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REVIEW ARTICLE

Pathogen detection in suspected spinal infection: metagenomic next-generation sequencing versus culture

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Abstract

Purpose The aim is to compare the pathogen detection performance of metagenomic next-generation sequencing (mNGS) and the culturing of percutaneous needle biopsy samples obtained from an individual with a suspected spinal infection.

Methods A retrospective study of 141 individuals with a suspected spinal infection was conducted, and mNGS was performed. The microbial spectra and detection performance between mNGS and the culturing-based method were compared, and the effects of antibiotic intervention and biopsy on the detection performance were assessed.

Results The microorganisms isolated most commonly via the culturing-based method were *Mycobacterium tuberculosis* (n = 21), followed by *Staphylococcus epidermidis* (n = 13). The most common microorganisms detected via mNGS were *Mycobacterium tuberculosis complex (MTBC)* (n = 39), followed by *Staphylococcus aureus* (n = 15). The difference in the type of detected microorganisms between culturing and mNGS was observed only in *Mycobacterium* ($P = 0.001$). mNGS helped identify potential pathogens in 80.9% of cases, which was significantly higher than the positivity rate of 59.6% observed for the culturing-based method ($P < 0.001$). Moreover, mNGS had a sensitivity of 85.7% (95% CI, 78.4% to 91.3%), a specificity of 86.7% (95% CI, 59.5% to 98.3%), and sensitivity gains of 35% (85.7% vs. 50.8%; $P < 0.001$) during culturing, while no differences were observed in the specificity (86.7% vs. 93.3%; $P = 0.543$). In addition, antibiotic interventions significantly lowered the positivity rate of the culturing-based method (66.0% vs. 45.5%, $P = 0.021$) but had no effects on the results of mNGS (82.5% vs. 77.3%, $P = 0.467$).

Conclusion The use of mNGS could result in a higher detection rate compared to that observed with the culturing-based method in an individual with spinal infection and is particularly valuable for evaluating the effects of a mycobacterial infection or previous antibiotic intervention.

Keywords Spinal infection · Metagenomic next-generation sequencing · Culture · Diagnosis · Pathogen · Percutaneous needle biopsy

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Introduction

Spinal infections continue to affect individuals worldwide [1]. It is a severe infectious disease of the spine or paraspinal structures caused by a wide variety of organisms, representing a heterogeneous group of disorders that includes discitis, spondylitis, spondylodiscitis, and epidural abscess [1, 2]. An increased incidence of spinal infection has been observed with an increase in the predisposing risk factors in recent years [3]. Three major agents responsible for causing spinal infections include bacteria, tuberculosis, and brucella. They are responsible for causing infections such as pyogenic and granulomatosis; other less common infections are caused by microbes such as fungi and parasites [4, 5]. Diagnosis is generally difficult because the symptoms are non-specific, leading to a significant delay in treatment [6]. Bacteriological analysis is crucial for the early diagnosis and prompt treatment of spinal infections, but the identification of the etiological agent is a major challenge in clinical practice [7].

Percutaneous needle biopsy (PNB) is a rapid, cost-effective, and safe procedure that has been recognized as a valuable method for achieving a microbiologic or pathologic diagnosis [8]. The culturing yield reportedly ranges from 30 to 75% and is higher in individuals not subjected to an antibiotic intervention [9, 10]. Metagenomic next-generation sequencing (mNGS) is an emerging diagnostic platform that enables the broad detection of organisms and facilitates the rapid identification of species in infectious diseases via high-throughput sequencing technology and bioinformatic analysis [11]. Theoretically, mNGS could identify all potential pathogens, and even rare microbiome components, in a sample without bias [12]. This technique has significantly improved microbial diagnostic efficiency in patients with neurological infections, osteoarticular infections, and periprosthetic joint infections [13–15].

To our knowledge, few studies have evaluated the use of mNGS for identifying pathogens in spinal infections. This study aimed to compare the pathogen detection performance of the mNGS and culturing methods using PNB specimens in cases of suspected spinal infection.

Materials and methods

Study population

We received approval from the ethics committee to perform a retrospective study of patients who were admitted to our center from August 2018 to August 2021, diagnosed with a suspected spinal infection, and underwent PNB. The diagnosis of suspected spinal infection was based on clinical symptoms, laboratory examination, and compatible imaging features [16, 17]. The following patients were included: (a) patients with a preliminary diagnosis indicating a suspected spinal infection who had undergone PNB; (b) patients whose age was ≥ 18 years; (c) patients whose biopsy specimens underwent bacterial culturing, histopathological examination, and mNGS analysis. Patients were excluded if they had spinal infections due to trauma or invasive examination, surgical site infections (SSIs), spinal tumors confirmed by histopathology, and incomplete clinical data.

Definition

Based on previous studies [16, 17], a suspected spinal infection was defined as a new or worsening spine pain and/or neurologic symptoms and at least one of the following presentations: fever, elevated erythrocyte sedimentation rate (ESR) or C reactive protein (CRP) levels, bloodstream infections or infective endocarditis, a recent episode of a *Staphylococcus aureus* bloodstream infection, imaging features compatible with discitis, spondylitis, or spondylodiscitis (vertebral end-plate destruction, disc inflammation, and presence of necrosis or pus in the disc space and paraspinal soft tissues and epidural spaces). SSIs are defined by the Centers for Disease Control and Prevention as superficial or deep infections that occur within 30 days after a surgical procedure or within 1 year if the hardware is retained [18]. The matching relationships between mNGS and culturing results were classified as a match, mismatch, and partial match at the species or genus level. A partial match was defined as at least one overlap of pathogens detected in polymicrobial results [19]. Histopathology positivity is defined as the demonstration of acute, chronic, or granulomatous inflammation, including inflammatory infiltration, congested or thrombosed blood vessels, bone necrosis, and/or infectious organisms [8].

Data collection

The data collected included age, gender, comorbidities, antibiotic intervention, previous history of tuberculosis, CRP and ESR levels, biopsy procedures (biopsy site, biopsy level), microbiological culturing results, histopathological reports, and mNGS results. All examinations were performed in the laboratory of our hospital.

Sample collection and microbiologic culturing

Anatomical imaging was performed via a computed tomography (CT) or magnetic resonance imaging (MRI) in all cases to evaluate the location of the infectious lesion. PNB

procedures were performed by experienced surgeons to obtain specimens subjected to local anesthesia under fluoroscopy or CT guidance. All fresh biopsy specimens were immediately transported to microbiology and pathology laboratories for microbiologic culturing and histopathological examination. A microbiology laboratory examination consisted of combinations of routine bacterial (aerobic and anaerobic), fungal, and mycobacterial cultureing processess. In addition, each biopsy sample was also sent for mNGS analysis.

Metagenomic next-generation sequencing analysis

The mNGS assay of all biopsy samples was performed by the Precision Medical Center laboratory of our hospital. Each specimen was processed immediately upon arrival at the laboratory, and DNA was extracted from specimens and used for library construction. The process of constructing a DNA library included ultrasonic DNA fragmentation, end-repair, ligation with barcode adapters, and polymerase chain reaction (PCR) amplification [12]. The Agilent 2100 Bioanalyzer was used to perform the quality control-related procedures for the DNA library, and DNA sequencing analysis was performed on the MGISEQ-50 platform.

Adapter sequences and low-quality reads were filtered out from the raw sequencing data to obtain clean reads for subsequent analysis. Subsequently, high-quality sequencing data were generated by subtracting the sequence reads mapped to the human reference genome (hg19) from clean reads using Burrows-Wheeler Alignment software [20]. Additionally, the remaining sequencing data were simultaneously aligned to RefSeq Microbial Genome Databases after the removal of low-complexity reads and host sequences and then classified as viruses, bacteria, fungi, and parasites. Reference sequences in the database assemblies were downloaded from the National Center for Biotechnology Information (NCBI, ftp://ftp.ncbi.nlm.nih.gov/genomes/). RefSeq contains 4945 whole genome sequences of DNA viral taxa, 6039 bacterial genomes or scaffolds, 1064 pathogenic fungi associated with human infections, and 234 parasites associated with human diseases.

Statistical analysis

Continuous variables were expressed as means ± standard deviation values, and categorical variables were expressed as numerical values (percentages). The detection rates of mNGS and culturing were counted. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and corresponding 95% confidence intervals (CIs) were calculated independently for mNGS and the culturing-based method from the reference standard using the etiological and histopathological results. The categorical data were analyzed using the χ^2 or Fisher's exact test. SPSS 22.0 software was used for statistical analysis, and a P value < 0.05 was considered statistically significant.

Results

Demographic characteristics

A total of 141 patients with a mean age of 54.18 ± 13.93 years were enrolled. Among these, 30 patients had diabetes, 29 had hypertension, 21 had a previous history of tuberculosis, and 44 were exposed to antibiotics prior to PNB. Preoperative CRP and ESR levels were 33.60 ± 45.10 mg/L and 54.52 ± 36.05 mm/h, respectively. The level of the lumbar spine and site of the intervertebral disc were the most common in patients who underwent biopsy procedures (Table 1).

Comparison of mNGS and culturing methods in detection of pathogens

The microbiological spectrum detected by mNGS and culturing methods is shown in Fig. 1. The most commonly isolated microorganisms in the culturing-based method were *Mycobacterium tuberculosis* (n = 21), followed by *Staphylococcus epidermidis* (n = 13), *Staphylococcus aureus* (n = 11), *Brucella* (n = 7), and *Escherichia coli* (n = 7), as shown in Fig. 1a. The most common microorganisms detected by mNGS were *Mycobacterium tuberculosis complex (MBTC)*

Table 1 Demographic characteristics of 141 cases enrolled

	Total (n = 141)
Demographic data and comorbidities	
Male; n(%)	86 (61.0%)
Age (years)	54.18 ± 13.93
Hypertension; n(%)	29 (20.6%)
Diabetes; n(%)	30 (21.3%)
Previous history of tuberculosis; n(%)	21 (14.9%)
Antibiotic intervention; n(%)	44 (31.2%)
Laboratory investigations	
CRP (mg/L)	33.60 ± 45.10
ESR (mm/h)	54.52 ± 36.05
Biopsy procedure	
Biopsy level	
Thoracic; n(%)	36 (25.5%)
Lumbar; n(%)	105 (74.5%)
Biopsy site	
Intervertebral disc; n(%)	100 (70.9%)
Vertebral body; n(%)	41 (29.1%)

WBC white blood cell, *CRP* c-reactive protein, *ESR* erythrocyte sedimentation rate

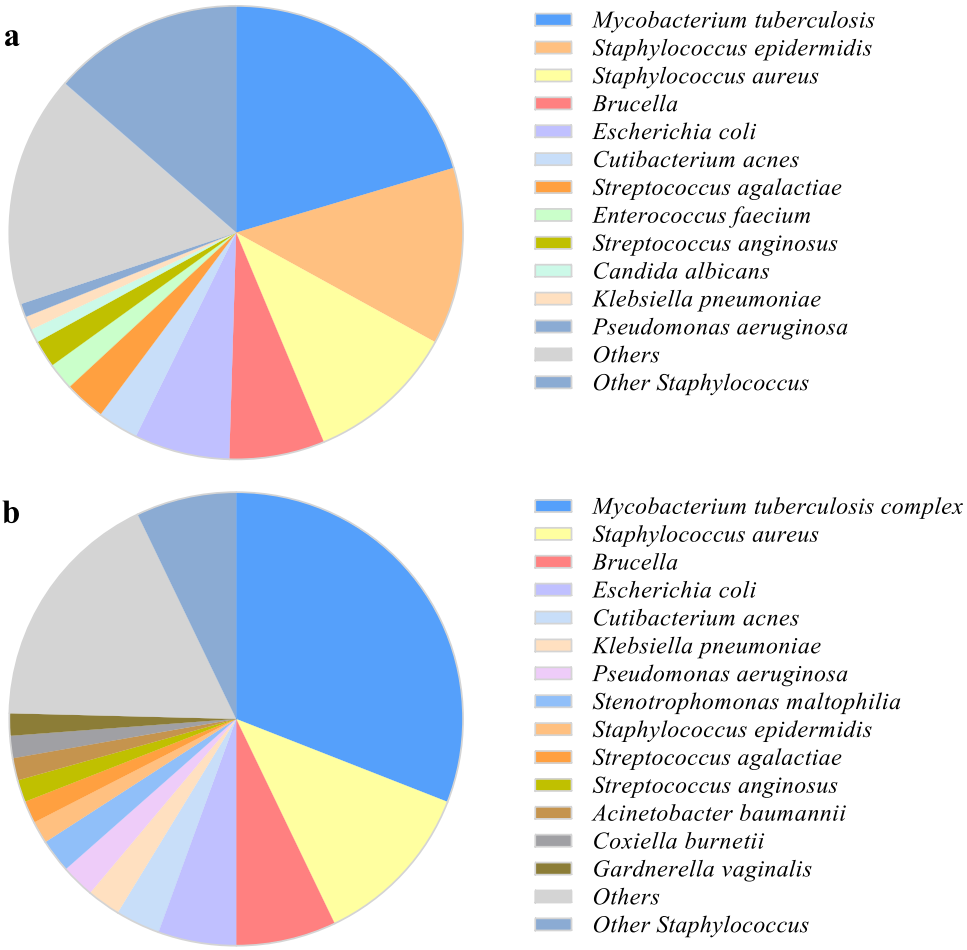

Fig. 1 The microbiological spectrum of culture (**a**) and mNGS (**b**)

(39 cases), followed by *Staphylococcus aureus* (15 cases), *Brucella* (9 cases), and *Escherichia coli* (7 cases), as shown in Fig. 1b.

The comparison of mNGS and the culturing-based method for microbiological stratification is shown in Fig. 2. Bacteria-causing pyogenic infections were detected most commonly, and a significant difference between culturing and mNGS was observed only for *Mycobacterium* ($P = 0.001$); see Fig. 2a. No significant difference was observed for all the other bacteria at the genus level (Fig. 2b).

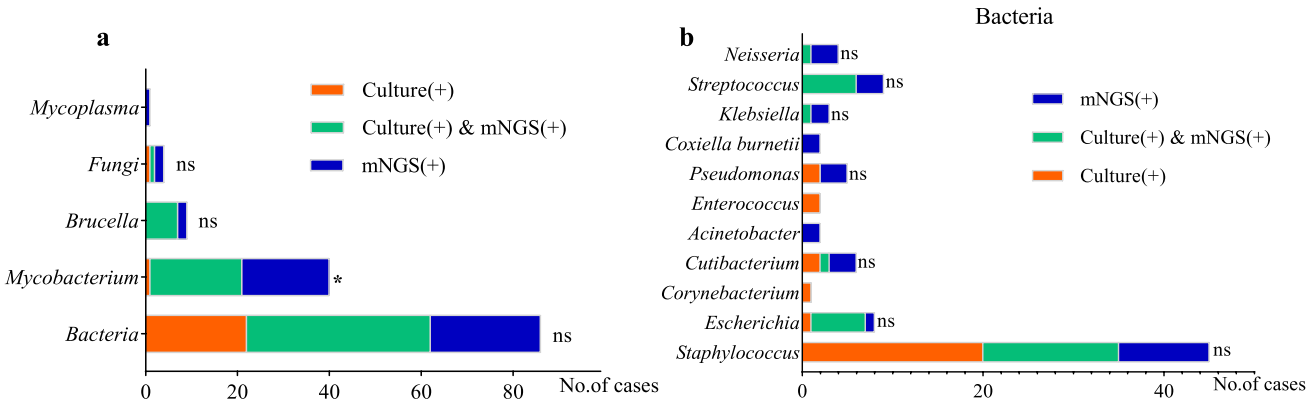

Fig. 2 Comparison of culture and mNGS in microbiological stratification **a** Comparison of pathogens stratified to bacteria, mycobacterium, brucella, fungi, and mycoplasma, **b** comparison of culture and mNGS in bacteria stratification. * $P < 0.05$

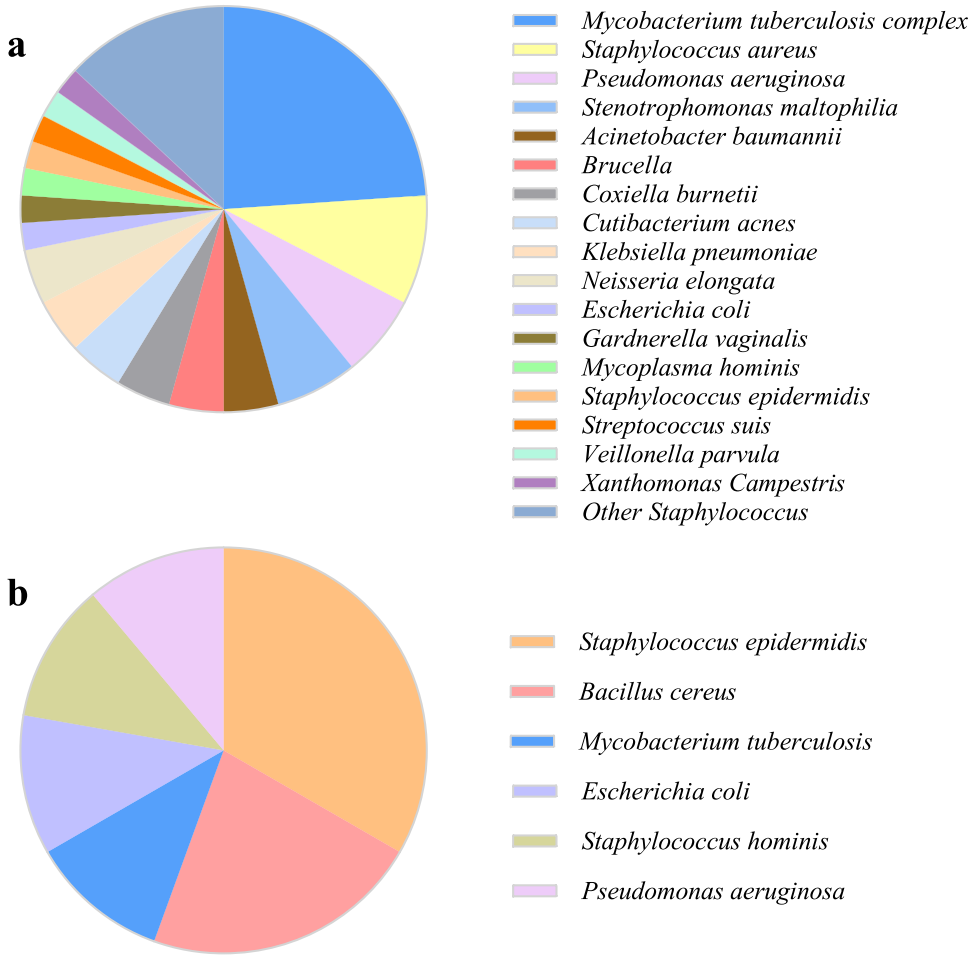

Fig. 3 Distribution of microbiology detected only by mNGS (**a**) or culture (**b**)

Moreover, 18 potentially pathogenic species were successfully detected solely by mNGS from culturing-negative samples (Fig. 3a). Among these species, *MBTC* was the most common (n = 11), whereas the other microorganisms included *Mycoplasma hominis*, *Gardnerella vaginalis*, *Coxiella burnetii*. However, 6 potential pathogens species were isolated only via the culturing-based method in 7 patients and could not be identified using mNGS, *Staphylococcus epidermidis* was the most common (n = 3); see Fig. 3b.

The detection rates and concordance between the mNGS and culturing processes

In this study, the detection rates of the culturing and mNGS processes were 59.6% (84/141) and 80.9% (114/141), respectively. The detection rate of mNGS significantly higher by 21%, compared to that observed for the culturinge-based method ($P < 0.001$). Additionally, positive results were obtained with both the culturing and mNGS methods in 77 cases (54.6%), and negative results were obtained with both the methods in 20 cases (14.2%); see Fig. 4a. Among the patients with double-positive results, 46 cases (59.7%) were matched, 13 cases (16.9%) were partially matched, and 18 cases (23.4%) were mismatched at the genus level (Fig. 4b). For the same set of samples, 42 cases (54.5%) were matched at the species level (Fig. 4c).

The detection performance of mNGS and culturing

Upon using the etiological and histopathological results as the reference standard, the culturing-based method exhibited a sensitivity of 50.8% (95% CI, 41.7% to 59.8%), specificity of 93.3% (95% CI, 68.1% to 99.8%), PPV of 98.5%, and NPV of 18.4%. Moreover, the mNGS exhibited a sensitivity of 85.7% (95% CI, 78.4% to 91.3%), specificity of 86.7% (95% CI, 59.5% to 98.3%), PPV of 98.2%, and NPV of 41.9%. In contrast, mNGS exhibited a gain in sensitivity of 35% (85.7% vs. 50.8%; $P < 0.001$) over the culturing method, while the specificity did not differ significantly between the two methods (86.7% vs. 93.3%; $P = 0.543$); see Table 2.

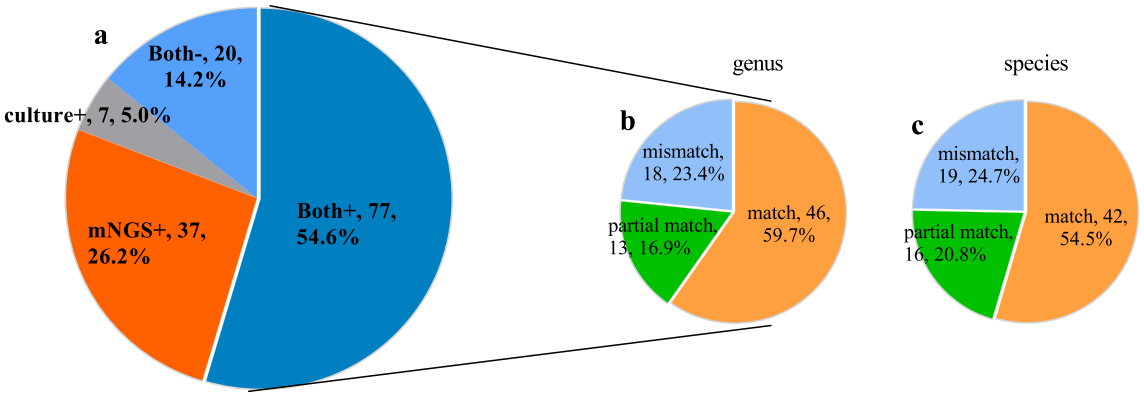

Fig. 4 The concordance of results between culture and mNGS, **a** the positivity distribution of mNGS and culture. The matching relationships between culture and mNGS at the genus level **b** and species level **c**. Both+, culture, and mNGS were both positive; Both−, culture and mNGS were both negative; mNGS+, only the mNGS was positive, culture was not; Culture+, only the culture was positive, mNGS was not

Table 2 Sensitivity and specificity of mNGS compared with culture

	mNGS	Culture	*P*
Sensitivity	85.7% (78.4%-91.3%)	50.8% (41.7%-59.8%)	<0.001
Specificity	86.7% (59.5%-98.3%)	93.3% (68.1%-99.8%)	0.543

Effects of antibiotic intervention and biopsy procedures on pathogen detection

In this study, 44 patients received antibiotic intervention before PNB. The detection rates of mNGS and the culturing-based method were 77.3% (34/44) and 45.5% (20/44), respectively, in patients subjected to antibiotic interventions ($P=0.003$). Among non-antibiotic interventions, the detection rates of mNGS and culturing-based method were 82.5% (80/97) and 66.0% (64/97), respectively ($P=0.002$). In comparison, the administration of antibiotics drugs significantly lowered the detection rate of the culturing-based method (66.0% vs. 45.5%, $P=0.021$), while having no effect on that of mNGS (82.5% vs. 77.3%, $P=0.467$), as shown in Fig. 5a. Moreover, the biopsy level and biopsy site had no significant effects on mNGS results. Interestingly, there was a significant difference between the detection rates of the culturing-based method (59.0%) and mNGS (81.0%) on the lumbar level ($P<0.001$), as shown in Fig. 5b. The site of intervertebral discs also significantly affected the detection rates of the culturing-based method and mNGS (59% vs. 82%, $P<0.001$), as shown in Fig. 5c.

Discussion

The mNGS method for pathogen detection is based on hypothesis-free sampling. It is a powerful tool that enables us to broadly recognize known as well as unexpected pathogens or even facilitate the discovery of new microorganisms and pathogens [12]. After applying this approach to PNB specimens obtained from individuals with a suspected spinal infection, this study compared the microbiological detection performance of mNGS and the culturing-based method. The results demonstrate that mNGS has several advantages. First, the microbial profiles generated using mNGS are more varied compared to the culturing-based method. Second, mNGS exhibited a significantly 21% higher detection rate and 35% higher sensitivity towards pathogens, compared to conventional culturing tests of PNB specimens. Moreover, the detection performance of mNGS was not affected by

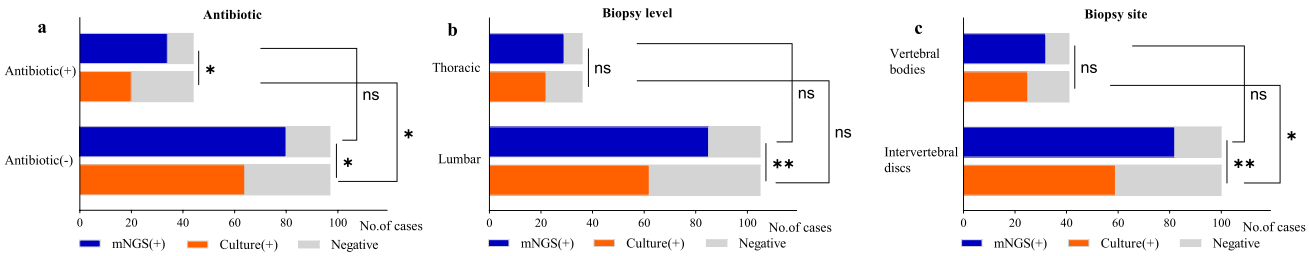

Fig. 5 Effects of antibiotic intervention (**a**), biopsy level (**b**), and biopsy site (**c**) on pathogen detection. * $P<0.05$, ** $P<0.01$

previous empirical antibiotic exposure, and especially for the detection of *Mycobacteria*.

Our findings showed that *Mycobacteria* and *Staphylococcus* are frequently isolated microorganisms, which is similar to those reported in other studies [2, 21]. The high detection frequency of *Mycobacteria* may be associated with the local tuberculosis epidemiology, and that of *Staphylococcus* may be associated with an increased incidence of pyogenic spinal infections. The microbiological spectrum distribution of mNGS is more complicated than that of the culturing-based method. In addition to the common causative microorganisms causing spinal infection, mNGS also detected rare or potentially opportunistic microorganisms, such as *Acinetobacter baumannii*, *Mycoplasma hominis*, and *Aspergillus fumigatus*, which provided additional microbial data. This observation may offer a valuable reference for establishing microbiological evidence of an infection. Culturing-based method requires specific culturing conditions, and some bacteria are difficult to culture and other bacteria grow slowly and, therefore, may not be isolated in culturing media [22]. However, mNGS, which is based on the nucleic acid of a specimen rather than active microorganisms, follows standardized procedures to parallelly facilitate sequencing and identify microorganisms to improve the diagnosis of infections [11].

The application value of PNB in the microbiological diagnosis of a spinal infection has been affirmed in previous studies [8, 9]. However, because of the low positivity and limitations in the breadth of pathogens detected by the culturing-based method, it is challenging to establish etiological evidence. mNGS theoretically overcomes the drawbacks of the culturing-based method as it involves the sequencing analysis of clinical specimens. MA C et al. found that mNGS may be useful as an adjunct method for diagnosing of spinal infections with a detection rate of 70%, sensitivity of 70.3%, and specificity of 75.0% [23]; however, the small sample size (n = 30) was a major limitation. This study with a relatively large sample size also resulted in similar findings; the detection rate of mNGS was 80.9%, and sensitivity was 85.7%, and detection rate was significantly improved by 21% (80.9% vs. 59.6%, $P < 0.001$), while the sensitivity was improved by 35% (85.7% vs. 50.8%, $P < 0.001$), compared to the culturing-based method. This study further confirmed that mNGS has significant diagnostic advantages over the conventional culturing method, with or without antibiotic intervention. In addition, this study found that the detection rate of mNGS was significantly higher than that of culturing-based method with a certain biopsy level of the lumbar spine or biopsy site of intervertebral space. This, might be because the most common site of a spinal infections is the lumbar or intervertebral space. A study of osteoarticular infections also indicated that mNGS could be a robust diagnostic tool for pathogenic detection, compared to the culturing method, and is particularly valuable for the identification of pathogens that are difficult to culture or treat with empiric antibiotics [14]. Simultaneously, the relatively high diagnostic performance of mNGS in central nervous system infections, periprosthetic joint infections, and bloodstream infections has already been affirmed in previous studies [13, 15, 24]. Furthermore, mNGS could provide the specific genomic information necessary for the prediction and evolution of drug resistance [12], along with valuable quantitative data on biological concentrations for polymicrobial infections [25]. Thus, the mNGS technique has promising clinical application prospects.

Notably, certain potential risks are associated with the use of mNGS including false-positive or false-negative results, and mismatches with the results of culturing-based method. In this study, 18 potentially pathogenic microorganisms were detected by mNGS alone, while 6 potential pathogens were detected only by the culturing-based method, and 23.4% of the detected microorganisms were mismatched at the genus level. On the one hand, the short length of sequencing reads, highly homologous genomes, and nucleic acid degradation during sample processing may lead to false-positive or false-negative results [26]. On the other hand, the analysis and interpretation of detailed mNGS data derived from background signals, potential microbiota, contamination of reagents, and disruption of laboratory environments may also potentially affect the results [12, 27, 28]. Microbial nucleic acids are dominated by the human host background, which is a key disadvantage inherent to unbiased mNGS and is partly mitigated by methods such as targeted sequencing or host depletion [12, 29]. In contrast to the risks of contamination of the skin microbiome that may exist during surgical sampling [30], PNB was selected as it could further lower the possibility of contamination of samples. Strict quality assurance and standard processes are imperative for clinical practice. Testing samples were harvested and processed under stringent sterile conditions to prevent microbial contamination. Additionally, negative controls and reagent assessments are needed to ensure that laboratory cross-contamination among samples does not lead to false-positive results [12]. When multiple pathogens are detected by mNGS, instead of immediately establishing a diagnosis of multiple infections, it is always necessary to first discriminate the true pathogens from background microorganisms by skilled professionals. It is feasible to switch to other methods such as PCR for further detection if necessary. Simultaneously, the cost-effectiveness of mNGS should be considered in clinical practice.

This study had several limitations. First, this is a retrospective study, and inevitable selection bias might have affected the conclusions. Second, this study is a single-center study conducted in a tertiary medical institution. The epidemiology of pathogens might reflect the local geographic distribution and incidence, hindering the generalizability

of these results to a larger, heterogeneous population. Prospective, multicenter studies and clinical trials are needed to further verify the diagnostic value of mNGS in individuals with spinal infections. Another limitation of this study is the absence of non-infected samples as a matched control group to assess the detection value of mNGS more comprehensively because mNGS analysis was performed only in the setting of suspected spinal infections at our center. Also, the false-positive results of culture and mNGS were not fully considered because the positive criteria was the isolation of the microorganisms in this study. In the next step, the value of mNGS for the diagnosis of spinal infection cases will be further validated and confirmed by etiological or histopathological evidence using non-infectious spinal disease samples as controls.

Conclusion

In conclusion, this study confirmed that mNGS exhibited a higher detection rate for pathogen identification in spinal infections, as compared to culturing methods, and was particularly valuable for the assessment of conditions in individuals who received a previous antibiotic intervention or had a mycobacterial infection. Thus, the use of mNGS as a supplementary diagnostic method might have broad prospects for the detection of pathogens in spinal infections.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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