



COMMENTARY

The Role of Metagenomic Next-Generation Sequencing as a Promising Technology for Diagnosing HIV-TB Coinfection

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Abstract

The human immunodeficiency virus (HIV) pandemic has caused a resurgence of tuberculosis (TB), thus increasing morbidity and mortality. Moreover, HIV-TB coinfection leads to difficulties in diagnosis. Sputum smear microscopy, mycobacterial culture and GeneXpert MTB/RIF assays are generally endorsed to detect *Mycobacterium tuberculosis* (*M. tuberculosis*) in HIV-TB coinfection. However, these methods cannot diagnose TB in an accurate and timely manner, thus increasing the rates of HIV-associated morbidity and mortality in patients with TB. Hence, a considerable need exists for better diagnostic tools for patients with HIV-TB coinfection. Metagenomic next-generation sequencing (mNGS) is a novel detection platform widely used to assess infectious disease, antimicrobial resistance, the microbiome and human host gene expression. Herein, we summarize the advantages of mNGS for infectious disease diagnostics. We then assess the efficiency of mNGS in the detection of *M. tuberculosis* in different specimens and several cases of HIV-TB coinfection. We conclude that mNGS is an acceptable diagnostic method for HIV-TB coinfection, although limited research is available.

Key words: Metagenomic next-generation sequencing (mNGS), HIV-TB coinfection, diagnosis

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INTRODUCTION

People living with HIV have a high risk (15–21 fold higher than that in the absence of HIV) of developing active tuberculosis (TB), which is the leading cause of death in this population. In 2020, approximately 214,000 people died from HIV-associated TB [1]. The diagnosis of HIV-TB coinfection poses challenges, owing to the high incidence of smear-negative TB and extrapulmonary TB (EPTB) [2,3]. Smear microscopy, mycobacterial culture and GeneXpert MTB/RIF assays (Xpert) are commonly used to diagnose TB. Theron et al. have reported that the sensitivity of smear microscopy is only 20–50%.

Although mycobacterial culture is considered the gold standard for detecting *Mycobacterium tuberculosis* (*M. tuberculosis*), it is costly and time consuming, requiring approximately 2–8 weeks [4]. In 2010, Xpert was initially approved for the diagnosis of HIV-TB coinfection by the World Health Organization. A retrospective study conducted in a high HIV prevalence setting has established that the overall sensitivity of Xpert in smear-positive culture-positive TB cases is significantly higher than that in smear-negative culture-positive TB cases (94.7% vs 46.8%) [4]. Therefore, an accurate diagnostic approach is highly important for patients with HIV-TB coinfection.

Metagenomic next-generation sequencing (mNGS) is a novel technique with infectious disease applications. In 2008, mNGS was first applied in human infectious diseases and used to successfully identify a then-new virus called arenavirus [5]. This emerging approach can detect all potential pathogens, including bacteria, viruses, fungi and parasites. It also offers unbiased sequencing and identification of microbial genetic material. The use of mNGS in infectious diseases has been reviewed by Chiu et al., who have reported that mNGS has irreplaceable diagnostic value for rare, novel, atypical and complicated infectious diseases [6]. The sensitivity of mNGS in smear-negative TB is significantly higher than that of MGIT 960 culture and Xpert [7]. In 2022, a comprehensive overview of neurological infections and coinfections in HIV-infected Ugandan adults with subacute meningitis detected through unbiased cerebrospinal fluid mNGS was reported [8]. However, mNGS has scarcely been used for diagnosis of HIV-TB coinfection, and only several studies are available. We believe that mNGS may have value in the diagnosis of HIV-TB coinfection. Therefore, we provide evidence suggesting that this technology is not only applicable for diagnosing TB single infection but also for HIV-TB coinfection.

DETECTION ADVANTAGES OF mNGS, A PROMISING TECHNOLOGY

With the advent of next-generation sequencing technology in 2005, metagenomics emerged in the field of pathogenic microorganism detection. In 2008, Palacios et al. discovered a new arenavirus, a deadly transplant-associated disease, by using mNGS. In that study, mNGS was first used for diagnosing human infectious diseases [5]. In 2014, mNGS was successfully used to diagnose *Leptospira* infection, whereas traditional diagnosis methods, such as bacterial and viral culture, serologic tests and polymerase-chain reaction (PCR), did not identify the causative pathogen [9]. Furthermore, mNGS also plays a crucial role in diagnosing newly emerging infectious diseases, such as in rapid identification of SARS-CoV-2 [10]. Thus, the widespread application and development of mNGS has promoted diagnosis of infectious diseases.

mNGS is a promising tool with many advantages in the diagnosis of infectious diseases. First, high-throughput sequencing allows for simultaneous independent detection of thousands to billions of DNA fragments. Second, mNGS can sequence full-length genomes of pathogens. Therefore, it can not only accurately detect pathogenic microorganisms in cases with suspected complications or immunosuppression, but also screen drug resistance pathogens and virulence genes. Third, mNGS can be applied directly to original specimens without microorganism culture. mNGS has been successfully applied in various types of samples, such as cerebrospinal fluid, respiratory secretions, feces, urine, blood and other types of tissue [6]. Finally, mNGS, because it is an unbiased and hypothesis-free approach that can detect all types of

pathogens, has considerable potential for the discovery of novel or unexpected pathogens. Detecting *M. tuberculosis* in HIV-TB coinfection has been intractable, but mNGS is expected to overcome this problem by virtue of its detection advantages.

APPLICATION OF mNGS IN DETECTING *M. TUBERCULOSIS*

Early studies evaluated the potential ability of mNGS to detect *M. tuberculosis* [11]. With the development of sequencing technology, the role of mNGS in TB became recognized in many clinical studies. Zhou and colleagues, in a prospective study evaluating several detection methods for *M. tuberculosis*, have discovered that the sensitivity mNGS (44%) is similar to that of Xpert (42%) but much higher than that of other conventional methods (29%) [12]. Chen et al. have reported that the sensitivity of mNGS in diagnosing various types of TB specimens is far superior to that of *M. tuberculosis* culture detection (66.7% vs 36.1%) and Xpert detection (76.9% vs 61.5%) [13]. Another retrospective study has indicated that the diagnostic accuracy of mNGS in detecting active TB is higher than that of culture on liquid medium (the MGIT 960 system) (49.6% vs 35.2%). In addition, the turnaround time of mNGS detection, 32–36 hours, is markedly shorter than the 2–6 weeks required for the MGIT 960 system [14].

mNGS can be used to not only diagnose pulmonary TB but also detect *M. tuberculosis* from different EPTB types or specimens (Table 1). Many clinical studies have shown the superiority of mNGS in diagnosing EPTB, such as hepatic tuberculosis and tuberculous meningitis. Ai et al. first reported that mNGS can be used to diagnose local hepatic TB [17]. mNGS has also been used to detect *M. tuberculosis* from cerebrospinal fluid, and has been found to have higher sensitivity (66.67%) than acid-fast bacillus, PCR and *M. tuberculosis* culture (33.33, 25 and 8.33%) [16]. A large cohort study has evaluated that the ability of mNGS to rapidly detect smear-negative extrapulmonary specimens in an area with high TB burden and has shown that mNGS has higher sensitivity (56.11%) than Xpert (36.11%) and MGIT960 culture (13.89%) [7]. Zhu et al. have reported that mNGS provides sensitive detection of *M. tuberculosis* in bronchoalveolar lavage fluid or lung tissue biopsy samples in smear-negative cases with suspected pulmonary TB [15].

APPLICABILITY OF mNGS IN HIV-TB COINFECTION

HIV-TB is a specific type of TB whose diagnosis remains difficult, owing to the paucibacillary nature of the disease. Existing methods such as smear microscopy, mycobacterial culture and Xpert MTB/RIF cannot achieve accurate and rapid diagnosis, particularly in cases of smear-negative TB and extrapulmonary tuberculosis. On the basis

TABLE 1 | The ability of mNGS to detect *M. tuberculosis* from different specimens/EPTB types.

Specimen/EPTB types	Sensitivity (%)	Specificity (%)	Refs.
Bronchoalveolar lavage fluid	55; 90.63	98.2; 97.83	[14,15]
Lung tissue	88.9; 85.71	97.8; 93.33	[14,15]
Sputum	52.3	98.5	[14]
TBM/CSF	88.4; 66.67; 44	100; 100; 98	[7,16,12]
Serious fluid	50	97.3	[14]
Pus	50	98	[14]
Extrapulmonary tissue	40.9	100	[14]
Tuberculous lymphadenitis	70.21	100	[7]
Osteoarticular/spinal tuberculosis	53.33	100	[7]
Genitourinary tuberculosis	45.45	100	[7]
Tuberculosis peritonitis	33.33	100	[7]
Tuberculosis pleurisy	26.19	100	[7]
Tuberculosis pericarditis	25	100	[7]

CSF, cerebrospinal fluid; TBM; tuberculous meningitis; EPTB; extrapulmonary tuberculosis.

The mNGS sensitivity markedly varied across different specimens /EPTB types.

mNGS showed excellent specificity across different specimens/EPTB types.

of the diagnostic ability and advantages of mNGS, we believe that this technology may overcome the diagnostic difficulties of HIV-TB coinfection. In HIV-TB coinfection, mNGS has been used for early, rapid and accurate diagnosis of HIV-infected individuals with tuberculosis meningitis, thus facilitating the diagnosis of aseptic meningitis and the treatment of acute HIV infection [18]. In another prospective study in 368 HIV-infected Ugandan adults, Ramachandran et al. first developed a combined diagnostic approach with high sensitivity (88.9%) and specificity (86.7%) for TB meningitis and its many mimics by exploiting the specificity of mNGS in host cerebrospinal fluid and the sensitivity of a machine learning classifier. Thus, comparable combined assay performance at sequencing depths was a novel and successful application of mNGS should be suitable for low-income countries [8]. Overall, although several studies are available, the utility of mNGS in diagnosing HIV-TB coinfection cannot be overlooked.

DISCUSSION

Diagnosis of HIV-TB coinfection remains a challenge, and better diagnostic technology is required. Therefore, we provided an overview of mNGS as a promising technology to diagnose HIV-TB coinfection. First, mNGS can diagnose smear-negative TB and EPTB in HIV-coinfected patients, through detection of *M. tuberculosis* from multiple specimen types. Second, mNGS can sequence full-length genomes of pathogens and provide more genetic information than 16S rRNA sequencing and quantitative PCR. Therefore, it can serve as a comprehensive diagnostic approach through in-depth sequencing of various

pathogenic microorganisms and identification of multiple drug resistance sites [6]. In patients with HIV-TB, mNGS can be used not only to identify other co-infection pathogens and determine *M. tuberculosis* recurrence or reinfection, but also to detect multidrug-resistant TB. Finally, mNGS can be used to guide clinical treatment regimens and improve antibiotic stewardship [19].

Although mNGS has made great achievements in diagnosing infectious disease, several challenges remain regarding quality control standards, bioinformatics analysis, testing cost and clinical application: i) Quality control standards must be strictly established. mNGS involves complex processes, such as sample collection, nucleic acid extraction, library preparation, sequencing and data analysis, all of which may be subject to contamination, thus causing false results. ii) The massive data generated by mNGS pose challenges in data storage and analysis with bioinformatics methods. Furthermore, public databases of microbial reference genomes must be updated and optimized. Although large databases such as the National Center for Biotechnology Information are currently relatively comprehensive, they also contain errors. iii) The detection cost is relatively high, thus limiting the widespread use of mNGS, particularly in resource-poor areas [20]. iv) Although mNGS has shown good performance in diagnosing TB and HIV-TB coinfection in some cases and samples, large-cohort, multi-center clinical studies of patients with HIV-TB coinfection are required to establish the use of mNGS for this application. With the improvement of existing technology, particularly the optimization of the sequencing process, the construction of a complete database and progress in bioinformatics, these challenges

can be overcome. In the future, mNGS is expected have the potential to become a more accurate, economical and universal diagnostic technology that can address the diagnostic dilemmas associated with complicated infectious diseases such as HIV-TB coinfection.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

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