  <https://orcid.org/0009-0008-0519-3901>

Supplemental material

Supplemental material for this article is available online.

References

1. Lener S, Hartmann S, Barbagallo GMV, et al. Management of spinal infection: a review of the literature. *Acta Neurochir (Wien)* 2018; 160: 487–496.
2. Liu X, Zheng M, Sun J, et al. A diagnostic model for differentiating tuberculous spondylitis from pyogenic spondylitis on computed tomography images. *Eur Radiol* 2021; 31: 7626–7636.
3. Tsantes AG, Papadopoulos DV, Vrioni G, et al. Spinal infections: an update. *Microorganisms* 2020; 8: 476.
4. Zhang G, Zhang H, Hu X, et al. Clinical application value of metagenomic next-generation sequencing in the diagnosis of spinal infections and its impact on clinical outcomes. *Front Cell Infect Microbiol* 2023; 13: 1076525.
5. Grammatico L, Baron S, Rusch E, et al. Epidemiology of vertebral osteomyelitis in France: analysis of hospital-discharge data 2002–2003. *Epidemiol Infect* 2008; 136: 653–660.
6. Fragió Gil JJ, González Mazarío R, Salavert Lleti M, et al. Vertebral osteomyelitis: clinical, microbiological and radiological characteristics of 116 patients. *Med Clin (Barc)* 2020; 155: 335–339.
7. Bhattacharyya S and Bradshaw MJ. Infections of the spine and spinal cord. *Continuum (Minneap Minn)* 2021; 27: 887–920.
8. Zheng YH, Cao P, Chen Z, et al. Spinal infection. *Int Orthop* 2014; 35: 24–26.
9. Li C, Xiao N, Ke B, et al. Application of metagenomic next-generation sequencing in suspected spinal infectious diseases. *World Neurosurg* 2024; 185: e542–e548.
10. Li N, Cai Q, Miao Q, et al. High-throughput metagenomics for identification of pathogens in the clinical settings. *Small Methods* 2021; 5: 2000792.
11. Hu T, Chitnis N, Monos D, et al. Next-generation sequencing technologies: an overview. *Hum Immunol* 2021; 82: 801–811.
12. Goodwin S, McPherson JD and McCombie WR. Coming of age: ten years of next-generation sequencing technologies. *Nat Rev Genet* 2016; 17: 333–351.
13. Nath K, Miao Z, Jiang M, et al. Comparative analysis of sequencing technologies for single-cell transcriptomics. *Genome Biol* 2019; 20: 70.
14. Liu L, Li Y, Li S, et al. Comparison of next-generation sequencing systems. *J Biomed Biotechnol* 2012; 2012: 251364.
15. CD Genomics. 16S rRNA sequencing vs. metagenome sequencing: a guide for selection, <https://www.cdgenomics.com/genome-research/> (2025, accessed 18 August 2025).
16. Lindahl BD and Nilsson RH. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. *New Phytol* 2013; 199: 288–299.
17. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; 464: 59–65.
18. Integrated DNA Technologies, Hybridization capture, <https://www.idtdna.com/pages/products-and-services/next-generation-sequencing/dna-sequencing/targeted-sequencing/hybridization-capture> (2025, accessed 18 August 2025).
19. Peay JF, Bruns TD and Taylor DL. Assessment of primer bias in high-throughput sequencing of fungal communities. *Fungal Ecol* 2010; 3: 116–124.
20. Tedersoo L, Bahram M and Põlme S. Challenges and perspectives in fungal metagenomics. *Curr Opin Biotechnol* 2014; 25: 89–94.
21. Mertes F, ElSharawy A and Sauer S. Targeted enrichment of genomic DNA regions for next-generation sequencing. *Brief Funct Genomics* 2011; 10: 374–386.
22. Li Z, Zhang Q, Wang H, et al. Multicenter study on detection of pathogens in primary spinal infectious diseases using metagenomic next-generation sequencing. *Chin J Surg* 2024; 62: 1128–1135.
23. Jin W, Li N, Zhuo X, et al. Etiological diagnostic value of metagenomic next-generation sequencing in spinal infection. *Chin J Clin Med* 2022; 27: 567–571.
24. Wilson MR, Naccache SN, Samayoa E, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med* 2014; 370: 2408–241.

25. Greninger AL, Waghmare A, Adler A, et al. Rule-out outbreak: 24-hour metagenomic next-generation sequencing for characterizing respiratory virus source for infection prevention. *J Pediatric Infect Dis Soc* 2017; 6: 168–172.
26. Zhang CF, Wang CX, Chen F, et al. Metagenomic next-generation sequencing technique helps identify cryptococcal infection in the rib: a report of 2 cases and review of the literature. *JBJS Case Connect* 2019; 9: e0367.
27. Wilson MR, Sample HA, Zorn KC, et al. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. *N Engl J Med* 2019; 380: 2327–2340.
28. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; 372: n71.
29. Shi T, Lin Y, Zheng X, et al. Metagenomic next-generation sequencing for the clinical identification of spinal infection-associated pathogens. *Front Cell Infect Microbiol* 2025; 14: 1437665.
30. Zhang Q, Li J, Zhang Q, et al. Influence factors of metagenomic next-generation sequencing negative results in diagnosed patients with spinal infection. *Diagn Microbiol Infect Dis* 2024; 109: 116278.
31. Huang H, Shi J, Zheng M, et al. Pathogen detection in suspected spinal infection: metagenomic next-generation sequencing versus culture. *Eur Spine J* 2023; 32: 4220–4228.
32. Qi M, Du Y, Guan J, et al. The clinical management and efficacy of metagenomic next-generation sequencing in patients with pyogenic spinal infection: a single-center cohort study. *J Orthop Surg Res* 2024; 19: 51.
33. Li Z, Zhang Q, Lian X, et al. Value of metagenomic next-generation sequencing in the diagnosis of native pyogenic spinal infections: a multicenter, retrospective observational study. *Spine J* 2025; 25: 640–648.
34. Liu F, Zu G, Shi S, et al. Application of metagenomic next-generation sequencing in pathogenic diagnosis of pyogenic discitis and vertebral osteomyelitis. *Chin J Bone Joint Inj* 2023; 38: 1–9.
35. Guo L, Yu L, Li L, et al. Diagnostic value of metagenomic next-generation sequencing technology for tuberculous and nontuberculous spinal infectious diseases. *Acad J Chin PLA Med Sch* 2024; 45: 457–480.
36. Jin Y, Hu S, Feng J, et al. Clinical value of metagenomic next-generation sequencing using spinal tissue in the rapid diagnosis of spinal tuberculosis. *Infect Drug Resist* 2023; 16: 3305–3313.
37. Ye Y, Yang N, Zhou J, et al. Case report: Metagenomic next-generation sequencing in diagnosis of disseminated tuberculosis of an immunocompetent patient. *Front Med* 2021; 8: 687984.
38. Li Y, Yao X, Tang L, et al. Diagnostic efficiency of metagenomic next-generation sequencing for suspected spinal tuberculosis in China: a multicenter prospective study. *Front Microbiol* 2022; 13: 1018938.
39. Du J, Tao Y, Yang J, et al. Case report: metagenomic next-generation sequencing confirmed a case of spine infection with *Brucella melitensis* in non-endemic area. *Infect Drug Resist* 2023; 16: 7219–7225.
40. Yin C, Cong Y, Wang H, et al. Pathogenic detection by metagenomic next-generation sequencing in spinal infections. *Spine (Phila Pa 1976)* 2025; 50: E70–E75.
41. Lv H, Liao S, Shi Z, et al. Application of metagenomic next-generation sequencing for rapid molecular identification in spinal infection diagnosis. *Front Cell Infect Microbiol* 2024; 14: 1382635.
42. Liu X, Yin C, Cong Y, et al. Value of metagenomic next-generation sequencing in conservative treatment of primary spinal infection. *Chin J Bone Joint Surg* 2023; 16: 433–438.
43. Wang G, Long J, Zhuang Y, et al. Application of metagenomic next-generation sequencing in the detection of pathogens in spinal infections. *Spine J* 2023; 23: 859–867.
44. Wang Z. The application of metagenomic next-generation sequencing technology in the pathogenic diagnosis of spinal infection. *Master's Thesis, Fujian Medical University, China*, 2021.
45. Wan J, Duan L, Chen Q, et al. Potential clinical impact of metagenomic next-generation sequencing of plasma for cervical spine injury with sepsis in intensive care unit: a retrospective study. *Front Cell Infect Microbiol* 2022; 12: 948602.
46. Peng M, Li W, Li F, et al. *Mycobacterium xenopi* related spine infections: a case report and systematic literature review. *One Health* 2023; 16: 100502.

47. Gu H, Cai Q, Dai X, et al. A case report of *Klebsiella aerogenes*-caused lumbar spine infection identified by metagenome next-generation sequencing. *BMC Infect Dis* 2022; 22: 512.
48. Wang C, Hu J, Gu Y, et al. Application of next-generation metagenomic sequencing in the diagnosis and treatment of acute spinal infections. *Heliyon* 2023; 9: e13951.
49. Zhang Y, Chen J, Yi X, et al. Evaluation of the metagenomic next-generation sequencing performance in pathogenic detection in patients with spinal infection. *Front Cell Infect Microbiol* 2022; 12: 967584.
50. Long SP, Liu ZL, Huang SH, et al. Application of metagenomics next-generation sequencing in the diagnosis and treatment of spinal infection. *J Pract Med* 2024; 40(1): 119–128.
51. Du C, Li Q, Wang Z, et al. Diagnosis of spinal fungal infection: analysis of 11 cases. *Chin J Bone Joint Surg* 2020; 13: 819–824.
52. Majmundar N, Patel PD, Dodson V, et al. Parasitic infections of the spine: case series and review of the literature. *Neurosurg Focus* 2019; 46: E12.
53. Xia Y and Chen LY. Letters to the editor: parasitic spinal infections present as a lobulated, multilocular, septated cystic mass. *Neurosurg Focus* 2019; 46(6): E13.
54. Mayday MY, Khan LM, Chen A, et al. Miniaturization and optimization of 384-well compatible RNA sequencing library preparation. *PLoS One* 2019; 14: e0206194.
55. He Y, Fang K, Shi X, et al. Enhanced DNA and RNA pathogen detection via metagenomic sequencing in patients with pneumonia. *J Transl Med* 2022; 20: 123.
56. Zhang D. *Research on the value of metagenomic next-generation sequencing technology in the diagnosis of etiology in patients with spinal infection*. Master's Thesis, Henan University, China, 2023.
57. Zhou J, Wang R, Huo X, et al. Incidence of surgical site infection after spine surgery: a systematic review and meta-analysis. *Spine (Phila Pa 1976)* 2020; 45: 208–216.
58. Cheng H, Wu H, Tan N, et al. Diagnostic efficacy of metagenomic next-generation sequencing in patients with spinal infections: a retrospective study. *Infect Drug Resist* 2023; 16: 7613–7620.
59. Lin W, Xie F, Li X, et al. Diagnostic performance of metagenomic next-generation sequencing and conventional microbial culture for spinal infection: a retrospective comparative study. *Eur Spine J* 2023; 32: 4238–4245.
60. Edmiston CE, Leaper DJ, Chitnis AS, et al. Risk and economic burden of surgical site infection following spinal fusion in adults. *Infect Control Hosp Epidemiol* 2023; 44: 88–95.
61. Lam KS and Webb JK. Discitis. *Hosp Med* 2004; 65: 280–286.
62. Hopkinson N, Stevenson J and Benjamin S. A case ascertainment study of septic discitis: clinical, microbiological and radiological features. *QJM* 2001; 94: 465–470.
63. Tali ET. Spinal infections. *Eur J Radiol* 2004; 50: 120–133.
64. Lin J, Lin Y, Luo J, et al. Diagnosis and treatment of pyogenic spondylitis (a report of 67 cases). *J Spinal Surg* 2021; 19: 172–176.
65. Zhang SC, Li YX, Mo L, et al. Analysis of clinical characteristics of 20 patients with pyogenic spondylitis [in Chinese]. *China J Orthop* 2016; 29: 923–927.
66. Mashabela GT, De Wet TJ and Warner DF. Mycobacterium tuberculosis metabolism. *Microbiol Spectr* 2019; 7: 665.
67. Wei S and Liu YH. Dedicated to innovation, practicing to achieve targets: interpretation of research & innovation part of the WHO Global Tuberculosis Report 2023 [in Chinese]. *Zhongguo Fang Lao Za Zhi* 2024; 46: 613–617.
68. Li T, Yan X, Du X, et al. Extrapulmonary tuberculosis in China: a national survey. *Int J Infect Dis* 2022; 128: 69–77.
69. Li Z, Wang J, Xiu X, et al. Evaluation of different diagnostic methods for spinal tuberculosis infection. *BMC Infect Dis* 2023; 23: 86.
70. Luan Y, Hu H, Liu C, et al. A proof-of-concept study of an automated solution for clinical metagenomic next-generation sequencing. *J Appl Microbiol* 2021; 131: 1007–1016.

Visit Sage journals online
journals.sagepub.com/
home/tai

 Sage journals