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ORIGINAL RESEARCH

Metagenomic Next-Generation Sequencing Improves the Diagnosis Efficiency of Mixed Periprosthetic Joint Infections

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Purpose: To explore the clinical significance of metagenomic next-generation sequencing (mNGS) in the diagnosis of mixed periprosthetic joint infections (PJI).

Methods: The data pertaining to patients suspected of PJI who underwent arthroplasty at our hospital between January 2020 and June 2024 were analyzed. Patients included in the study were subjected to microbial culture and mNGS analyses to evaluate the efficacy of mNGS in diagnosing mixed PJIs.

Results: Among the 44 PJI patients included, 20 (45.45%) were culture-positive, and 35 (79.55%) were mNGS-positive. Compared to microbial culture, mNGS demonstrated significantly higher sensitivity, negative predictive value, and accuracy (79.55% vs 45.45%, 55.00% vs 35.14%, and 80.70% vs 57.89%, respectively; all $P<0.05$). However, the specificity of mNGS was significantly lower than culture (84.62% vs 100.00%, $P<0.05$). For mixed PJIs, the sensitivity of mNGS was notably higher, albeit with lower specificity and positive predictive value compared to microbial culture (72.23% vs 27.27%, 85.19% vs 100.00%, 66.67% vs 100.00%, respectively; all $P<0.05$). mNGS enables more sensitive detection of co-pathogens in mixed PJI, accelerating targeted therapy and reducing inappropriate broad-spectrum therapy. While its lower specificity requires clinical integration, it clarifies complex diagnoses and streamlines stewardship for improved outcomes.

Conclusion: mNGS is a promising technique for rapidly and accurately detecting co-pathogens in mixed PJI.

Keywords: metagenomic next-generation sequencing, periprosthetic joint infections, mixed infection, diagnosis, microbial culture

Introduction

Arthroplasty effectively treats end-stage bone disease, but periprosthetic joint infection (PJI), a devastating postoperative complication, occurs in 0.5–3% of primary surgeries and up to 35% of revisions.[1–4] With aging populations and increased arthroplasty use, PJI incidence continues to rise.[5] Early identification of pathogens and timely targeted anti-infection treatment are of considerable importance to PJI patients. In the majority of cases, PJI is typically caused by a single pathogen; however, some patients present with mixed PJIs.[6] The prognosis of PJI patients is frequently determined by pathogen characteristics, including virulence and drug resistance.[7] Numerous clinical studies have shown that patients with mixed PJIs have poorer prognosis than those with PJI induced by single pathogen.[8,9] At the same time, it is often difficult to diagnose mixed PJIs. As a result, antibiotic treatment proves ineffective, often necessitating multiple revision surgeries, thereby amplifying the economic strain on patients and significantly impacting their physical and mental well-being.

Conventional microbial culture is the gold standard for PJI diagnosis and the main tool for the diagnosis of mixed PJIs.[1] Nonetheless, this method has a number of disadvantages. Firstly, the wrapping of pathogens by bio-membranes easily leads to false-negative culture results.[10] Secondly, numerous microorganisms exhibit competitive inhibition effects during the culture process, potentially leading to missed detection.[11] Thirdly, certain microorganisms with strict culture

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requirements may go undetected due to inadequate cultivation conditions.[12] PCR can quickly and accurately identify pathogenic microorganisms, but it cannot distinguish mixed infections.[13]

mNGS is an advanced technology for pathogen detection, offering the capability to identify all nucleic acids present in a sample without bias.[5] This technique enables simultaneous detection of bacteria, fungi, viruses, and parasites.[5] While conventional microbial culture may focus on just one pathogen and ignore others, mNGS can provide comprehensive coverage of all possible pathogens and is particularly suitable for the detection of complex pathogens in mixed infections. The detection cycle of mNGS is relatively short, typically completed within 24–48 hours, allowing clinicians to develop targeted anti-infection treatment plans early. This helps avoid the uncertainty of empirical therapy, enhances treatment efficacy, and reduces potential side effects. The existing research has shown that the sensitivity of mNGS for PJI diagnosis attains over 90%, and mNGS greatly improves the diagnosis rate of PJI.[7,14] However, there is a scarcity of reports on the diagnosis of mixed PJIs by mNGS. In the present study, the application value of mNGS in mixed PJI diagnosis was assessed.

Materials and Methods

Study Population Selection

The data of patients suspected of having PJI and aseptic loosening (AL) after undergoing arthroplasty in our Hospital from January 2020 to June 2024 were reviewed. The inclusion criteria were as follows: (1) patients who needed revision surgery; (2) classification of patients into PJI or AL categories based on the Musculoskeletal Infection Society (MSIS) diagnostic criteria for PJI;[15] (3) submission of both microbial culture and mNGS tests with specimens meeting mNGS testing requirements; and (4) provision of informed consent by patients. The exclusion criteria were as follows: (1) incomplete clinical and experimental data to diagnose PJI or AL; (2) patients with mNGS sequencing failure due to sample quality problems; (3) patients with autoimmune diseases, malignant tumours, or other inflammatory diseases. The present study was approved by the Medical Ethics Committee of Zhengzhou Orthopaedics Hospital (20220510001). The study is in line with the Declaration of Helsinki.

Sample Collection

The intraoperative samples of suspected PJI patients, including periprosthetic tissues and puncture fluid, were collected. Joint fluid sampling: Puncture fluid collection occurred post-skin incision but prior to opening the joint capsule during surgery, aimed at minimizing contamination of the puncture fluid resulting from incision exposure. Tissue sampling: In cases where the collected puncture fluid amounted to less than 10mL, tissue samples from the interface between the prosthesis and bone were extracted during surgery and placed in a sterile container for testing purposes.

Conventional Microbial Culture

The puncture fluid collected was divided and added into separate aerobic, anaerobic, fungal and tuberculosis culture bottles. These bottles were subsequently placed into the Bactec fully automated blood culture system (BD, USA) for incubation and detection of microbial growth. Tissue samples were ground with a tissue grinder and the tissue homogenates were inoculated into blood agar plates, MacConkey plates, chocolate plates and *Sabouraud dextrose* plates, respectively, and then the inoculated media were placed in the Thermo scientific CO2 incubator (USA) at 35°C. The bacteria cultivated were all identified using the Phoenix100 microbial automatic identification system (USA).

mNGS

The mNGS was performed according to the previously described method.[7,16,17] The main steps are as follows: (1) DNA Extraction: 500μL of puncture fluid or homogenized tissue was taken, and total DNA was extracted using the TIANamp Micro DNA Kit (DP316, Tiangen, Beijing, China), according to the instructions of the reagents.(2) Library Conduction and Sequencing: The construction of DNA library followed the protocol of the Nextera XT library construction Kit (Illumina, San Diego, CA, USA), and the quantified libraries sequenced on the Illumina Nextseq550DX sequencing platform. (3) Bioinformatics analysis: Initial filtering of the raw data was conducted using FastQC software (version

0.11.7, http://bioinformatics.babraham.ac.uk/projects/fastqc/), including filtering out the low-quality data, repeated sequences, sequences shorter than 35 bp, and adaptor contamination to produce high-quality sequencing data. Human reference genome (hg19)-mapped human host sequences were afterward computationally subtracted using Burrows-Wheeler Alignment technique. The remaining data were classified by simultaneously aligning to four Microbial Genome Databases, consisting of viruses, bacteria, fungi, and parasites. The classification reference databases were downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/). (4) Interpretation of mNGS results: the interpretation was carried out by the PJI expert group, which included at least one senior microbiologist and orthopedic expert.

Aseptic techniques were strictly followed throughout the experiment to prevent external contamination. Sample exposure time to the environment was minimised to reduce contamination risk. Each experimental batch included negative controls (nucleic acid-free water), positive controls, and internal host parameters to monitor potential contamination from reagents or the environment and to validate experimental sensitivity. Sequencing of key samples was repeated to ensure result consistency, and routine environmental monitoring was conducted to maintain laboratory integrity.

Clinical Diagnostic Criteria for Mixed PJI

The patients included in the present study were diagnosed with PJI or AL according to the MSIS diagnostic criteria. Subsequently, cases classified as PJI were further assessed for mixed PJI based on the following criteria: (1) Detection of two or more pathogens through culture or mNGS, with these pathogens being previously reported as causative agents in the literature; (2) Involvement of at least two orthopaedic specialists and one microbiologist in the analysis of detected pathogens and formulation of antibiotic regimens, ultimately resulting in successful infection eradication. The diagnostic process for mixed PJI is shown in Figure 1.

Diagnostic Index

Sensitivity refers to the proportion of truly diseased individuals correctly identified as positive by a diagnostic test, reflecting the test's ability to detect true cases. Specificity denotes the proportion of truly non-diseased individuals

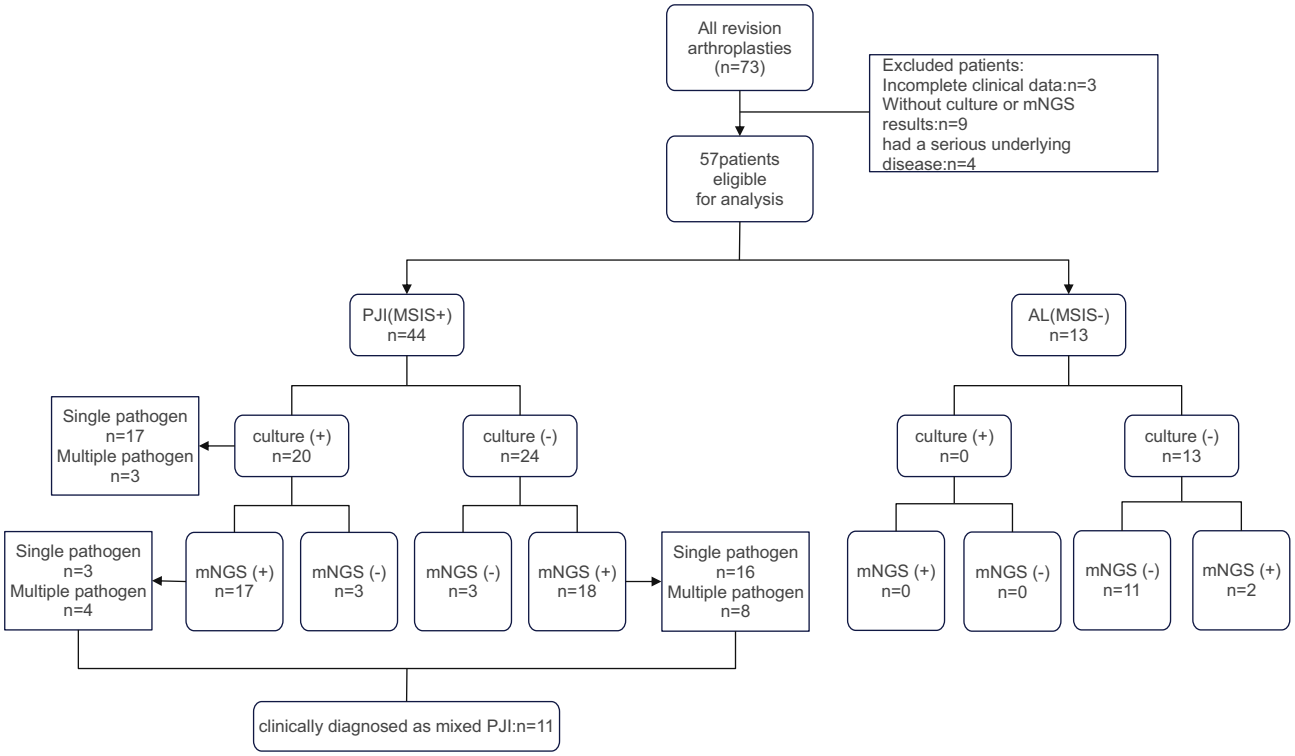

Figure 1 Diagnostic flow chart for mixed PJI.

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correctly identified as negative, representing the test's capacity to exclude non-cases. PPV indicates the proportion of test-positive individuals who are truly diseased, reflecting the probability of actual disease among positive results. NPV represents the proportion of test-negative individuals who are truly disease-free, demonstrating the probability of being truly non-diseased among negative results. Accuracy measures the overall correct classification rate, calculated as (true positives + true negatives) / total tested population.

Data Analysis

The SPSS 19.0 (SPSS Inc, Chicago, IL, USA) was used for data analysis. Differences between PJI and AL were assessed using the Chi-squared test or Fisher's exact test for categorical variables. McNemar test was used to compare Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV). Differences were expressed as statistically significant at $P<0.05$.

Results

Demographic Characteristics

According to the inclusion and exclusion criteria, a total of 57 patients were included in the present study. Based on the MSIS criteria, 44 patients (77.19%, 20 males/24 females, median age 64.77 ± 10.54 years) were classified as PJI, while 13 patients (22.81%, 5 males/8 females, median age 61.15 ± 13.08 years) were classified as AL. There were no statistically significant differences in age and gender between the PJI and AL groups (both P>0.05). The postoperative erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level of PJI patients were significantly higher than those of AL patients (both P<0.05). The characteristics of all the included patients are showed in Table 1.

Comparison of Results Between the Conventional Microbial Culture and mNGS

Among 44 PJI patients, 20 (45.45%) were culture-positive and 35 (79.55%) were mNGS-positive. All 13 AL patients tested negative for microbial culture, yet 2 of them yielded positive results via mNGS. Microbial culture analysis detected a total of 13 pathogens, whereas mNGS identified 28 pathogens in total. Both the microbial culture and mNGS results show that *Staphylococcus epidermidis* had the highest proportion among the pathogens detected (Figures 2 and 3). Among the 20 patients with positive microbial culture in the PJI group, 17 tested positive for mNGS, of which 11 had completely consistent results with the two. Four had more pathogens detected in mNGS than in microbial culture, 1 had more pathogens detected in microbial culture than in mNGS, and 1 had completely inconsistent results.

Comparison of Diagnostic Efficiency of PJI Between Microbial Culture and mNGS

A comparison of microbial culture and mNGS for PJI diagnosis is presented in Table 2. The results reveal that mNGS had significantly higher sensitivity, negative predictive value, and concordance rate, and lower specificity than the microbial culture (all P<0.05). This notable improvement in sensitivity and NPV by mNGS suggests that it is a more

Table 1 Comparison of Preoperative General Data Between PJI and AL Groups

Characteristics	PJI (n=44)	AL (n=13)	*p*-value
Age, years (mean±SD)	64.77±10.54	61.15±13.08	0.308
Gender, female, n (%)	24 (54.55%)	8 (61.54%)	0.655
Location, n (%)			
Knee	39 (88.64%)	11 (84.62%)	0.151
Hip	5 (11.36%)	2 (15.38%)	0.698
Laboratory findings			
CRP (mg/l, mean±SD)	46.20±32.18	17.59±12.40	0.003
ESR (mm/h, mean±SD)	40.68±20.57	14.92±8.14	0.001
Antibiotics prior to surgery, n	15	0	0.001

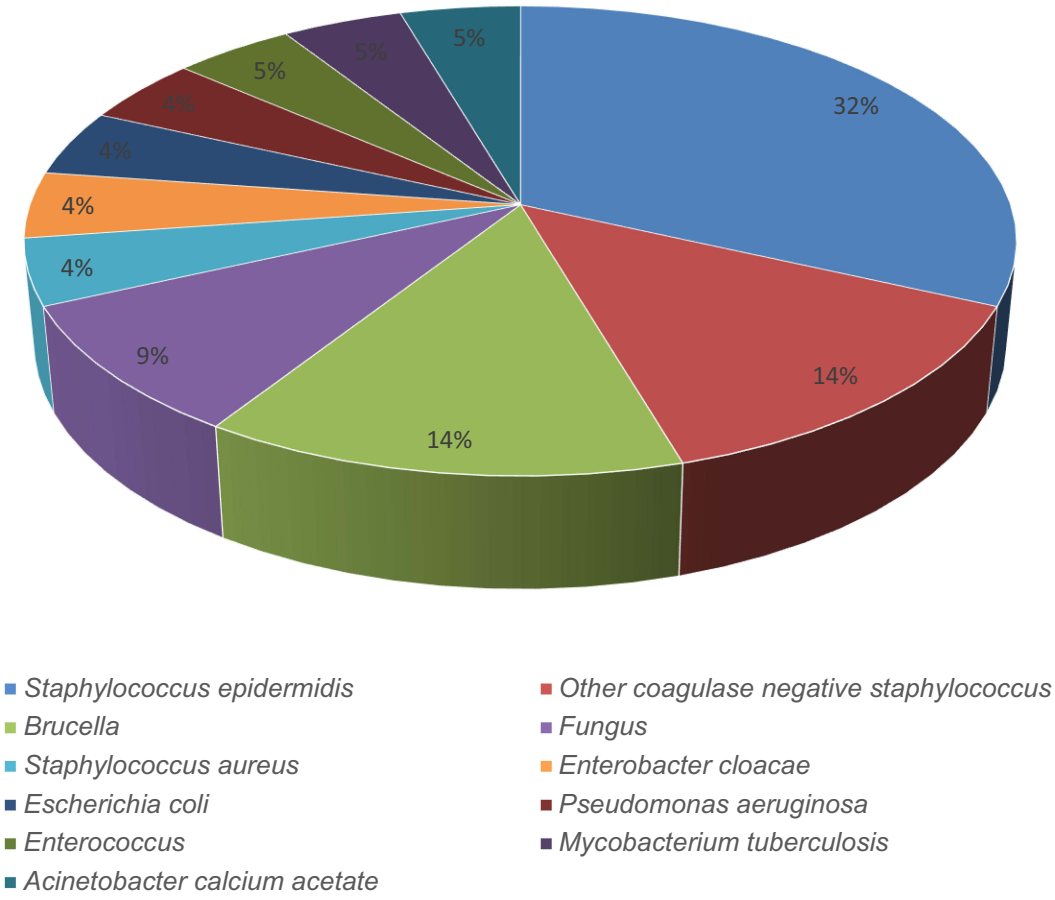

Figure 2 Results of pathogen detection by culture method.

effective diagnostic tool, especially when it comes to identifying low-abundance pathogens that are frequently overlooked in conventional cultures. Moreover, mNGSs took a shorter time to detect all pathogens than the bacterial culture method ($P<0.05$). It has significant advantages in the early diagnosis of PJI.

Comparison of Diagnostic Efficiency of Mixed PJI Between Microbial Culture and mNGS

In Table 3, detailed data regarding the detection of multiple pathogens by microbial culture and mNGS are presented. Specifically, microbial culture identified a total of 3 cases with multiple pathogens, all of which were deemed mixed infections. On the other hand, mNGS detected 12 cases with multiple pathogens, among which 8 were confirmed as mixed infections.

A comparison of microbial culture and mNGS for mixed PJI diagnosis is presented in Table 4. The results show that the sensitivity of mNGS in diagnosing mixed PJI was significantly higher than that of microbial culture ($P<0.05$), which could avoid incomplete treatment caused by missed diagnosis, shorten the treatment time and improve the prognosis of patients. The specificity and PPV of mNGS were significantly lower than that of microbial culture ($P<0.05$). The most common misidentified organisms for mNGS include human symbiotic bacteria (eg, *Staphylococcus epidermidis, Propionibacterium*) and environmental microorganisms (eg, *Corynebacterium, Aspergillus*). These misidentifications are mainly due to contamination, sequence similarity, database limitations, and experimental problems. Clinical interpretation should be combined with patients' immune status, infection site characteristics and multi-modal detection results to avoid misdiagnosis or over-treatment.

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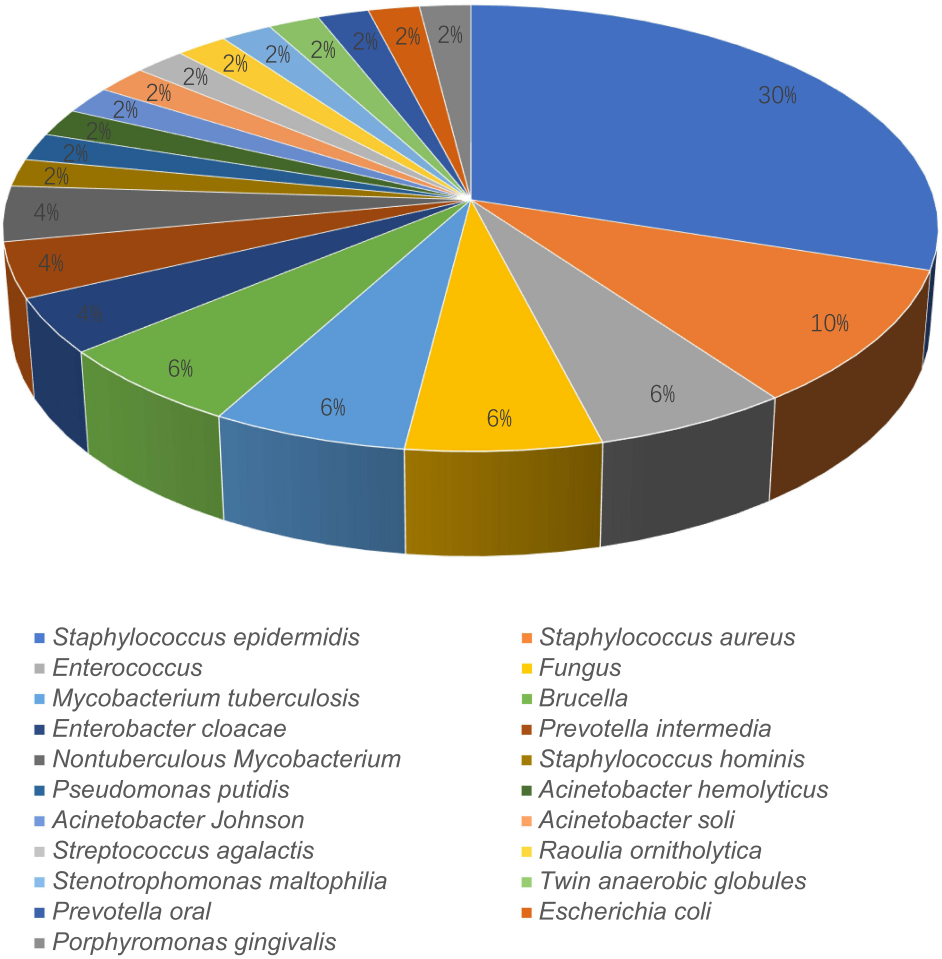

Figure 3 Results of pathogen detection by mNGS.

Statistical Adjustments and Effect Size Analysis

To account for multiple comparisons, we applied a post-hoc Benjamini-Hochberg correction (false discovery rate, FDR = 0.05). The original p-values for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 0.001, 0.026, 0.542, and 0.031, respectively. Using the ranking-based critical value formula (i/m)×Q (where i is the rank, m=4 is the total number of comparisons, and (Q=0.05), the adjusted significance thresholds were 0.0125, 0.025, 0.0375, and 0.05. After correction, sensitivity (p = 0.001) and NPV (p = 0.031) remained statistically significant,

Table 2 Comparison of Diagnostic Efficiency Between Microbial Culture and mNGS for PJI

Methods	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Concordance Rate% (95% CI)	Detection time
culture	45.45 (31.70–59.93)	100 (45.64–100)	100 (56.17–100)	35.14 (21.83–51.25)	57.89 (43.52–75.35)	5.23±1.87d
mNGS	79.55 (65.50–88.85)	84.62 (57.77–95.68)	94.59 (82.29–98.50)	55.00 (35.06–73.45)	80.70 (68.40–89.14)	1.72±0.52d
p-value	0.001	0.026	0.542	0.031	0.016	0.001

Abbreviations: PPV, Positive predictive value; NPV, Negative predictive value; CI, Confidence interval.

Table 3 Cases Clinically Diagnosed as Mixed PJI

Patient No.	Infection Site	Antibiotics Prior to Surgery (Yes, Y/No, N)	Culture Results	mNGS Results
No.1	Knee	N	*Staphylococcus warneri, Mycobacterium tuberculosis*	*Mycobacterium tuberculosis complex*
No.2	Knee	N	Negative	*Staphylococcus epidermidis, Mycobacterium avium*
No.3	Knee	Y	Negative	*Acinetobacter Johnson, Pseudomonas putida*
No.4	Knee	N	*Brucella*	*Brucella, Staphylococcus hominis, Acinetobacter haemolyticus*
No.5	Knee	Y	Negative	*Prevotella intermedia, Staphylococcus epidermidis*
No.6	Knee	N	Negative	*Raoulia ornitholytica, Prevotella intermedia, Staphylococcus epidermidis*
No.7	Knee	Y	Negative	*Staphylococcus epidermidis, Stenotrophomonas maltophilia*
No.8	Hip	N	*Acinetobacter calcoaceticus, Candida albicans*	*Porphyromonas gingivalis*
No.9	Knee	N	*Staphylococcus haemolyticus, Candida tropicalis*	Negative
No.10	Hip	Y	Negative	*Staphylococcus epidermidis, Enterobacter cloacae*
No.11	Knee	N	*Enterobacter cloacae*	*Enterobacter Howe, Staphylococcus aureus*

Table 4 Comparison of Diagnostic Efficiency Between Microbial Culture and mNGS for Mixed PJI

Methods	Sensitivity% (95% CI)	Specificity% (95% CI)	PPV% (95% CI)	NPV% (95% CI)	Concordance Rate% (95% CI)
culture	27.27 (9.75–56.5)	100.00 (89.57–100)	100.00 (43.85–100)	77.14 (60.98–87.93)	78.95 (62.43–88.57)
mNGS	72.73 (43.44–90.25)	85.19 (69.08–93.35)	66.67 (39.06–86.19)	88.46 (71.02–96.00)	81.57 (67.70–90.80)
p-value	0.001	0.028	0.001	0.429	0.898

Abbreviations: PPV, Positive predictive value; NPV, Negative predictive value; CI, Confidence interval.

while specificity (p = 0.026) and PPV (p = 0.542) did not. These results confirm the robustness of our primary finding that mNGS exhibits superior sensitivity for PJI diagnosis.

To further contextualize these findings, we calculated effect sizes. The risk difference (RD) for sensitivity was 34.1% (95% CI: 16.8–51.4%), with an odds ratio (OR) of 4.69 (95% CI: 1.89–11.62), indicating that mNGS detects 34% more infections than culture. The specificity RD was −15.38% (95% CI: −30.2–0.0%), suggesting a modest trade-off, underscoring the need to integrate mNGS with culture to minimize false positives. Fisher's exact test for specificity yielded a non-significant p-value (p = 0.198), further supporting a complementary diagnostic approach.

Discussion

PJI represents a critical complication following arthroplasty, with timely identification of the causative bacteria being paramount for effective diagnosis and treatment.[5] Delayed detection of the true pathogenic bacteria frequently results in suboptimal or unsuccessful treatment outcomes, alongside an elevated risk of developing drug resistance.[18] This scenario exacerbates the economic burden on patients and contributes to increased postoperative morbidity and mortality rates.[18] Research has shown that mNGS has a high value in the diagnosis of PJI14.[19] In the present study, the performances of mNGS and the conventional microbial culture in PJI diagnosis were also compared. The results suggest that mNGS had significantly higher sensitivity than the microbial culture (P<0.05), meaning that it can greatly improve the pathogen detection rate in PJI patients. A total of 28 pathogens were detected by mNGS, while only 13 pathogens were identified

by the microbial culture in the present study. mNGS offers the advantage of detecting a broader range of latent pathogens compared to microbial culture. This capability is particularly valuable in diagnosing culture-negative patients with PJI caused by uncommon bacteria. Further, mNGS facilitates rapid diagnosis, with results for all patients in this study obtained and reported within 48h. By contrast, the microbial culture took 4 to 17d. mNGS can greatly shorten the detection time, provide early targeted treatment, and reduce the risk of exacerbation of PJI infection. This substantial decrease in diagnostic time raises the possibility of an early intervention as well as a change in standard procedure that would prioritize mNGS as the main instrument for the quick detection of complex infections such as PJI.

According to existing research, 17%~39% of PJI patients are mixed PJI. The detection rate of mixed PJI in the present study was 25%, which is within the range reported by previous studies.[7,20] The detection rate of mNGS may be influenced by factors such as sample quality and sequencing depth. For instance, Mei et al's[7] study enrolled 91 patients and included tissue, synovial fluid, and sonicate fluid samples, whereas the current study collected 57 tissue and synovial fluid samples. The small sample size of the present study may reduce statistical power, increase the risk of missing true effects, and lack sensitivity to identify subtle differences. Additionally, differences in detection rates could also be attributed to variations in patient characteristics, infection types, and sample processing methods. To more accurately validate the diagnostic value of mNGS in polymicrobial PJIs, future studies should expand the sample size, compare the diagnostic value of different sample types, and conduct a more detailed statistical analysis of patient data.

Compared with PJI patients induced by single pathogens, patients with mixed PJI may have a different antibiotic spectrum, more severe clinical symptoms and poorer prognosis.[7,8] The conventional microbial culture exhibits several limitations, including compromised sensitivity, restricted detection range, and prolonged time requirements for pathogen identification, Moreover, it demonstrates suboptimal performance in detecting mixed PJI.[1,7,18] Pathogens that remain undetected by conventional microbial culture methods may contribute to relapses and escalate the incidence of antibiotic-related complications.[18,21] mNGS, independent of conventional microbial culture, represents an unbiased, broad-spectrum detection technique for microorganisms.[1] Given its capability to identify multiple microorganisms, mNGS holds significant potential and offers distinct advantages in diagnosing mixed infections.[7,22] In the present study, the sensitivity of mNGS in diagnosing mixed PJI was found to be significantly higher than that of the bacterial culture method (72.73% vs 27.27%, $P<0.05$). This finding is consistent with the results reported by Mei et al.[7] These findings have significant ramifications since they suggest that mNGS may be a key component of updated guidelines for the diagnosis of mixed PJI, which could result in improved management strategies. If solely reliant on microbial culture in this study, 8 patients with mixed PJI, constituting 72.73% of cases, would have been erroneously misdiagnosed. The high sensitivity of mNGS can improve the diagnostic efficiency of mixed PJI, and through early and accurate identification of pathogens, it can quickly guide targeted therapy, reduce the abuse of broad-spectrum antibiotics, and reduce the revision surgery rate. Although the cost of a single detection is higher than that of traditional culture, it can reduce the economic burden of repeated surgery, hospital stay and ineffective treatment. It is especially cost-effective in complex, high-risk cases.

Although mNGS can improve the detection rate of mixed PJI, the application of mNGS alone may also lead to false positives. Among the 44 PJI included in the present study, 12 patients were classified as mixed PJI according to mNGS, but 4 of them were confirmed as false positives. Several pathogens detected in the patients, such as *Aspergillus niger* and *anaerobic Gemella*, were not previously reported as causative agents of bone and joint infection. Despite specialized culture attempts, these pathogens yielded negative results. Although mNGS boasts high sensitivity, it remains susceptible to failure in detecting certain pathogens, potentially resulting in false negatives. In the present study, *Candida albicans* and *Candida tropicalis* were separately detected by the microbial culture in 2 patients, but they were not detected by mNGS. These differences underline the necessity of integrating comprehensive diagnostic techniques and continuously improving mNGS protocols, with a focus on striking a balance between sensitivity and specificity to reduce the possibility of misdiagnoses. This discrepancy may stem from the challenge of lysing the robust cell walls of fungal organisms, hindering effective extraction of nucleic acids and resulting in false negatives. In addition, the microbial culture method identified *Staphylococcus haemolyticus* in one patient and *Staphylococcus epidermidis* in another patient, but these two pathogens were not detected by mNGS. The reason may be that there was cross contamination during the operation, destroying the microbial DNA sequences and resulting in missing data.[23]

The false positives and false negatives of mNGS are mainly caused by contamination, low micro-biomass and database errors, and we can adopt the following solutions to alleviate these problems: We optimize the experimental procedures and strictly follow aseptic procedures. Host cells and non-target microorganisms were removed by filtration, centrifugation and other methods to improve the detection efficiency of target pathogens. Improve data analysis by using proven, high-quality reference databases (eg NCBI, RefSeq) to ensure the accuracy of sequence matching. Bioinformatics filtering, based on sequence coverage, abundance and specificity set a reasonable threshold to reduce false positive results. The results of mNGS were verified by traditional methods such as culture, PCR and serological detection. The suspected pathogenic bacteria detected by mNGS were further confirmed by targeted sequencing. Positive results are repeated to ensure their repeatability. The results of mNGS were evaluated by combining clinical symptoms, imaging and other laboratory results. Sequencing individual microbial cells to reduce background noise and pollution.

To summarise, mNGS has a clear advantage in the diagnosis of mixed PJI due to its exceptional pathogen detection efficiency. However, relying solely on mNGS may result in numerous false diagnoses. As such, it is recommended to integrate both microbial culture and mNGS results when diagnosing mixed infections caused by multiple microorganisms. Pathogens can be confirmed if the microbial culture and mNGS results align. In cases of discrepancy, clinicians should carefully evaluate the patient's medical history and condition, as well as the characteristics and pathogenicity of the microorganisms involved, to make a comprehensive diagnosis.

Conclusion

In the present study, mNGS has demonstrated its value as a diagnostic tool for the management of PJI. The findings indicate that mNGS offers greater sensitivity than traditional microbial cultures, particularly in detecting co-pathogens in mixed infections. Its rapid turnaround time enables early diagnosis and intervention, which helps avoid unnecessary antibiotic use, reduces the need for revision surgeries, and plays a critical role in improving outcomes for patients with complex infections. Although mNGS shows lower specificity and positive predictive value compared to culture, it provides notable advantages in overall diagnostic accuracy and speed. Future improvements in specificity may be achieved through optimised experimental protocols, rigorous contamination control, efficient host DNA depletion, enhanced bioinformatics pipelines, integration of clinical data, and the use of multiple validation techniques. Additionally, machine learning and artificial intelligence hold promise for processing the large datasets generated by mNGS, identifying pathogen-specific sequences, and refining comparisons with established pathogen databases, thereby enhancing diagnostic precision and consistency. These results support the integration of mNGS into routine diagnostic workflows to accelerate and improve the diagnosis of PJI. Nonetheless, microbial culture is still the "gold standard" for the diagnosis of PJI, which can guide precise antibacterial treatment and is irreplaceable. mNGS should be viewed as a complementary technique that works in synergy with traditional culture methods, combining their respective strengths to advance clinical diagnostics.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing financial interests.

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