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RESEARCH ARTICLE

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Clinical application of metagenomic next-generation sequencing technology in the diagnosis and treatment of pulmonary infection pathogens: A prospective single-center study of 138 patients

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Abstract

Introduction: Rapid and accurate pathogen identification is essential for the treatment of pneumonia. Metagenomic next-generation sequencing (mNGS) is a newly developed technology to obtain microbial nucleic acid sequence information quickly, efficiently, and without bias.

Methods: We performed shotgun metagenomic next-generation sequencing (mNGS) of bronchoalveolar lavage fluid (BALF) for pathogen identification in pneumonia in a prospective study with 138 patients from a single center. We compared the results of mNGS with standard methods including culture, staining, and targeted PCR and evaluated the clinical applicability of mNGS.

Results: Most of the patients (128/138, 92.75%) were cured or improved. One patient (1/138, 0.72%) died because of acute gastrointestinal bleeding, and 9 patients (9/138, 6.52%) showed no improvement. mNGS identified more bacteria (53 versus 27), fewer fungi (8 versus 31), and more viruses (16 versus 1) than standard methods. In total, treatment in 34 out of 138 cases (24.64%) was adjusted and optimized because of mNGS results. Positive mNGS results contributed to a definitive diagnosis in 23 cases (16.67%), which helped guide treatment decision by either adjusting the antibiotics without de-escalation or continuing the empirical treatment. mNGS also confirmed no active infection in 11 cases (7.97%) allowed for antibiotic de-escalation.

Conclusion: This prospective clinical study evaluated the clinical utility of mNGS for the diagnosis of pneumonia and showed that mNGS of BALF provides valuable information for effective treatment.

KEYWORDS
bronchoalveolar lavage fluid, metagenomic next-generation sequencing, pulmonary infection

1 | INTRODUCTION

Infectious diseases are a major cause of human death. Pulmonary infection is the most deadly of all infectious diseases due to its rapid onset, severe illness, and many complications.[1,2] The early and rapid identification of pathogenic agents for the selection of ideal anti-infective drugs is the key to improving clinical diagnosis and treatment outcomes. However, the identification of the causative pathogen in infectious diseases, including pulmonary infections, is difficult and often inaccurate. The main reason for this is the diversity of pathogenic agents, including bacteria, fungi, viruses, and parasites, and the limited existing microbiological detection methods. Depending on epidemiological characteristics, pneumonia is mainly divided into community-acquired pneumonia and hospital-acquired pneumonia, and the causative pathogens of the two are different. Bacterial and respiratory viral infections are common in community-acquired pneumonia, in which *Streptococcus pneumoniae* and atypical pathogens are more common, while pneumonia caused by fungi and parasites is affected by geographical location and host factors.[3] Ventilator-associated pneumonia (VAP),[4] common hospital-acquired pneumonia, mainly occurs due to *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Hospital-acquired pneumonia is prevalent in China and other countries around the world, especially in intensive care units, and is closely related to the patient's underlying disease state, the source of pathogenic agent, the route of transmission, and the empirical anti-infective treatment before the pathogen is identified. There exist a number of difficulties in the detection of the causative pathogens of pneumonia, including the following: (1) due to empirical treatment and the misuse of anti-infective drugs, pathogen isolation and identification rate is often low, the sensitivity is poor, and is time-consuming; (2) severe pneumonia is often caused by multiple infections, and it is not easy to simultaneously detect them by conventional microbial culture; (3) traditional pathogen detection techniques have certain limitations. Currently, the identification of pathogenic agents in pneumonia mainly relies on microbial culture and nucleic acid amplification techniques. However, microbial culture is limited in that many pathogens cannot be cultured and many pulmonary infections present as multi-pathogen infections. The pathogenic agents may also not grow due to the ongoing empirical anti-infective treatments. Although nucleic acid amplification technology is sensitive, rapid, and non-culture dependent, it can only target known pathogens and has a narrow detection range. Therefore, new strategies for efficient and accurate diagnosis are being explored.

Metagenomics next-generation sequencing (mNGS) is a newly developed technology to obtain microbial nucleic acid sequence information quickly, efficiently, and without bias. In recent years, it has greatly benefited human microbiome research and improved our understanding of the impact of the microbiome on human health. Its diagnostic value is increasingly being recognized ever since a case of leptospirosis infection diagnosed by mNGS was first reported in 2014.[5] As common pathogenic agents contain nucleic acid as genetic material, NGS can identify the type of pathogen, as well as drug resistance gene information by nucleic acid sequence information. In addition, due to its high sensitivity, fast turn-around time, and unbiased, culture-independent nature, NGS has been used in medical microbiology as an emerging and powerful diagnostic technology and may become a rapid and universal diagnostic method for the diagnosis of infectious diseases. Metagenomic NGS includes 16S rRNA-based targeted-amplicon sequencing and shotgun metagenomic sequencing.[6] Targeted-amplicon sequencing is mainly used for bacterial pathogen detection and can also be used for the detection of eukaryotes and fungal pathogens based on 18S rRNA[7] or internal transcribed spacer sequencing,[8] respectively. The shotgun method can be used to detect a wider range of pathogenic agents including bacteria, fungi, viruses, and parasites as well as identify some drug resistance genes and is more suitable for the detection of pathogenic agents that cannot be identified using conventional protocols. Currently, there is a lack of a complete set of standard procedures from sample collection to amplification and library construction, quality control processing, data analysis, and the other steps to facilitate NGS application in clinical settings.

Bronchoalveolar lavage fluid (BALF) is easily obtained during bronchoscopy in patients with respiratory diseases. It is a pertinent clinical sample for pathogen evaluation in patients with respiratory infections.[9] Bronchoalveolar lavage contains high concentrations of nucleic acid, making it suitable for metagenomic sequencing, especially for the detection of pathogenic viruses and fungi.[9,10]

This study is a prospective single-center observational study involving BALF collection from patients clinically diagnosed with pulmonary infection in Jiaxing First People's Hospital from May 2018 to July 2021, and pathogen detection using traditional and secondary mNGS to compare their efficacy in pathogen detection and evaluate the application value of mNGS in pulmonary infection diagnosis.

2 | METHODS

2.1 | Patient enrollment

A total of 138 patients with pulmonary infections were enrolled in this study. The inclusion criteria were as follows: adults with an initial clinical diagnosis of pneumonia based on positive radiographic findings, such as chest X-ray or lung CT, and clinical presentations including the new onset of fever, cough, increased sputum production, shortness of breath, and hemoptysis; patients that could tolerate bronchoscopy and had adequate BALF for collection. The patients could read and understand, provide informed consent, were willing to cooperate with the research plan, and signed relevant documents.

The exclusion criteria were as follows: patients that were diagnosed as non-infectious diseases on being clinically suspected for infections caused by RNA viruses, such as influenza virus infection.

This research was approved by the ethics committee of Jiaxing First People's Hospital. All patients or authorized family members provided informed consent.

2.2 | Samples and laboratory testing

All patients were subjected to bronchoscopy. The BALF was collected and aliquoted for both standard laboratory testing and mNGS. The standard diagnostic methods for testing BALF including bacterial, mycobacterial, and fungal culture; gram staining, fluorescent staining, acid-fast bacilli (AFB) smear stain for mycobacteria, modified AFB stain for Nocardia, Gomori methenamine silver stain for fungi, direct fluorescence antibody stain (DFA) for *Pneumocystis jirovecii* (PJP), galactomannan antigen test, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, PCR assays for *Mycobacterium tuberculosis* (MTB) complex (GeneXpert, Cepheid), and cytomegalovirus (CMV) (Zhejiang IngeniGen XunMinKang Biotechnology, Hangzhou) were conducted. Besides, Cryptococcus capsular antigen tests were also performed using serum samples.[11]

2.3 | mNGS testing

BALF samples were collected, stored in a cryogenic storage container, and immediately transported to a commercial laboratory (Zhejiang IngeniGen XunMinKang Biotechnology, Hangzhou) for mNGS testing within 8 hr. Briefly, DNA was extracted from 300 μl of BALF and a sequencing library was prepared using the Total Nucleic Acid Extraction Kit and the mNGS-DNA Library Prep Kit (Zhejiang IngeniGen XunMinKang Biotechnology). Sequencing was performed using the 75-bp paired-end protocol on the Illumina Nextseq550 platform. At least 2.5 million reads (75 bp) were obtained from each sample after sequencing. IngeniSeq-MG v1.0 mNGS software (IngeniGen XunMinKang Biotechnology, Hangzhou, China) was used to analyzed the sequence data which contains a proprietary curated database of more than 20,000 microbial reference genomes.[11] Shotgun metagenomic sequencing was performed by an Illumina MiniSeq sequencer. The bioinformatics followed were as described previously by Fang et al.[12] All reports were reported to the doctor within 36 hr.

2.4 | Data analysis and quality control

Quality control measures to ensure the validity of the mNGS results were used as follows: (1) each mNGS run has a negative control to detect background microbial DNA contaminants. A true positive result was reported valid to the doctor only when the sequence reads were ten times more than the corresponding reads in the negative control; (2) a true positive result was considered valid only when the sequence reads of a species in a sample exceeded 10% of the total reads of the same species in all samples in the same run to avoid false-positive result; (3) a negative result was considered valid only when the internal control (a unique marine bacteria spiked in each sample) was detected at more than 100 reads; (4) the IngeniSeq-MG v1.0 mNGS software were used to determine the contaminants, which are species detected with more than 10% frequency in the negative controls over past 100 runs; (5) species normally colonizing in human respiratory tract, pre-determined by the IngeniSeq-MG v1.0 mNGS software, were also filtered out as contaminants if their reads were lower than a specific cutoff built in this software. The specific thresholds for filtering out normally colonizing in human respiratory tract were determined by a metadata analysis of >5000 BALF samples (proprietary data of Zhejiang IngeniGen XunMinKang Biotechnology).[11]

TABLE 1 Enrolled patient demographics and clinical manifestation

	N (%)
Gender	
Male	88 (63.77)
Female	50 (36.23)
Age (in years)	
18–49	30 (21.74)
50–69	78 (56.52)
≥70	30 (21.74)
Primary clinical symptoms	
Radiographic findings	138 (100)
Fever	32 (23.19)
Cough	69 (50.00)
Increased sputum production	36 (26.09)
Shortness of breath	29 (21.01)
Hemoptysis	9 (6.52)
Other	14 (10.14)

2.5 | Determination of clinical impact

A positive impact was defined by (1) a definitive diagnosis made depending on the mNGS results, or (2) a change in management due to the mNGS results that led to a favorable clinical outcome. A negative impact was defined by an inaccurate diagnosis made using the mNGS results that led to unnecessary or suboptimal treatment.

3 | RESULTS

3.1 | Patient summary

The study was conducted among 88 males and 50 females with ages ranging from 25 to 92 years (mean = 58.96; Table 1). All patients had radiographic findings consistent with pulmonary infection, with the most common symptoms being cough (50.00%), increased sputum production (26.09%), fever (23.19%), and shortness of breath (21.01%; Table 1). The condition of most of the patients (128/138, 92.75%) improved. One patient (1/138, 0.72%) died because of acute gastrointestinal bleeding, and nine patients (9/138, 6.52%) showed no improvement.

3.2 | Comparison of mNGS results to standard methods

mNGS data and results of standard methods had a complete agreement in 55 cases (55/138, 39.86%) with 47 negative cases (47/138, 34.06%) and 8 positive cases (8/138, 5.80%), respectively.

Standard methods were unable to identify any pathogens in 82 of the 138 cases (59.42%), while mNGS identified the following number of unique pathogens per sample: 1 (n = 26), 2 (n = 5), or 3+ (n = 4; Table 2). In contrast, mNGS was negative in 74 cases (53.62%), of which standard methods identified 1 organism (n = 14), 2 organisms (n = 4), or 3+ organisms (n = 9) in a total of 27 cases (Table 2). Overall, mNGS identified more bacteria (53 versus 27), fewer fungi (8 versus 31), and more viruses (16 versus 1) than standard methods (Figure 1).

3.3 | Clinical Impact of mNGS results on diagnosis and treatment

Clinical treatment decisions are made by the doctor after summarizing test data. In 35 cases, pathogens were detected by mNGS but none by standard methods. Nine of the 35 patients received adjusted appropriate treatment because of mNGS results. In 27 cases, pathogens were detected by standard methods and none were detected by mNGS.

One of these patients, therefore, received adjusted treatment. The mNGS result of this patient helped to exclude active infection which allowed for antibiotic de-escalation. Of another 76 cases, the treatment of 26 was appropriately adjusted treatment because of mNGS results. In total, 34 out of 138 cases (24.64%) received adjusted treatment because of mNGS results. Treatment was not adjusted for 104 out of 138 (75.36%) cases.

Of the 34 cases detected positive by mNGS, mNGS results contributed to a definitive diagnosis in 23 cases (Table 3), which helped guide treatment decision by either adjusting the antibiotics without de-escalation or continuing the empirical treatment. While the 11 cases where mNGS confirmed no active infection allowed for antibiotic de-escalation (Table 4). In the other 104 cases, 73 of them did not detect additional pathogens. 31 cases detected additional pathogens, but the treatment did not adjust (Table 4).

TABLE 2 Comparison of pathogens detected by mNGS and standard methods for each specimen

		Standard methods			
		Negative	1	2	3+
mNGS	Negative	47	14	4	9
	1	26	7	2	4
	2	5	5	2	3
	3+	4	4	2	0

4 | DISCUSSION

In this study, we compared the application of traditional methods and mNGS in pathogenic detection in pulmonary infection using BALF and found that mNGS has certain advantages over traditional diagnostic methods. First, mNGS has a significantly higher detection rate for bacterial infection, mixed infection, viral infection, and pneumocystis infection. This is mainly because of the non-culture dependence and unbiased nature of mNGS in diagnosing pathogens, which makes the simultaneous detection of several different targets possible. mNGS, although was largely advantageous, showed less sensitivity in diagnosing fungal infection, which may be because of nucleic acid extraction due to inefficient fungal wall breaking, leading to high false-negative results.[13] The fungal cell wall is thicker and tougher, and its wall-breaking difficulty is greater than that of bacterial cells. In some cases, no fungi were detected by mNGS, but Cryptococcus neoformans was detected by conventional methods (ink staining and large capsular observed) and confirmed by the high-titer cryptococcus capsular antigen test. This example suggests that mNGS has certain limitations. However, in this study, there were also patients with mNGS testing that suggested Pneumocystis jirovecii infection, but standard diagnostic methods did not find it, and the patient's condition has improved significantly after Bactrim treatment, indicating that mNGS still has high-diagnostic value in certain cases of fungal infection.

The proportion of cases wherein pathogens were not diagnosed by both traditional methods and mNGS is relatively small, and the number of cases in which pathogens were diagnosed by mNGS alone is more than that by traditional methods alone. These features suggest the strong diagnostic utility of mNGS, especially for the detection of pathogens that are difficult to identify by traditional methods. Moreover, in cases where broad-spectrum antibiotics are

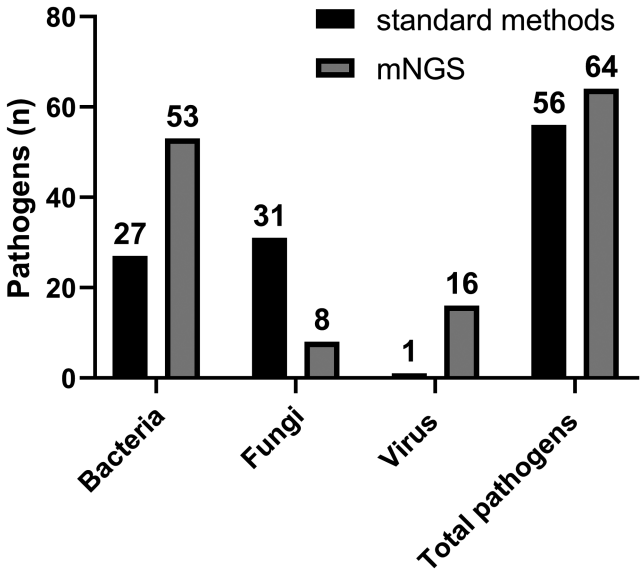

FIGURE 1 mNGS identified more bacteria (53 versus 27), fewer fungi (8 versus 31), and more viruses (16 versus 1) than standard methods

TABLE 3 Cases in which mNGS led to definitive diagnosis

Treatment changes	Pathogens newly detected by mNGS	Detailed information
Combine antiviral treatment ($n = 8$)	*Herpes simplex virus 1* ($n = 6$)	Combine Acyclovir treatment
	Human cytomegalovirus ($n = 2$)	Combine Ganciclovir treatment
Combine antifungal treatment ($n = 5$)	*Aspergillus fumigatus* ($n = 3$)*Exophiala xenobiotica* ($n = 1$)*Cryptococcus neoformans* ($n = 1$)	Combine Itraconazole treatment
Antibiotics adjusted ($n = 2$)	*Mycobacterium tuberculosis* ($n = 2$)	Combine Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol treatment
Empirical treatment continued ($n = 8$)	*Mycoplasma pneumoniae* ($n = 1$)*Moraxella catarrhalis* ($n = 1$)*Pseudomonas aeruginosa* ($n = 1$)*Legionella pneumophila* ($n = 1$)*Klebsiella pneumoniae* ($n = 2$)*Mycobacterium kansasii* ($n = 1$)*Haemophilus influenzae* ($n = 1$)	Empirical treatment has covered these pathogens

TABLE 4 Clinical impact and role of mNGS results

Clinical treatment	Role of mNGS results	Treatment changes due to mNGS results
Adjusted ($n = 34$, 24.64%)	Contributed to definitive diagnosis ($n = 23$, 16.67%)	Combine antiviral treatment ($n = 8$)Combine antifungal treatment ($n = 5$)Antibiotics adjusted ($n = 2$)Empirical treatment continued ($n = 8$)
	Helped to exclude active infection ($n = 11$, 7.97%)	Antibiotics de-escalated
Not adjusted ($n = 104$, 75.36%)	No additional pathogen detected ($n = 73$, 54.1%)	No changes
	Additional pathogen detected ($n = 31$, 22.46%)	

empirically used before detection, mNGS can be used as an effective supplement to traditional methods to further improve the efficiency of pathogen diagnosis in pulmonary infections and optimize treatment.

In this study, mNGS had an average turnaround time of 48 hr. Compared with traditional methods, including pathogen culture, mNGS is rapid and therefore helpful for clinicians to diagnose and make treatment decisions, especially in mixed infection. Furthermore, mNGS has obvious clinical utility in the detection of pathogens that are difficult to culture, including *Streptococcus*, anaerobic bacteria, cytomegalovirus, *Pneumocystis jirovecii*, and non-tuberculous mycobacteria. Some scholars have proposed that a shorter detection time is the key factor for mNGS to be widely used in clinical practice.[14] Moreover, some studies have shown that high-throughput sequencing to diagnose pathogens can be completed in as little as 9 hr,[15] however, 48 hr is the average time needed. This is advantageous over traditional methods that are completed an average of approximately 3–5 days.[13]

Generally, culture methods are considered as gold standard for pathogen detection. For example, the diagnosis of *Mycobacterium tuberculosis* mainly relies on acid-fast staining and GeneXpert, the diagnosis of Pneumocystis infection relies on silver staining, and that of fungal infection relies on fluorescent staining, G test, GM test, and Cryptococcus capsular polysaccharide antigen detection. The diagnosis of viral infections mainly relies on respiratory virus screening, CMV-DNA, etc. mNGS has some diagnostic limitations. Although mNGS has a higher rate of diagnosis than traditional methods, previous studies have shown that the diagnostic sensitivity of mNGS is between 36%[16] and 100%.[17] This wide range of sensitivity may be explained by the fact that there is currently no unified standardized protocol for mNGS testing. The process of clinical specimen collection, storage, and transportation may need to be further optimized to avoid inaccurate test results.[13,18,19] Therefore, for improved sensitivity, mNGS results should be evaluated based on clinical presentation and concurrent laboratory findings, including bacterial and fungal cultures, direct smear staining, histology, and serology. In cases where disparities arise in the results of the gold standard method and mNGS, clinicians must use their clinical judgment, as in this study, to decide on the optimum treatment.

In the case of pathogenic infections that are difficult to clinically detect or cases in which broad-spectrum antibiotics have been used empirically before testing, mNGS has unique diagnostic advantages and can be prudently applied in clinical practice.

In conclusion, we presented a prospective clinical study to evaluate the utility of mNGS in clinical settings for the diagnosis of pneumonia and found that mNGS of BALF provided valuable information for treatment optimization. We postulate that although mNGS cannot replace the existing traditional methods, it is indispensable as a supplementary method.

AUTHORS CONTRIBUTIONS

Na Li, Xiaolong Ma and Feng Tao designed the study. Jiaqi Zhou, Jingjing Deng, Chao Gu, Chunyuan Fei, Linfeng Cao, and Qi Zhang were involved in the patient enrollment. Na Li, Jiaqi Zhou, Jingjing Deng, and Feng Tao performed the analyses and drafted the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

Na Li, Xiaolong Ma, Jiaqi Zhou, Jingjing Deng, Chao Gu, Chunyuan Fei, Linfeng Cao, Qi Zhang, and Feng Tao declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used in this study cannot be shared for legal reasons. The data included participant's private information and can only be used for the purpose stated in the informed consent granted and not be shared to the public. However, the data can be obtained from the corresponding author due to reasonable request.

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REFERENCES

1. Kalil AC, Metersky ML, Klompas M, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis*. 2016;63:e61-e111.
2. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin Infect Dis*. 2007;44(Suppl 2):S27-S72.
3. Remington LT, Sligl WI. Community-acquired pneumonia. *Curr Opin Pulm Med*. 2014;20:215-224.
4. Nair GB, Niederman MS. Nosocomial pneumonia: lessons learned. *Crit Care Clin*. 2013;29:521-546.
5. Wilson MR, Naccache SN, Samayoa E, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. *N Engl J Med*. 2014;370:2408-2417.
6. Forbes JD, Knox NC, Ronholm J, Pagotto F, Reimer A. Metagenomics: the next culture-independent game changer. *Front Microbiol*. 2017;8:1069.
7. Hadziavdic K, Lekang K, Lanzen A, Jonassen I, Thompson EM, Troedsson C. Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. *PLoS One*. 2014;9:e87624.
8. Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. *BMC Microbiol*. 2010;10:189.
9. Leo S, Gaia N, Ruppe E, et al. Detection of bacterial pathogens from Broncho-alveolar lavage by next-generation sequencing. *Int J Mol Sci*. 2017;18(9):2011.
10. Pan T, Tan R, Qu H, et al. Next-generation sequencing of the BALF in the diagnosis of community-acquired pneumonia in immunocompromised patients. *J Infect*. 2019;79:61-74.
11. Zhou H, Larkin PMK, Zhao D, et al. Clinical impact of metagenomic next-generation sequencing of bronchoalveolar lavage in the diagnosis and Management of Pneumonia: a multicenter prospective observational study. *J Mol Diagn*. 2021;23:1259-1268.
12. Fang X, Xu M, Fang Q, et al. Real-time utilization of metagenomic sequencing in the diagnosis and treatment monitoring of an invasive adenovirus B55 infection and subsequent herpes simplex virus encephalitis in an immunocompetent young adult. Open forum. *Infect Dis*. 2018;5:ofy114.
13. Simner PJ, Miller S, Carroll KC. Understanding the promises and hurdles of metagenomic next-generation sequencing as a diagnostic tool for infectious diseases. *Clin Infect Dis*. 2018;66:778-788.
14. Motro Y, Moran-Gilad J. Next-generation sequencing applications in clinical bacteriology. *Biomol Detect Quantif*. 2017;14:1-6.
15. Pendleton KM, Erb-Downward JR, Bao Y, et al. Rapid pathogen identification in bacterial pneumonia using real-time metagenomics. *Am J Respir Crit Care Med*. 2017;196:1610-1612.
16. Langelier C, Zinter MS, Kalantar K, et al. Metagenomic sequencing detects respiratory pathogens in hematopoietic cellular transplant patients. *Am J Respir Crit Care Med*. 2018;197:524-528.
17. Parize P, Muth E, Richaud C, et al. Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study. *Clin Microbiol Infect*. 2017;23(574):e1-e6.
18. Goldberg B, Sichtig H, Geyer C, Ledeboer N, Weinstock GM. Making the leap from research laboratory to clinic: challenges and opportunities for next-generation sequencing in infectious disease diagnostics. *MBio*. 2015;6:e01888-15.
19. Martin TC, Visconti A, Spector TD, Falchi M. Conducting metagenomic studies in microbiology and clinical research. *Appl Microbiol Biotechnol*. 2018;102:8629-8646.

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