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Review of the Clinical Applications of Metagenomic Next-Generation Sequencing in Bloodstream Infection

Jiajun Zhang*, Guomin Zhang#, Shiman Jin

Department of Infectious Diseases, Affiliated Hospital of Chengde Medical College, Chengde, China
Email: *1432132008@qq.com, #cyfyzgm@163.com, 1721511763@qq.com

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Abstract

Bloodstream infection (BSI) is an important cause of morbidity and mortality worldwide. If we can make early diagnosis and start effective antibiotic treatment in time, the hospitalization time of patients with bloodstream infection can be significantly shortened. However, the current diagnosis of bloodstream infection cannot achieve the ideal therapeutic effect to a large extent because of the matrix effect of blood and the long turnaround time of blood culture. Therefore, a new detection method with a short turnaround time and high sensitivity is needed for the early diagnosis and timely treatment to improve the prognosis of patients. Metagenomic next-generation sequencing (mNGS) is a recently developed method for the comprehensive analysis of all microorganisms and genetic materials in clinical samples and is expected to be the main method for the early diagnosis of bloodstream infections. This review discusses the clinical application of mNGS in bloodstream infections. We also discuss technical challenges that need to be addressed to improve the diagnostic applicability of mNGS.

Keywords

Metagenomic Next-Generation Sequencing (mNGS), Bloodstream Infection (BSI), Bacteremia, Sepsis, Diagnosis

1. Introduction

Bloodstream infection is a serious systemic infection, which may lead to septic shock, disseminated intravascular coagulation, multiple organ failure, and even death. The annual mortality rate of patients with bloodstream infections is be-

*First author.
#Corresponding author.

tween 8% and 48% [1]. For patients with severe sepsis or septic shock, the mortality rate can reach 46%, the hospitalization time is long and hospitalization costs are high [2]. Therefore, appropriate empirical antimicrobial therapy should be provided early to prevent casualties [3]. Studies have confirmed that with every hour delay in the use of empirical antibiotics, the risk of death increases linearly [4]. However, studies have also reported that unnecessary empirical treatment increases the risk of *Clostridium labile* infection by 26%, which is a more serious trend of kidney injury and may also lead to an increased risk of antibiotic resistance [5]. In summary, rapid and accurate detection of BSI pathogens is of great significance to guide early antibacterial treatment, better manage the use of antibiotics, and improve clinical efficacy. MNGS has the characteristics of no preference, high sensitivity, and strong specificity, therefore, it can be considered as a promising molecular biological detection method for early diagnosis of bloodstream infection.

2. The Development of Sequencing Technology

Since the advent of first-generation sequencing technology in 1970, sequencing technologies have continuously developed and have now been updated to fourth-generation sequencing. The development of sequencing technologies has brought remarkable progress in many fields, including medical research, drug development, infectious disease diagnosis [6]. The main feature of first-generation (Sanger) sequencing technology is that it can read DNA with a length of about 1000 bp with an accuracy of 99.999%, and is mainly used for whole-genome sequencing of small genome pathogens, as well as bacterial 16S rRNA gene and fungal 18S rRNA gene sequencing. However, its large-scale application is seriously hindered because of its shortcomings of low throughput and high costs. Large-scale parallel sequencing technology makes high-throughput sequencing possible, ushering in the development of second-generation sequencing [7]. Second-generation sequencing technology can quickly detect all DNA or RNA in samples, and requires less blood and has a high positive rate, however, it cannot detect drug resistance genes and has the shortcomings of high cost, cycle length and high professional requirements. The reduction of sequencing costs promotes the popularity of mNGS technology. MNGS is a method for parallel sequencing of all nucleic acids (DNA and/or RNA) in clinical samples. It has a wide range of applications, including in studying the microbiome, antimicrobial resistance, human host gene expression, and oncology. MNGS simply extracts a small amount of DNA from a sample, identifies the pathogen by correlating the sequencing reads with an accurate reference genome database using bioinformatics tools, and based on the sequence depth, the pathogen's susceptibility to antibiotics can be inferred. Currently, the most widely used second-generation sequencers in clinical settings include the Illumina sequencer and BGISEQ sequencer. The Illumina sequencer has the highest throughput of all sequencers on the market, but the technology has the disadvantage of barcode index switching

and may be misallocated during flow cell scans, which may cause microbial readings from one sample containing high titers of pathogens to cross-contaminate other samples in the same operation, resulting in false-positive results [8]. BGISEQ sequencer has the advantages of low cost and short sequencing time, therefore, it is most commonly used to detect pathogens. BGISEQ sequencer is therefore increasingly being used for mNGS for pathogen detection in clinical settings [9]. Most sequencers have a long sequencing time and cannot provide real-time sequencing data. Third-generation sequencing technology is thus advantageous in that it can detect ten nucleotides per second, which greatly shortens the sequencing time [10]. Moreover, it can directly sequence original DNA/RNA samples without PCR amplification, has no preference for CG nucleotides, and can directly detect and obtain methylation information [11] [12]. Nanopore sequencing technology is not only the third-generation sequencing technology, but also considered a fourth-generation technology, because it can carry out real-time data collection and analysis, and is widely used in the field of outbreak investigation to detect infectious pathogens and antimicrobial resistance [13]. However, although third and fourth generation sequencing has many advantages, it has not been widely used in clinical settings because of its high error rate and cost [14].

3. Application Value of mNGS in Different Samples

3.1. Application of mNGS in Respiratory Diseases

Common samples for pathogenic detection for diagnosis in respiratory infectious diseases include nasopharyngeal swabs, sputum, broncho-alveolar lavage fluid (BALF), and solid lung tissue.

The theoretical research of mNGS in respiratory infectious diseases has become mature, and its sensitivity for the diagnosis of respiratory diseases is 50.7%, which is significantly better than that of traditional culture methods, especially in the detection of *Mycobacterium tuberculosis*, fungi, and anaerobes [15].

Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease (COVID-19) infection has spread rapidly around the world. mNGS can detect and analyze SARS-CoV-2 without the need for virus target enrichment or amplification and respiratory microbiome analysis, facilitating the detection of SARS-CoV-2 from nasopharyngeal swabs, which is easily obtained [16]. Huang performed mNGS on 240 patients with suspected pulmonary infection and found that the sensitivity of mNGS detection (88.30%) was much higher than that of traditional methods (25.73%), especially in patients with low immunity, while the specificity of mNGS detection (81.16%) was slightly lower than that of traditional methods (88.41%) [17]. This is consistent with the conclusions of Li and Qian [18] [19]. The reason for the low specificity of mNGS compared to traditional methods may be that traditional methods cannot detect all pathogens, increasing the true negative rate of non-pulmonary

infections. Lung biopsy is the gold standard for the diagnosis of pulmonary invasive fungal infection. Li predetermined the threshold of unique readings and relative abundance needed to identify infectious pathogens and then used a new mNGS-based data manager to identify pathogens in lung biopsies of 15 patients with pulmonary diseases, most of which were fungi [20]. The deficiency of this experiment is that the positive rate of *Mycobacterium tuberculosis* complex (MTBC) is low, which may be due to the high homology of MTBC, or it may be related to its predetermined threshold. In summary, the sensitivity of mNGS in detecting pathogens is better than that of lung biopsy Furthermore, mNGS also has a short turnaround time for pathogen detection, which is especially valuable in the selection of effective antibiotic therapy and improvement in the prognosis of patients.

3.2. Application of mNGS in Nervous Disorders

Cerebrospinal fluid (CSF) is a dynamic and metabolically active secretion that can provide important information related to cerebral inflammation. Wilson performed mNGS on the CSF of a 14-year-old boy with meningoencephalitis and successfully diagnosed him with neuroleptospirosis [21], which confirmed the effectiveness of mNGS in clinical diagnosis for the first time. Subsequently, more and more clinicians used mNGS to improve the rate of pathogen detection. A study compared mNGS with traditional methods and concluded that mNGS could detect bacteria, fungi, and other pathogens in culture-negative CNS, with a detection rate of 90% in patients without empirical treatment and 66.67% in patients receiving empirical treatment [22]. This indicates that empirical antimicrobial therapy before detection can significantly reduce the detection rate of traditional methods but does not affect mNGS. Therefore, mNGS detection can promote the adjustment of antibiotic treatment in time to improve prognosis and survival.

3.3. Application of mNGS in Other Samples

Osteoarthritis (OA) is one of the most crippling diseases in the West. Early diagnosis of OA can significantly improve the prognosis of patients. Akhbari sequenced the synovial fluid of 92 patients with osteoarticular infection and found that the sensitivity of mNGS (86.7%) was significantly higher than that of traditional methods (68.7%), and mNGS was of special value in the diagnosis of difficult-to-culture pathogens and patients who had been treated with antibiotics before detection [23]. Li sequenced the urine sample of a patient with suspected urinary tract infection whose results of traditional culture and serological tests were negative and found *Enterococcus faecalis* by mNGS [24]. Therefore, mNGS can be used in the etiological diagnosis of urinary tract infection. Wang performed mNGS on the skin and soft tissue samples of 96 patients with skin and soft tissue infection and found that mNGS was superior to culture in identifying viruses, anaerobes, and mixed pathogens [25]. In addition, mNGS can also be

used in the study of infective endocarditis and eye diseases [26] [27]. In conclusion, although mNGS cannot completely replace traditional methods in the short term, it plays an irreplaceable role in microbial detection and may be especially beneficial in certain cases and for use in conjunction with traditional methods.

4. The Diagnosis of Bloodstream Infection

To date, blood culture is still the gold standard for the diagnosis of bloodstream infection in clinical practice, and most pathogens of BSI can be diagnosed within 48 hours of blood culture [28]. However, there are many limitations of blood culture, including long turnaround time, high risk of contamination, limited sensitivity to antimicrobials, and inability to identify specific organisms [29]. To make up for the above shortcomings, molecular diagnostic technology is gradually on the rise. It includes pathogen detection based on blood culture positive samples (such as fluorescence in situ hybridization, DNA microarray-based hybridization, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, etc.) and direct detection of pathogens in whole blood (such as nucleic acid amplification, PCR combined with T2 magnetic resonance detection, second-generation sequencing, etc.). Fluorescence in situ hybridization (FISH) can identify common clinical bacteria, but because of its limited number of probes it cannot identify all pathogenic bacteria; it is also time-consuming (0.5 - 2.5 h) [30]. DNA microarray can simultaneously detect multiple pathogens and their antibiotic resistance genes and takes about 2.5 hours, whereas, in the growth of mixed bacteria blood culture, the association between drug resistance marker genes and specific organisms cannot be observed. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry can identify bacteria by comparing the protein spectrum of bacteria with the standard spectrum of known bacteria in the database [31]. It can accurately identify 87.60% of microorganisms in blood culture within 20 minutes, however, it cannot identify all the microorganisms in the mixed medium, and there are defects in the species-level identification of Acinetobacter and Enterobacter. Because their species and genera have similar phenotypes and protein spectrum [32].

The nucleic acid amplification technique can detect less than 1 ml of blood volume, which takes about 8 - 12 hours, making up for the defect of blood culture which requires a large quantity of blood sample. Moreover, mNGS is not affected by the use of antibiotics. Nucleic acid amplification may prove unsuccessful in the case of mutations and polymorphisms in the binding area of primers and probes, which may affect pathogen detection. PCR combined with T2 magnetic resonance imaging can be used to detect bloodstream infections caused by bacteria and Candida and takes about 3 - 7 hours, but the number of pathogens detected is limited, and there is often a lack of detection of drug resistance markers associated with ESKAPE bacteria [33]. Second-generation sequencing technique is used to amplify the 16S and 18S rRNA genes of bacteria and fungi, respectively, by primers, and then the amplifiers are sequenced to identify the

taxonomic map of the isolates. Second-generation sequencing can be suitably performed in whole blood, the turnaround time is 8 - 12 h, 48% and 86% for sensitivity and specificity，respectively [34]. Although this method can detect multi-bacterial infection, it cannot detect drug resistance genes and has low sensitivity, which limits its clinical application.

5. Application of mNGS in Bloodstream Infection

For blood samples, mNGS is more commonly used for clinical diagnosis and prognosis of hematological neoplastic diseases. Moreover, the pathogens leading to transfusion-related septicemia can be accurately detected to quickly evaluate the identity and abundance of pathogens, to reduce the risk of transfusion-transmitted infection [35]. Because all infectious pathogens contain DNA or RNA genomes, mNGS has become an attractive method for detecting pathogens in bloodstream infections. Studies have confirmed that the consistency between mNGS and blood culture test results of sepsis patients can be as high as 93.7%, but the overall diagnostic sensitivity of mNGS is significantly higher than that of blood culture and provides better detection results for culture-negative sepsis cases [35] [36]. Adult bloodstream infection is more common in middle-aged and elderly patients with more underlying diseases and low immunity [37]. Xu performed mNGS on plasma samples from 118 patients with sepsis and found that the detection rate of mNGS pathogens (35.59%) was higher than that of traditional microbial culture (16.10%) [38]. At the same time, difficult to detect pathogens such as viruses and chlamydia could be identified, which corroborates the results of Long [39]. Because the immune system and skin and mucous membrane barrier in children have not been completely established, their resistance is poor, and children are more likely to suffer from bloodstream infection. Moreover, their symptoms are atypical, disease progression is rapid, the need for mNGS is more urgent. He performed blood culture and mNGS pathogen detection in 25 children with suspected blood flow infection and found that the average reporting time of mNGS detection was significantly shorter than that of blood culture, and the positive rate (52%) was significantly higher than that of blood culture (4%), which provided a theoretical basis for the diagnosis and treatment of children [40]. Although mNGS has significant advantages in the diagnosis of bloodstream infection, it also has some shortcomings. Some studies have reported a remarkable rate of false-negative results associated with mNGS diagnosis [41]. Therefore, negative results of mNGS must be interpreted carefully. In addition, interpreting mNGS results is also challenging, and experts in mNGS laboratories are usually required to assist in diagnosis and treatment [42]. The biggest limitation of mNGS is the high costs of mNGS, and the turnaround time ranges from 1 day to 2 weeks depending on the type of sample used [43]. In the future, it is necessary to establish the detection efficiency of mNGS through large clinical studies, reducing detection costs, and formulating specific etiological detection methods. In conclusion, mNGS has undeniable advantages over culture

in the diagnosis and treatment of bloodstream infections because it can significantly shorten the time required for pathogen identification to less than 24 hours regardless of the type of microorganism and is not affected by antibiotic administration. In addition, mNGS may prove invaluable in patients infected with rare fungi, mycobacteria, and parasites, enabling doctors to accurately diagnose and regulate treatment in unique cases [44]. Furthermore, mNGS can detect the presence of viral or mixed infections to avoid antibiotic abuse [22].

6. Conclusion

mNGS is an unbiased diagnostic method, which can effectively detect all pathogens in clinical samples. It is especially suitable for patients with bloodstream infections in certain situations, such as negative blood culture results, treatment with antibiotics prior to testing, and identification of rare pathogens. However, its applicability in clinical settings has been limited mainly because of its high cost, imperfect reimbursement system, complex interpretation of sequencing results, and high professional requirements. It can provide more feasible basis for the application of mNGS in bloodstream infection by reducing the cost and establishing a more perfect bioinformation manager library. MNGS detection technology is expected to be carried out in more clinical hospitals in the future.

Authors' Contributions

All authors contributed to the conception of the Review. Zhang Jiajun conceptualized the study and drafted the manuscript; Zhang Guomin reviewed the literature and drafted the manuscript. Jin Shiman drafted the manuscript.

All authors agree to be accountable for all aspects of the work.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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