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The clinical management and efficacy of metagenomic next-generation sequencing in patients with pyogenic spinal infection: a single-center cohort study

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Abstract

Objective This study aims to evaluate the clinical management and effectiveness of metagenomic next-generation sequencing (mNGS) in patients with pyogenic spinal infections.

Methods We conducted a retrospective review of 17 patients diagnosed with pyogenic spinal infections and treated at our institution between October 2022 and February 2024. The cohort included 8 males and 9 females, with a mean age of 63.59 ± 10.18 years (range: 41–71 years). The infections comprised 9 epidural abscesses, 6 intervertebral space infections, and 2 deep abscesses. All patients underwent open surgical procedures and mNGS-based bacterial identification using intraoperative pus or tissue specimens, in addition to conventional blood bacterial cultures. Clinical outcomes were assessed using CRP, PCT, WBC inflammatory markers, and VAS scores postoperatively.

Results All 17 patients with pyogenic spinal infections underwent open surgery and mNGS bacterial detection at our institution. Among the 17 patients, mNGS yielded positive results in 14 cases (82.4%), significantly higher than the 5.9% positivity rate of conventional bacterial cultures ($p < 0.001$). The mNGS test time was notably shorter than conventional cultures (1.0 vs. 5.88 days, $p < 0.001$). Postoperative antibiotic therapy was adjusted based on mNGS findings. There were significant reductions in postoperative VAS, WBC, PCT, and CRP values compared to preoperative levels ($p < 0.01$).

Conclusion Metagenomic next-generation sequencing is effective in managing pyogenic spinal infections by facilitating rapid and sensitive detection of pathogens. This technique improves the timeliness and accuracy of diagnosis, highlighting its potential for broader clinical use.

Keywords Metagenomic next-generation sequencing, Diagnosis, Spinal infection, Pyogenic infection

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Introduction

Pyogenic spinal infections manifest as spondylitis, epidural abscesses, psoas abscesses, retropharyngeal abscesses, intervertebral abscesses, and spinal osteomyelitis [1]. The overall prevalence of these infections ranges from 0.2 to 2 per 100,000 annually. These infections can spread to neighboring tissues through spinal surgeries, lumbar punctures, open trauma, or hematogenous routes from infected skin lesions. Presently, the symptoms exhibited by patients with spinal infections lack specificity which include fever, intense localized pain, and spinal dysfunction. This lack of specificity can lead to misdiagnosis in the early stages. Consequently, preoperative diagnosis primarily relies on a combination of peripheral blood tests and imaging examinations, which can result in considerable diagnostic delays or even misdiagnosis, allowing the infection to spread and cause severe complications [2, 3].

The diagnostic process for pyogenic spinal infections involves patient history, clinical symptoms, imaging examinations, and laboratory analyses. While MRI and CT scans have limited discriminatory power, laboratory investigations provide significant diagnostic utility [4]. Conventional microbiological approaches are reported to have low sensitivity and delayed results [5]. Staphylococcus aureus is the primary pathogen, with occasional infections by Staphylococcus epidermidis, Streptococcus, and Pseudomonas aeruginosa. Blood cultures and biopsies have detection rates below 60% and 75%, respectively [6, 7]. Metagenomic next-generation sequencing (mNGS) offers comprehensive microbial identification, effectively identifying pathogens along with their resistance profiles and virulence factors [8–12]. Despite the limited availability of literature, this methodology is increasingly recognized for diagnosing spinal infections.

In this study, we analyzed 17 patients with pyogenic spinal infections. MNGS-guided antimicrobial therapy led to a favorable prognosis for patients, which provides a clinical basis for the application of mNGS in spinal infections.

In this study, we analyzed 17 patients with pyogenic spinal infections. The use of mNGS-guided antimicrobial therapy resulted in favorable prognoses for these patients, providing a clinical basis for the application of mNGS in the treatment of spinal infections.

Materials and methods

Patients enrollment

We conducted an analysis in a cohort of 17 patients who suffered from spinal infections and underwent surgical intervention at Xuanwu Hospital hospital from October 2022 to February 2024. The cohort consisted of 8 males and 9 females, with an average age of 63.59 ± 10.18 years, ranging from 41 to 71 years. Approval for this study was obtained from the Ethics Committee of Xuanwu Hospital, and all patients provided written informed consent.

Eligibility criteria included: (1) Patients undergoing primary treatment; (2) Magnetic resonance imaging reveals inflammation-induced destruction of the vertebral body or paravertebral region; (3) Definitive pathological examination confirming spinal infection on septic tissue and pus specimens. Exclusion criteria encompassed: (1) Patients with neurological deficits due to pronounced spinal cord compression; (2) Patients with significant concurrent diseases, systemic infections, or immune deficiencies; (3) Biopsies suggestive of neoplastic processes, non-infective inflammatory states, or inconsequential tissue (Fig. 1).

Clinical and radiological assessment

Clinical data include detailed clinical histories, laboratory examinations, and treatments. Laboratory tests comprised standard preoperative peripheral blood tests, such as blood culturing and perioperative inflammatory indices including white blood count (WBC), C-reactive protein (CRP), and procalcitonin (PCT), specifically at seven-day and three-month postoperative intervals. Imaging examinations included CT scans and MRIs. MRI seems to be the most reliable method for diagnosing spinal infections, while CT is specifically used to accurately identify bony changes and bone necrosis. All patients with suspected spinal infections underwent surgical intervention and given routinely commence empiric antibiotic therapy immediately after cultures are obtained, in line with standard guidelines. The obtained tissue and pus samples were subjected to histopathological examination and sent for mNGS (PathoXtract®, WillINGMED) and microbial culture for etiological examination (Fig. 2). Only those patients finally diagnosed with spinal infection were enrolled in this study. The antibiotics were used according to the examination results after surgery. The effect of medication mNGS-guided was evaluated according to the improvement of postoperative symptoms and regular serological tests. The criteria for a clinical cure included the normalization of inflammatory markers like the PCT and CRP, alleviation of local symptoms, and absence of postoperative infection-related complications. Two neurosurgeons extracted and reviewed patients' medical records, and the results were compared and validated.

Statistical analyses

We utilized SPSS Statistics (version 26.0, IBM, Chicago, USA) for data analysis. Continuous variables were shown as means and standard deviations. Changes in Visual Analog Scale scores from baseline to follow-ups were

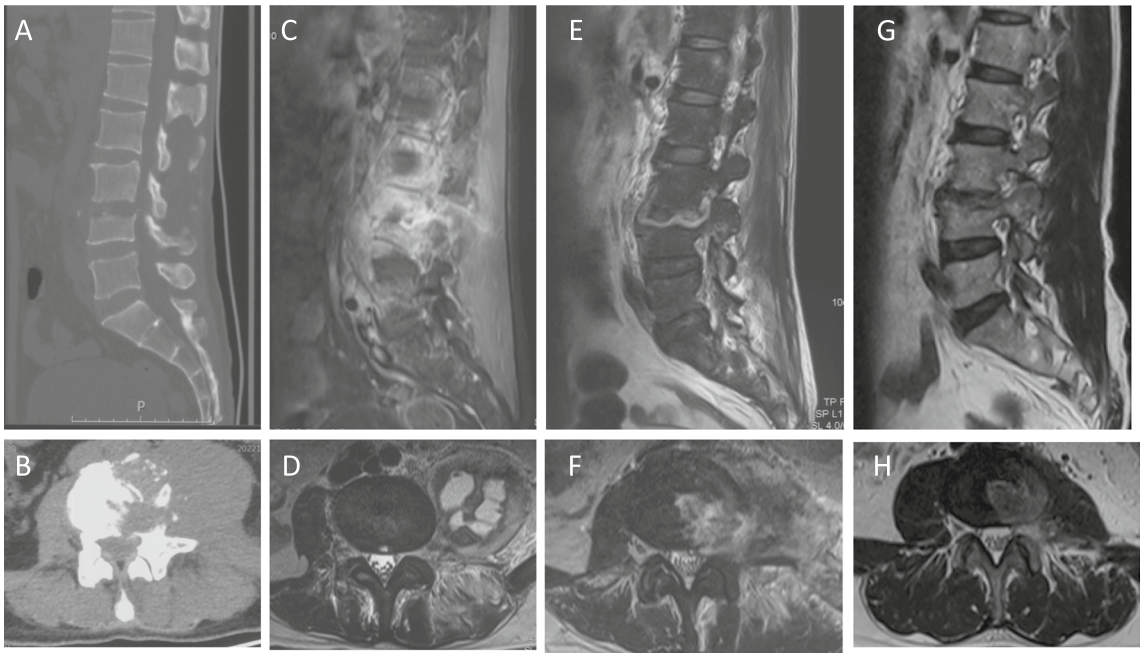

Fig. 1 A 52-year-old male patient presented left lower extremity pain and weakness persisting for over two months. **A**, **B**: Lumbar spine sagittal and horizontal CT images revealed signal alterations in the L3/4 intervertebral space, accompanied by significant destruction of the superior endplate of L4; **C**, **D**: Enhanced magnetic resonance imaging exhibited a substantial fluid signal in the left psoas major region; **E**: Postoperative MRI, conducted three days after the operation, demonstrated successful excision of the abscess in both sagittal and axial imaging; **F**, **H**: Subsequent MRI evaluations, conducted three months post-surgery, revealed complete resolution of the abscess and no sign of recurrence

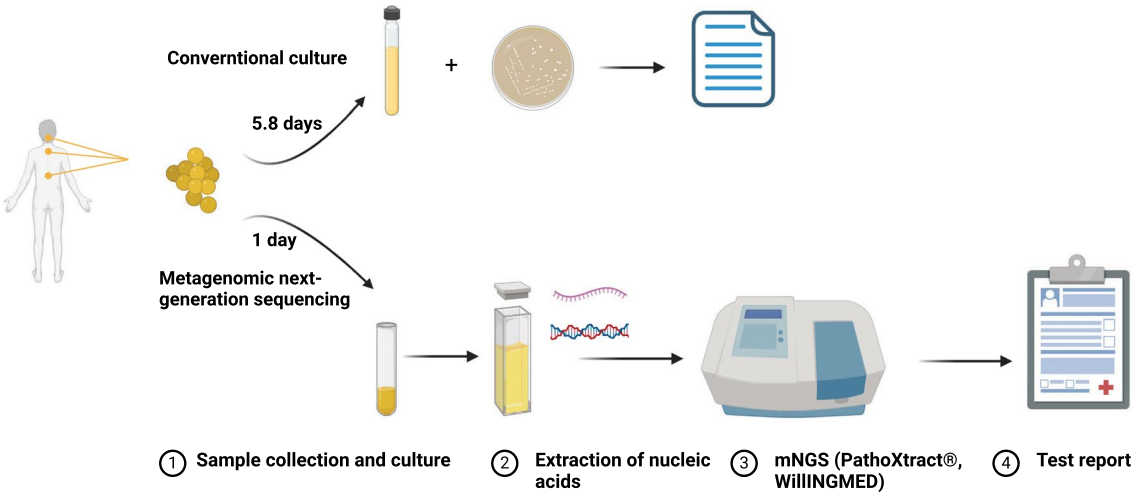

Fig. 2 The workflow and time differences between conventional culture-based methods and metagenomic next-generation sequencing (mNGS) for organism identification. Conventional culture methods, which involve sample collection, culturing for up to 5.8 days, and subsequent identification, are depicted in the top pathway. In contrast, the lower pathway shows the mNGS method, where sample collection is followed by nucleic acid extraction and sequencing. mNGS can provide results within 1 day by directly identifying pathogens from the sample without the need for prior culturing

assessed using the Wilcoxon signed-rank test. One-way ANOVA was used to analyze disparities in positive detection rates between mNGS and conventional microbiological cultures including WBC, CRP, and PCT. $p < 0.05$ was defined as statistical significance.

Table 1 Demographic characteristic of the patients with spinal infection

Characteristics	Number
Sex	
Male	8 (47.1%)
Female	9 (52.9%)
Age (y)	63.59 ± 10.18
Treatment with surgery	17
Types	
Discitis	6 (35.3%)
Paravertebral infection	2 (11.8%)
Epidural abscess	9 (52.9%)
Location	
Cervical	5 (29.4%)
Thoracic	1 (5.9%)
Lumbosacra	11 (64.7%)
Follow-up month/mediam	10

Table 2 Comparison of the positive rate and test time of mNGS (with tissue and pus samples) and conventinal microbial culture (with blood and pus samples)

Methods	Cases	Positive cases	Positive rate	Test time/median
mNGS	17	14	82.40%	1
Conventinal microbial culture	17	1	5.90%	5.8
P value			< 0.001	< 0.001

Results

All 17 patients diagnosed with pyogenic spinal infections successfully underwent invasive surgery, complemented by metagenomic next-generation sequencing (mNGS) for the microbiological examination of septic tissue and pus specimens at our medical institution (Fig. 1). Despite presenting with negative cultures for septic tissue and pus specimens, blood culture analysis identified Staphylococcus aureus in only one case, indicating a detection rate of 5.9%. In contrast, mNGS demonstrated a significantly higher detection rate of 82.4% for tissue and pus specimens, compared to the 5.9% detection rate with blood specimen culturing (Table 1). The diagnostic efficacy of mNGS revealed its vastly superior sensitivity at 82.4%, compared to the modest 5.9% sensitivity of conventional microbiological culturing ($p < 0.001$) (Table 2). Among the samples, one (5.9%) confirmed the presence of pathogens through both mNGS and conventional culturing methods, while three samples (17.6%) yielded negative results in both tests. The culture-positive sample matched precisely with mNGS findings, influencing subsequent antibiotic therapy and enhancing treatment effectiveness.

In total, 17 surgical specimens were obtained for this study, comprising 6 pus and 11 tissue samples. Rigorous mNGS testing yielded detectable pathological outcomes in 14 samples, while three tissue samples tested negative. However, each specimen consistently exhibited evidence of inflammatory cellular infiltration on pathological examination. The analysis identified intervertebral abscesses in 6 samples (35.3%), paravertebral infections in 2 (11.8%), and epidural abscesses in 9 (52.9%). Anatomically, the infections originated from the cervical vertebrae in 5 cases (29.4%), thoracic vertebrae in 1 case (5.9%), and lumbosacral vertebrae in 11 cases (64.7%). A single pathogen was identified in 12 samples (85.7%), while multiple infecting pathogens were found in two samples (14.3%). The mNGS results implicated common pathogens underlying pyogenic spinal infections, identifying Staphylococcus aureus in 5 instances (35.7%), streptococci in 2 instances (14.2%, with 1 presenting a mixed infection), Escherichia coli in 1 instance (7.1%), B. melitensis in 2 instances (14.2%), and various bacilli in 4 instances (23.1%, with 1 reflecting mixed infections). Additionally, 1 sample showed a viral co-infection (7.7%) (Table 3). Conventional culturing isolated Staphylococcus aureus in a single instance.

Pathogen tests were crucial for the prudent selection of antibiotics. For the patient diagnosed with a Staphylococcus aureus infection by both mNGS and culturing, appropriate antibiotics were administered. Additionally, for the 14 mNGS-positive patients, potential pathogens were inferred from a synthesis of clinical symptoms, guiding targeted antibiotic therapy. The effectiveness of antibiotic treatment supported diagnostic accuracy, as all patients exhibited positive health trajectories, highlighted by the success of mNGS-directed antimicrobial intervention during the three-month follow-up (Table 2).

Administration of antibiotics in the postoperative period led to significant decreases across all three monitored indicators: WBC counts decreased from a preoperative mean of 11.91 to a postoperative average of 8.59, stabilizing at 6.06 after 3 months ($p = 0.000$). CRP levels decreased from a preoperative high of 61.07 to a postoperative median of 39.86, ultimately reaching 16.61 after 3 months ($p = 0.003$). PCT values decreased from an initial 0.25–0.15 postoperatively, further decreasing to 0.07 at the 3-month mark ($p = 0.000$). The postoperative VAS scores also demonstrated a decrease from the preoperative average of 6.59 to a postoperative mean of 3.12, ultimately reaching 0.29 after 3 months ($p = 0.000$) (Table 4).

Table 3 mNGS and normal microbial culture for the patients with spinal infection

No	Sample types	mNGS	Disease triggers	Symptoms	Antibiotic use	Duration of medication/ Days	Prognosis	Time (mNGS)	CMC	Time (CMC)
1	Pus	*E. coli*	Disc ablation	Low back pain	Cefperazone–Sulbactam	7	Low back pain improved	1	NA	7
2	Pus	*S. aureus*	Skin Inflammation	Pain and weakness of lower limb	Cefperazone–Sulbactam	7	Pain and weakness improved	1	NA	5
3	Pus	*Veillonella*	NA	Neck pain	Cefperazone–Sulbactam	7	Neck pain improved	1	NA	5
4	Tissue	*S. aureus*	NA	Neck pain	Cefepime	7	Neck pain improved	1	NA	5
5	Tissue	*G. bacillus*	Pneumonia	Limb weakness	Cefperazone–Sulbactam	7	Limb weakness improved	1	NA	5
6	Tissue	Str. intermedius	NA	Pain and weakness of lower limb	Cefperazone–Sulbactam	7	Pain and weakness improved	1	NA	6
7	Tissue	*S. haemolyticu*, *C. striatum*	Lumbar surgery	Fever	Cefuroxim	10	Recovery	1	NA	5
8	Pus	*S. aureus*	Lumbar surgery	Low back pain	Cefperazone–Sulbactam	8	Low back pain improved	1	NA	5
9	Tissue	EBV, CMV, winkianeuii, *E. faecalis*, Slackiaexigua, *S. anginosus*	NA	Neck pain	Imipenem + vancomycin + tigacycline + fosfomycin	15	Neck pain improved	1	NA	5
10	Tissue	*S. aureus*	NA	Low back pain	Cefperazone–Sulbactam	6	Low back pain improved	1	E.coli	5
11	Tissue	*B. melitensis*	NA	Low back pain	Doxycycline + streptomycin	8	Low back pain improved	1	NA	8
12	Tissue	–	NA	Low back pain	Amikacin plus ceftazidime–avibactam	20	Low back pain improved	1	NA	6
13	Tissue	–	Cervical surgery	Limb weakness	Cefperazone–Sulbactam	8	Limb weakness improved	1	NA	7
14	Tissue	–	NA	Left lower limb pain	Cefuroxim	30	Left lower limb pain improved	1	NA	6
15	Pus	*S. aureus*	NA	Neck pain	Vancomycin + ceftriaxone	8	Neck pain improved	1	NA	6
16	Pus	*E. homaechei*	Disc ablation	Low back pain	Doxycycline + azithromycin	20	Low back pain improved	1	NA	7
17	Tissue	*B. melitensis*	NA	Pain and weakness of neck	Cefuroxime + rifampicin	9	Neck pain improved	1	NA	8

Table 4 Clinical and laboratory evaluation for the patients with spinal infection

Characteristics	Preoperation	7 days after the treatment	3 months after the treatment	F	*P* value
VAS	6.59 (0,10)	3.12 (0,8)	0.29 (0,1)	30.72	0.000
WBC (10*9·0L-1)	11.91 (5.56, 19.38)	8.59 (5.07,13.2)	6.06 (3.93, 10.01)	9.88	0.000
PCT (ng/ml)	0.25 (0.08, 0.51)	0.15 (0.07, 0.41)	0.07 (0.01, 0.12)	9.36	0.000
CRP (mg/l)	61.07 (7, 167)	39.86 (6,115)	16.61 (2, 67)	6.91	0.003

Discussion

Diagnostic techniques for spinal infections

Previous research has shown that the diagnosis of spinal infections can be delayed by 2–6 months after the onset of symptoms [13]. This delay in recognition and appropriate treatment can result in serious consequences. While pathogen identification by culture techniques is essential for the diagnosis and management of spinal infections, obtaining an accurate pathogenetic diagnosis can be hard because of its low sensitivity [14]. Conventional culture techniques are often challenging and time-consuming for identifying many pathogenic microorganisms. The earliest application of such technology dates to 2015 when Arisa Tsuru et al. proposed their potential [15]. While quantitative real-time PCR has been reported to improve the sensitivity of pathogen identification, it is limited to detecting specific known pathogens and cannot identify unknown ones (Table 5).

Metagenomic next-generation sequencing (mNGS) has emerged as a non-culture-based technology for identifying pathogenic bacteria with high sensitivity and specificity, and fast detection in spinal infections [16, 17]. 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 [18]. Scholarly literature has highlighted its higher diagnostic sensitivity and precision than traditional culture-based methods in the detection of pathogens in bloodstream infections, lung infections, meningoencephalitis, prosthetic joint infections, and other conditions [19–23]. In 2014, the use of mNGS to accurately diagnose intracranial leptospirosis infection for the first time, while 38 conventional tests were negative [21]. Chiyuan Ma pioneered the utilization of mNGS in diagnosing spinal infections in 2022 and 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%. Yi Zhang et al. reported that 78.95% of simples were identified to be positive by the mNGS method, which was higher than that of microbial culture (17, 44.74%) [11, 24]. Subsequently, Guang Zhang et al. analyzed 80 cases using mNGS, demonstrating its significant advantages over conventional microbiological cultures [10]. The detection rate for pathogens of mNGS was 71.8% (28/39), which was significantly higher than that of microbial culture. Wentao Lin et al. and Chen et al. found

Table 5 The summary of literature in mNGS for the diagnosis of spinal infection

References	Patients (N)	m NGS /positive rate (%)	Culture /positive rate (%)	Year	(mNGS versus Convebtional culture) Sensitive; Specificity
Yi Zhang et al	38	78.79	44.74	2022	84.2% versus 42.1%; 100.0% versus 100.0%
Chiyuan Ma et al	30	61.20	12.90	2022	75% versus 100%; 70.3% versus 14.8%
Liang Xu et al	108	81.48	43.51	2022	90.7% versus 52.17%; 81.82% versus 56.25%
Chen Wang et al	114	84.90	30.19	2023	–
Hanwen Cheng et al	78	84.00	32.00	2023	90.91% versus 0; 100% versus 100%
Haihong Huang et al	141	80.90	59.60	2023	87.5% versus 50.8%; 86.7% versus 93.3%
Wentao Lin et al	39	71.80	23.10	2023	87.1% versus 25.8%; 87.5% versus 87.5%
Yuan Li et al	100	71.9	23.10	2023	89% versus 28.1%; 88% versus 100%
Guang Zhang et al	158	86.96	19.57	2023	–
Cheng Li et al	23	85.19	48.15	2024	–
Qi-Chen Zhang et al	56	75.00	55.30	2024	–
Hui Lv et al	76	77.60	18.40	2024	82.3% versus 17.5%; 75% versus 27.6%
Chuqiang Yin et al	120	88.42	43.16	2024	–
Jiayi Chen et al	108	61.20	30.80	2024	79.4% versus 25.0%; 80% versus 100%

that mNGS demonstrated greater sensitivity (71.8% and 79.41%m, respectively) than cultures in their spinal infection cohorts in 2023 [25, 26].

This study investigates the use of metagenomic next-generation sequencing (mNGS) to identify pathogens from spinal infection samples, comparing its diagnostic efficacy and impact on patient prognosis to those of conventional microbiological cultures. The findings show that mNGS achieved a positivity rate of 82.4% (14 /17), significantly higher than the 5.9% (1 /17) sensitivity observed with conventional cultures, consistent with findings from other studies [10]. The use of mNGS for pathogen identification guided targeted antibiotic treatments, resulting in uniformly favorable patient outcomes. These results highlight the effectiveness of mNGS in diagnosing and managing spinal infections.

Advantages of mNGS in spinal infection diagnosis

Identifying pathogens early is crucial for patients with spinal infections before starting standard treatments. The specificity and speed of metagenomic next-generation sequencing (mNGS) surpass conventional methodologies, yielding results typically by the second day, significantly enhancing diagnostic timeliness compared to conventional cultures [27–30]. In this study, the average time to obtain culture results was 5.8 days, whereas mNGS results were obtained in just 1 day, consistent with findings from other studies [12, 31–33]. This quicker diagnosis allows for timely treatment decisions, improving patient outcomes. Opportunistic and low-virulence pathogens, such as Streptococcus intermedius, have been identified in pyogenic spinal infections using mNGS [34]. The study also found better positive rates when testing pus specimens [11]. The early identification of appropriate antibiotic regimens through mNGS simplifies their use and prevents overuse. Even when mNGS tests are negative, they are clinically valuable. Negative mNGS results help discern infective presentations lacking fever symptoms or elevated inflammatory markers, highlighting mNGS's potential as a superior diagnostic tool for spinal infection pathogens [30]. Moreover, in cases of negative microbiological cultures, mNGS provides foundational pathogenic taxonomy, enhancing precision in antibiotic regimens. Specifically, mNGS is crucial for guiding therapy for atypical pathogens like Brucella [35, 36]. Conversely, negative laboratory and mNGS findings prompt considerations of non-infectious etiologies and potential antibiotic overuse.

Challenge and future of mNGS in spinal infection diagnosis

Conventional microbial cultures are essential to spinal infection diagnostic and therapeutic protocols, offering unmatched insights into antibiotic susceptibility. However, as a diagnostic adjunct, mNGS could reduce the rates of misidentification in such infections. Currently, the prohibitive cost and vulnerability to environmental contaminants, such as Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus mansoni, somewhat limit the widespread clinical use of mNGS [11]. However, meticulous interpretation of mNGS findings, coupled with clinical correlation, can address these challenges, fostering broader technological advancements [19]. This is particularly significant for culture-negative spinal infections, where mNGS provides insights into pathogen etiology and potential pathogenesis.

There are limitations in comparing results in such a retrospective study, the most significant being selection bias. In addition, the sample size of this study was relatively small due to the rarity of spinal infections and the fact that mNGS was only performed on economically advantaged patients. Moreover, the result of histopathology cannot directly confirm spinal infections, and we used inflammation as the diagnostic criterion. In future research, multi-center studies with larger sample sizes and extended follow-up periods are essential for obtaining more robust clinical evidence.

Conclusion

Metagenomic next-generation sequencing for patients with pyogenic spinal infections proves effective in managing inflammation. Offering a shorter pathogen identification time and increased sensitivity compared to conventional bacteriological cultures, mNGS plays a significant role as a diagnostic tool for the application in spinal infections.

Author contributions

Jiao Ma and Wenwen Li: data collection; Maoyang Qi and Yueqi Du: design of the study, results interpretation, manuscript writing and editing; Jian Guan: conception and manuscript editing; Zan Chen and Wanru Duan: conception, design of the study, results interpretation.

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Availability of data and materials

The sequence data that support the findings of this study can be obtained through the appropriate application.

Declarations

Ethical approval and consent to participate
The studies involving human participants were reviewed and approved by Xuanwu Hospital Review Board, Capital Medical University. The patients provided their written informed consent to participate in this study.

Consent for publication
All authors agree to publish "Analysis of failed posterior fossa decompression and an effective revision surgery for patients with basilar invagination and atlantoaxial dislocation" in the Journal of Orthopaedic Surgery and Research.

Competing interests
The authors declare no competing interests.

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