



Review Article

Progress in Application of Metagenomic Next-Generation Sequencing in Diagnosis and Treatment of Periprosthetic Knee Joint Infection

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Abstract: Background: With the development of total knee arthroplasty, patients benefit from the occurrence of postoperative complications that affect the efficacy of the procedure. Among them, periprosthetic knee joint infection is a catastrophic complication of total knee arthroplasty, which is more difficult to diagnose and treat in the clinic and has low patient satisfaction. The ability to clearly diagnose and identify the pathogen at an early stage is an important guide to treatment and is the key to successful treatment. Objective: Traditional detection techniques have the disadvantages of low detection rate, complicated operation and time consuming in the detection of pathogens in periprosthetic knee joint infection, which are difficult to meet the needs of disease detection. Metagenomic next-generation sequencing technology is used to extract the genetic material of pathogens in samples and combine with high-throughput sequencing technology and bioinformatics analysis for detection, which has the advantages of efficient, accurate and sensitive detection of pathogens. Conclusion: Metagenomic next-generation sequencing technology has high application value in the diagnosis and treatment of infectious diseases, and its application in the field of periprosthetic knee joint infection is becoming more and more widespread, and the application of this technology in the diagnosis and treatment of periprosthetic knee joint infection is reviewed.

Keywords: Metagenomic Next-Generation Sequencing, Periprosthetic Knee Joint Infection, Pathogen

1. Introduction

1.1. Periprosthetic Knee Joint Infection

Osteoarthritis (OA) of the knee is a common and highly prevalent disease worldwide, and surveys have shown that about 4% – 13% of the world's population is afflicted with osteoarthritis [1]. The main symptoms were joint pain and limitation of motion, which seriously affected the patient's life. In terms of treatment, patients with OA in the early and middle stages can be treated conservatively by reducing joint weight-bearing, functional exercise, use of non-steroidal drugs, and intra-articular injection. However, patients with advanced OA tend to respond poorly to conservative treatment, and most patients eventually require surgical treatment [2].

Total knee arthroplasty (TKA), as one of the most successful surgeries since the 20th century, has been widely carried out in the treatment of OA and has become one of the final treatment modalities for patients with advanced OA because of its definite surgical efficacy and high patient satisfaction [3]. With the continuous improvement of surgical techniques and biomechanical materials, the excellent and good rate of total knee arthroplasty is also increasing.

Surgical complications are the key to successful perioperative treatment, and periprosthetic joint infection (PJI) is a catastrophic complication of TKA. According to surveys, the incidence of PJI following primary TKA ranges from 0.7% to 2.0% [4, 5] while the incidence following revision surgery ranges from 5.6% to 35.0% [6, 7]. Although the incidence of PJI of the knee is relatively low, the total number of patients

who develop PJI after surgery is gradually increasing with the continuous development of TKA due to the current large base of OA patients [8].

The treatment of PJI of the knee is a great test for patients, who usually have a low quality of life. It is a huge economic burden in terms of treatment, and studies have shown that the cost of treatment for PJI is as much as five times that of TKA alone [9]. In addition, some studies have reported that the mortality rate of patients with repeated infections is even as high as 50% in the short term after two-stage revision [10]. The treatment of PJI of the knee joint is also a great challenge for clinicians. Some patients have no obvious early symptoms of PJI and are not easy to be diagnosed clinically. Early diagnosis and pathogen can help timely treatment plan, which is the key to successful treatment [11]. It has been reported in the literature that timely and accurate identification of pathogens in PJI can improve the control rate of infection [12]. However, it is still difficult to diagnose PJI of the knee in clinical practice, and traditional culture techniques are still the "gold standard" for the diagnosis of PJI [13]. However, the positive rate of pathogen detection is not ideal due to the shortcomings of traditional detection technology, such as time-consuming, complex operation, single detection, high culture requirements, and weak detection of low-virulence pathogens [14]. Fail to meet the clinical requirements for the diagnosis and treatment of knee PJI disease.

1.2. Metagenomic Next-Generation Sequencing Technology

In recent years, metagenomic Next-generation sequencing (mNGS), also known as high-throughput sequencing technology, has been continuously developed and widely used in clinical practice [15]. mNGS enables millions to hundreds of millions of nucleic acid fragments to be sequenced at a time by combining high-throughput sequencing technologies with bioinformatics analysis methods. mNGS does not rely on the isolation and culture of pathogens. Its principle is to extract nucleic acid sequences of pathogens in samples, construct sequencing libraries for detection, compare and analyze the results with databases, and finally identify the types and abundance of pathogens [16]. Compared with traditional detection techniques, mNGS has the advantages of high efficiency, accuracy, and sensitivity. It can not only significantly improve the detection rate of pathogens in patients with knee PJI, but also detect other potential pathogens without deviation, which is more conducive to the diagnosis and treatment of infection. It has a high application value in the diagnosis and treatment of infectious diseases, and the application of this technique in the diagnosis and treatment of PJI of the knee joint is reviewed.

2. Application of Traditional Detection Techniques in Diagnosis and Treatment of Knee PJI

At present, the traditional detection techniques used for the diagnosis of PJI of the knee include traditional culture

methods, serological methods, PCR methods, gene chip methods and other methods. Among them, traditional culture techniques remain the main method used in clinical practice to identify the pathogen of PJI in the knee joint. However, the detection rate of PJI pathogens in the knee joint is not high in traditional culture techniques due to factors such as prosthetic biofilm formation, low bacterial content in joint tissues, harsh culture conditions for some microorganisms, antibiotic use, and immunosuppression. It has been pointed out that the detection rate of pathogens by traditional culture techniques often does not exceed 50% [17, 18]. Even in patients with negative cultures, the possibility of PJI cannot be completely ruled out. Some patients with PJI of the knee joint may have multiple infections at the same time. Due to mutual competition between pathogens, weak pathogens are limited in growth and cannot be detected by traditional culture techniques, which is not conducive to the guiding selection of antibiotics and affects the therapeutic effect [19]. In addition, it has also been reported in the literature that because some pathogens need to grow in a specific environment, traditional culture methods reduce the detection rate of pathogens due to the inability to meet the culture conditions [20]. In addition, traditional culture techniques are cultured for a long time, often taking several days, which may delay the optimal timing of treatment of infection [21]. Serological methods are difficult to meet the needs of diverse pathogen detection due to the current limited number of antigen and antibody research and development. The PCR algorithm needs to predict the possible presence of pathogens in advance, cannot complete high-throughput detection, and has low detection efficiency. While gene chips can only detect known pathogens and cannot detect unknown pathogens, their application in the identification of PJI in the knee joint is limited.

In summary, traditional detection techniques fail to meet the need for rapid and efficient detection of knee PJI pathogens, which may delay the timing of treatment. Detection of PJI pathogens in the knee requires more efficient detection techniques.

3. Application of mNGS Technology in the Diagnosis and Treatment of Infectious Diseases

Infectious diseases refer to local or systemic inflammatory reactions caused by pathogens such as bacteria, fungi, viruses, parasites, tuberculosis and their products in human tissues [22]. The clinical manifestations are various and the condition is complex, which is one of the main causes of morbidity and mortality in patients worldwide. Timely identification of pathogens and targeted treatment measures are the key to successful treatment [23]. Due to the large use of antibiotics, immunosuppressive agents, hormones, etc. in clinical practice, combined with the increase of patients with malignant tumors, immunodeficiency, etc., the detection of pathogens faces complex and diverse problems, and traditional detection techniques are difficult to meet the needs of efficient diagnosis

and treatment of infectious diseases. Various emerging and recurrent infectious diseases and multiple infections also pose a great threat to human health, so higher requirements are put forward for the accuracy and timeliness of infectious disease diagnosis in clinical practice.

As a high-throughput sequencing technology, mNGS has the advantages of high efficiency, accuracy, and sensitivity. And because mNGS uses the method of random primer amplification of all nucleic acid sequences, all potential pathogens including bacteria, fungi, viruses, parasites and other microorganisms can theoretically be detected [15], which can significantly improve the early clinical diagnosis and treatment of infectious diseases, mNGS has received increasing attention from scholars in the neighborhood of infectious diseases [24].

3.1. Application of mNGS Technology in Diagnosis and Treatment of Bacterial Infection

Bacteria are still one of the most common pathogens of infectious diseases in clinical practice [25], and traditional culture techniques are standard methods for bacterial identification. However, the difficulty of bacterial culture, long culture time, single detection and low positive rate make it flawed in the diagnosis and treatment of bacterial infection diseases. As an efficient detection technology, mNGS can break through the limitations of traditional detection methods and be used for the rapid identification of bacteria in samples. The use of mNGS in bacterial infectious diseases has been reported in clinical practice. *Listeria monocytogenes* infection can cause meningitis, sepsis and other diseases, and traditional bacterial detection techniques are difficult to diagnose in clinical practice. Yao [26] et al. detected multiple meningitis patients with clinically suspected *L. monocytogenes* infection but negative traditional bacterial culture by mNGS, and finally the mNGS test results confirmed the diagnosis of *L. monocytogenes* infection, which rapidly improved after symptomatic treatment. It is also difficult to diagnose fever of unknown origin in time in clinical practice. Studies have reported that a 34-year-old man presented with acute symptoms of high fever, but the etiology remains unknown after perfecting laboratory, imaging and other relevant examinations. It was finally confirmed by mNGS that it was caused by *Coxiella burnetii* infection, and the patient's symptoms were significantly improved after timely treatment with tetracycline [27].

In summary, mNGS has the advantages of accuracy and high efficiency in the diagnosis of difficult to diagnose bacterial infections, fever of unknown origin caused by bacteria and other diseases, and mNGS can be used as an important supplement to the diagnostic methods for bacterial infections.

3.2. Application of mNGS Technology in the Diagnosis and Treatment of Fungal Infections

Fungal infection diseases are usually complicated and refractory. With the massive use of hormones,

broad-spectrum antibiotics and immunological agents, the infectious diseases caused by fungal infection are becoming more and more severe. Fungal detection in clinical practice mainly relies on conventional microscopic examination or traditional culture, and similarly, such detection techniques have weak detection ability for fungi. There have also been previous reports on the application of mNGS in the diagnosis and treatment of fungal infections. In a report of 310 immunocompromised patients with suspected pulmonary fungal infection, both conventional culture and mNGS testing were performed on samples. As a result, 20 fungi could be detected by mNGS, which was significantly higher than the traditional culture method in terms of sensitivity and specificity of detection [28]. Histoplasmosis is an endemic disease mainly prevalent in North America, and there are many misdiagnoses and missed diagnoses in China. A study reported a 27-year-old Chinese man who presented with progressive pharyngeal pain after chronic progressive lung disease without any symptoms for more than 1 year. Bronchoalveolar lavage fluid, epiglottic tissue culture, epiglottis and lung pathology were negative, and fungal infection with histoplasmosis was finally diagnosed after mNGS detection, with good results after treatment with itraconazole [29].

In summary: mNGS has rapid, sensitive and accurate pathogen identification ability in the diagnosis of immunocompromised patients and rare types of fungal infections, which can improve the diagnosis and treatment of infectious diseases.

3.3. Application of mNGS Technology in the Diagnosis and Treatment of Viral Infections

Viral infection develops rapidly and the symptoms are often serious. For patients, timely diagnosis and virus type are the key to successful treatment. At present, the diagnosis of viral infection mainly relies on immunological methods such as PCR method and gene chip method, but the detection of such methods requires first judging the virus type and cannot detect the unknown virus. mNGS is more convenient, efficient and comprehensive in detection because there is no need to predict the pathogen type in advance. In recent years, it has been favored by more and more physicians in the detection of viral infectious diseases. The novel coronavirus (Covid-19) is highly toxic and infectious, and is a viral infectious disease that has seriously affected the life and health of people all over the world so far, and the continuous generation of its variants has also increased the difficulty of prevention and control. Earlier studies have reported that the genomic sequences of novel coronavirus and various variants can be rapidly detected using the high efficiency of mNGS, which can provide a theoretical basis for clinical vaccine research and development [30]. Ebola hemorrhagic fever, which has been rampant in Africa and other countries, is caused by Zaire Ebola virus infection, and timely identification of the pathogen helps save the patient's life, but clinical diagnosis is difficult. Li [31] Equivalently detected Ebola virus in the blood of 70 patients suspected of having Ebola hemorrhagic fever by mNGS, and

also detected the remaining potential viruses including hepatitis B virus, human Peggy virus, Epstein-Barr virus, and Olengo virus.

In summary, mNGS has a more comprehensive and efficient detection ability for the detection of viral pathogens compared with traditional pathogen detection techniques. Because unknown pathogens can be detected and emerging viruses can be detected, it is helpful for public health institutions to monitor the epidemic.

3.4. Application of mNGS Technology in the Diagnosis and Treatment of Other Infections

At present, the detection methods for infectious diseases such as parasites, tuberculosis, chlamydia, mycoplasma and spirochetes mainly include culture method, microscopic examination method, immunological method and laboratory examination method, but such methods have high requirements for sample quality, collection time, culture conditions and experience of inspectors. mNGS It can compensate for the shortcomings of traditional detection techniques and is used for the diagnosis and treatment of such infectious diseases. Qu, J [32] et al reported a 51-year-old man who presented with low back pain lasting 3 years and right upper abdominal pain and discomfort for 4 months, abdominal CT examination revealed abdominal cysts, and the patient had a previous history of tuberculosis, but the symptoms were not relieved after receiving standardized anti-TB treatment. Abdominal cyst aspirate was eventually sequenced using mNGS and disseminated alveolar echinococcosis was diagnosed and the patient improved with albendazole. Tuberculous infection of skin wounds is now a rare condition. Kong, M [33] et al. reported a case of infected tuberculosis in a wound following injury. The patient suffered from wrist injury and was discharged after debridement. However, the wound showed persistent swelling and pain 2 months after surgery. Routine culture was negative and wound secretion was eventually mNGS performed Testing, Cutaneous tuberculosis was confirmed, and after taking anti-tuberculosis drugs, the wound healed and the symptoms disappeared. In addition, mNGS has also previously helped High detection rate of Mycobacterium tuberculosis in tuberculous meningitis Reported, considered mNGS Yes An alternative method for the detection of tuberculous meningitis [34]. Mycoplasma, Leptospira and other infections are difficult to culture and identify by conventional detection methods, and a case was reported on N Engl J Med using mNGS Testing cerebrospinal fluid to finally confirm the difficult cases of Leptospira infection is the first case in the world in which high-throughput sequencing technology is used for the diagnosis of infectious diseases [35].

In summary, mNGS can be used for the diagnosis of infections caused by rare pathogens and infections caused by pathogens that are difficult to diagnose compared with traditional pathogen detection techniques. When pathogens cannot be determined by traditional detection methods, mNGS can be used as an effective supplementary method and is a change in the way pathogens are diagnosed.

4. Application of mNGS Technology in the Diagnosis and Treatment of Knee PJI

As an emerging efficient detection technology, mNGS has been reported in the diagnosis of multiple systemic infectious diseases such as lung, brain, abdominal cavity, hematological system, and skin, and the advantages of efficient, accurate, and sensitive detection make it gradually adopted by clinicians in the field of knee PJI with difficult diagnosis.

4.1. Efficiency of mNGS Technology in the Diagnosis and Treatment of Knee PJI

At present, the detection ability of traditional detection techniques in knee PJI is still limited, and it has been reported in the literature that patients with suspected PJI who have negative preoperative traditional culture results are detected by mNGS in intraoperative tissue samples, and potential pathogens are found to be detected in up to 81.8% of patients [36]. Multiple infections may exist in knee PJI, and multiple pathogenic bacteria infection is one of the reasons for the failure of PJI treatment. Tarabichi [37] et al. tested 86 synovial fluid samples using mNGS and found that mNGS not only detected pathogens consistent with traditional culture results, but also detected a variety of other microorganisms. In addition, multiple infections also limit the ability of traditional detection techniques to detect pathogens to some extent. Mei, J [38] et al. performed traditional testing and mNGS on specimens from patients with multiple PJI and found that mNGS also had a good diagnostic effect in multiple PJI. In addition, mNGS has a fast running speed and can usually complete the detection of pathogens within 24-48 hours, greatly reducing the time required for detection and facilitating the early diagnosis and treatment of infection [39].

In summary, mNGS detection is efficient, comprehensive and rapid, allowing rapid detection of known pathogens and discovery of potential pathogens and can be combined with traditional culture techniques for diagnosis and treatment of knee PJI.

4.2. Accuracy of mNGS Technology in the Diagnosis and Treatment of Knee PJI

Tarabichi et al [40] first applied mNGS in the diagnosis of knee PJI in 2014, and they detected *S. canis* in all patients with knee PJI who had negative culture results using mNGS, which coincided with the patient 'history of dog scratch and provided guidance for the selection of antibiotics while confirming the diagnosis, showing excellent diagnosis and treatment results.

In addition, a study including 44 patients with suspected PJI who underwent surgery collected tissue samples from the periprosthetic area during surgery, along with conventional microbial culture and mNGS. The detection results showed that the accuracy of mNGS detection was significantly better than that of traditional microbial culture [41]. In addition, previous studies have also reported that mNGS was used again to detect PJI specimens with positive traditional bacterial

culture, and it was found that pathogens consistent with traditional bacterial culture were detected in all specimens, with an accuracy of 100%.

In summary, mNGS detection results are accurate and reliable and can be considered for routine application in the diagnosis and treatment of knee PJI.

4.3. Sensitivity of mNGS Technology in the Diagnosis and Treatment of Knee PJI

For patients who require revision surgery for suspected PJI of the knee, it is particularly important that the diagnosis be confirmed preoperatively. However, the sensitivity of traditional microbial culture is also low when faced with limited preoperative sample size, history of antibiotic treatment before sample collection, etc. Fang, X [42] et al performed mNGS on a small sample (1 ml synovial fluid) obtained preoperatively from patients with suspected PJI undergoing revision surgery, and the results showed that the mNGS test still had superior sensitivity. showed that even with only a small number of samples, mNGS still has good detection ability. Yu, Y [43] et al. evaluated the ability of mNGS to detect PJI patients who had received antibiotics within two weeks, and included a total of 52 cases of suspected PJI who underwent surgical treatment. mNGS and bacterial culture were performed on intraoperative tissue samples, and the results showed that mNGS was more sensitive than bacterial culture (69.5% vs. 23.1%, $P = 0.03$). showed that mNGS has a higher sensitivity for the detection of pathogens of PJI compared to traditional bacterial cultures, and mNGS detection is also less affected by antibiotic exposure. RUPPE [44] et al performed a study of traditional bacterial culture and mNGS detection in samples from 24 patients with osteoarticular infections, and the results showed that the predictive value of mNGS antimicrobial susceptibility was 94.1%, which could provide a basis for the selection of antibiotics.

In summary, mNGS has high assay sensitivity and can predict antimicrobial susceptibility, which helps to develop individualized medication regimens. It can also provide a new detection tool for special knee PJI cases such as less preoperative sample acquisition and preoperative use of antibiotics.

4.4. Application of mNGS Technology in the Diagnosis and Treatment of Rare Pathogens of Knee PJI

A retrospective analysis by Zhang, C [45] et al found that fungal PJI of the knee is uncommon in the clinic and represents a significant challenge for orthopaedic surgeons. Complications, history of multiple surgeries, and history of chronic antibiotic use are risk factors for fungal PJI of the knee. Compared with traditional microbial culture techniques, mNGS is more helpful for the diagnosis and treatment of fungal knee PJI, and antifungal cement interval therapy developed based on the test results is a supplementary option for the traditional treatment of knee PJI. Mycoplasma is a rare pathogen in PJI of the knee and is

difficult to diagnose, and there is no standardized diagnostic method for mycoplasma in PJI. Cai, Y [46] and others have continuously optimized the microbial culture method and 16S PCR method on the basis of mNGS standardization and studied the detection method that can improve the detection rate of mycoplasma.

In summary, standardized and optimized methods based on mNGS can be used to improve the diagnostic efficacy of mycoplasma PJI and are a novel diagnostic strategy for rare pathogens PJI.

4.5. Selection of Sample Types for mNGS Technology in the Diagnosis and Treatment of Knee PJI

Currently, the types of samples used for mNGS detection include periprosthetic tissue, synovial fluid and ultrasonic lysate of prosthesis. However, the optimal sample type has not been defined, and sample selection in clinical practice is blind. He, R [47] et al collected 177 specimens including periprosthetic tissue, synovial fluid, and ultrasonic lysate of the prosthesis from 59 joint prostheses undergoing revision surgery. Each sample was divided into two aliquots for culture and mNGS. The results showed that PJI could be detected efficiently using all three types of samples. However, the pathogen detection rate, sequencing reads and genome coverage of ultrasonic lysate samples of prostheses were superior to those of other samples. It has the potential to replace traditional tests such as bacterial culture.

In summary, mNGS was applied to detect PJI in knee joints, and the ultrasonic lysate of this prosthesis could be preferred for the selection of samples, which helped to improve the detection rate of pathogens.

5. Shortcomings of mNGS Technology in the Diagnosis and Treatment of Knee PJI

Although mNGS has superior detection ability in the detection of pathogens and has more successful applications in the diagnosis and treatment of knee joint PJI, its application in clinical practice still has many shortcomings, restricting its routine use.

5.1. Sample Contamination

There is a possibility of contamination during the extraction, transportation and treatment of samples. In the detection process, microbial contamination in the reagents may also cause false positive mNGS results due to cross-contamination. Therefore, due to regular evaluation of reagents, negative controls should be set. In addition, because most pathogens are easy to be degraded in accounting, they can be degraded by failing to strictly implement the standards or sequence operation in the process of extraction, transportation and detection, resulting in data loss and false negative results [48].

5.2. Host Nucleic Acid and Normal Microbial Flora Interference

The existence of host nucleic acids in samples is a major problem in detection. Because mNGS detection is highly sensitive, coexisting knee tissues in samples can interfere with host information and affect the accuracy of detection results [49]. In addition, detection is complicated by microbial flora that normally settle on the surface or deep tissues of the knee joint. Currently, mNGS can detect microorganisms in healthy human samples, and data information on pathogens can interfere with normal colonizers. It has been documented that an organism was identified in 6 of 17 patients following knee arthroplasty, but these patients did not have any clinical symptoms or laboratory evidence of knee infection [36]. It is difficult to clearly distinguish knee joint infection, colonization and contamination in clinical practice.

5.3. Effects of Antibiotic Exposure

The mNGS detects genetic material in a sample, so nucleic acids can be detected even in pathogens that have died. The detection of mNGS is theoretically unaffected by antibiotic use. This is consistent with the conclusions of most previous scholars' studies. However, because most pathogens have a short half-life of free nucleic acids, the number of nucleic acids is mainly determined by changes in the infectious condition, and the use of antibiotics is bound to intervene in the outcome of the disease. It has been found that the number of sequences detected by mNGS decreased significantly after antibiotic treatment in patients with concentrated toxemia, indicating the effect of antibiotic use on sequencing results [50]. Therefore, it is worth exploring whether serial numerical criteria should be lowered in patients with PJI of the knee who have undergone trial antibiotic therapy.

5.4. Database Construction Improvement and Data Processing Problems

At present, the database used for mNGS detection is huge and comprehensive, but there are still gaps and errors, so that some detection knots cannot be carried out or even provide wrong results, and the database needs to be continuously constructed and improved. In addition, mNGS has ultra-fast detection speed, and the generated macroscopic data also needs rapid and accurate analysis and comparison and convenient and feasible storage. Current technologies are still difficult to meet the increasing demand for mNGS in knee PJI [51]. On the other hand, most current pathogen tests are based on qualitative analysis, and quantitative detection of pathogen abundance is difficult [52].

5.5. Criteria for Result Analysis and Guidelines for Interpretation of Reports

The mNGS test results for knee PJI are complex and diverse, and the results need to be interpreted according to the actual situation. Testing variation among individuals also requires different criteria to be considered. However, there is still a

lack of uniform criteria for the analysis and comparison of mNGS test results and guidelines for the interpretation of reports [53]. In clinical, there is also a lack of uniform criteria for relative abundance, sequence readout cut-off for pathogens. The results of data processing, analysis and interpretation were different in different degrees.

5.6. Test Fee

Although the cost of high-throughput sequencing has declined in recent years, the cost of mNGS for PJI testing of the knee remains high. Compared with traditional detection technologies, mNGS has no advantage in economy [54], which is also one of the main reasons restricting its routine use. At present, most hospitals in clinical practice do not have the ability of mNGS detection and need to send samples for detection, but the cost of routine DNA and RNA detection usually varies from thousands of RMB, and patients mostly need to pay at their own expense, which is not a small economic burden.

6. Conclusion

The use of mNGS for knee PJI detection breaks through the limitations of traditional detection technology, has the advantages of efficient, accurate and sensitive identification of pathogens, has a good identification effect for rare, antibiotic-exposed, traditional culture-negative, unknown and other special pathogens, and can guide the use of antibiotics to some extent based on drug sensitivity prediction. The application of mNGS improves the success rate of knee PJI treatment.

It is anticipated that with the rapid development of mNGS, it will also be continuously optimized in terms of sequencing throughput, time, and cost, and will hopefully be routinely applied in the diagnosis and treatment of knee PJI. However, it is still in its initial stage, and it is necessary to continuously improve and optimize sample contamination, interference of host nucleic acid and normal microbial flora, antibiotic exposure, database construction and improvement, data processing standardization and report interpretation guidelines, detection costs, traditional methods result verification, drug resistance gene and drug susceptibility testing, and related software applications in the future to further improve the detection efficiency and better serve the clinic.

The treatment of PJI of the knee joint is difficult and requires interdisciplinary cooperation among orthopedic surgeons, infectious physicians, and microbiology experts in order to obtain the ideal therapeutic effect.

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