Metagenomic Next-generation Nequencing Aids the Diagnosis of Co-infection In HIV-infected Patients

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Abstract

Background HIV-infected patients are easy to combine with various opportunistic infections due to their low immunity. Improving microbiological diagnosis in these patients is of paramount clinical importance.

Methods Thirty-six HIV-infected patients with suspected infection were retrospectively reviewed from April 2018 to December 2019. The diagnostic performance of pathogens was compared between traditional microbiological methodology and mNGS.

Results The sensitivity of mNGS for diagnosing infectious disease was outperformed of culture, especially for virus (mNGS only; $P < 0.001$), fungus (odds ratio [OR], 1.1[95% confidence interval {CI}, 0.9–1.5]; $P < 0.05$), parasite (mNGS only; $P < 0.01$) and Mycobacterium tuberculosis (OR, 1.7 [95% CI, 1.0 – 2.8]; $P < 0.05$). Moreover, for mNGS-positive cases where the conventional method was inconclusive, 27 (69.2%) cases led to diagnosis modification.

Conclusions mNGS was more sensitive and comprehensive for pathogen identification therefore a promising method for microbiological diagnosis in AIDS patients with infection.

1. Introduction

Globally, 36.9 million people are living with human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS), with 1.7 million new infections in 2018 [1]. HIV virus infection reduces the number and functionality Of CD4 helper lymphocytes that direct and coordinate acquired immunity against most pathogens [2]. The gradual decrease in CD4 T lymphocytes cells ultimately results in a loss of control over immune response and the development of various opportunistic infections [3, 4], including bacteria, virus, parasites and fungi [5]. Although, the incidence of opportunistic infections has decreased after the discovery of antiretroviral drugs, it continued to be a serious issue which may have a significant impact on their well-being, quality of life, health care costs and their survival [6–8]. Pathogen identification of infectious disease is always difficult, leading precision diagnosis challenging in these patients. The low positivity rate and long time-consuming of conventional culture methodology increased difficulty of accurate and timely diagnosis, while the culture-independent techniques such as nucleic acid amplification tests needed for target specific primers. By combining unbiased sequencing, rapid data analysis and comprehensive reference databases, Metagenomic next-generation sequencing (mNGS) can be applied for hypothesis-free, universal pathogen detection, promising to improve diagnostic yield for syndromic testing of these infections [5, 9–12]. It is revolutionizing the field of medical diagnosis and paves the way for personalized medicine [13]. The technology of mNGS is rapidly becoming routine and shared between diagnostic specialties [14]. However, literature relevant to clinical applications for HIV-infected patients is lacking. With this objective in mind, here we assess the performance of mNGS testing in HIV-infected patients with suspected infection in real-life clinical practice.

2. Material And Methods

2.1 Study patients

We retrospectively reviewed 36 HIV-infected patients with suspected infection. at The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China, between April 2018 and December 2019. Peoples with positive antigen/antibody test of blood and confirmed by western blot or viral load testing were considered HIV-infected. Fifty-four specimens from the patients were tested by mNGS testing (BGI China) as well as regular clinical microbiological assay to evaluate if they were co-infected with other pathogens.

2.2 Metagenomic Next-generation Sequencing and Analysis

The protocol of Metagenomic Next-generation Sequencing and criteria for a positive mNGS result

refer to previous publications [15]. Samples, including blood, bronchoalveolar lavage fluid (BALF) and cerebrospinal fluid (CSF), were analyzed using a commercially available mNGS assay, which sequences cell-free DNA, reporting bacteria, fungi, DNA viruses, and parasites present at levels greater than a predefined threshold after removal of human sequences. Any organism identified by either mNGS or conventional infectious diagnostic testing was retrospectively classified as “clinically relevant” if the treating physician made management decisions based on the result; for cases in which this was unclear from the medical records, clinical relevance was determined by expert opinions from 2 pediatric infectious diseases (ID) physicians not directly involved in the patient’s care, with the opinion of a third ID physician used to resolve any discrepant opinions. Patients were classified as positive for an infectious disease if conventional and/or mNGS testing revealed a clinically relevant pathogen [16].

2.3 Statistical Analysis

Comparative analysis was conducted by Pearson χ 2 test, Fisher exact test, or the McNemar test

for discrete variables where appropriate. Data analyses were performed using SPSS 22.0 software.

P values < 0.05 were considered significant, and all tests were 2-tailed.

3. Results

3.1 Patient Characteristics and Sample

Demographic features of the patients in the current study are provided in Table 1. These patients were either diagnosed with HIV-infected previously or after admission to the hospital. The patients had a median age (range) of 37.5 (26–59) years, with 83.3% (30/36) males. Five of them were previously diagnosed with hepatitis B, nephritis syndrome or epilepsy, and the rest were healthy in the past. Of those, twenty-three contracted HIV through sexual transmission, only one through intravenous drug addiction, and for the remaining patients, the route of HIV infection were unknown. The median frequency of CD4 + T cells in the peripheral blood of 97.5 (ranging from 2 to 549/ul), while the HIV viral load was ranging

from undetectable to 1.0E + 07 copies/ml. Of the 36 patients, 25 were hospitalized for once, 7 for twice, 2 for three times, 1 for four times and 1 for five times, bringing the total number of hospitalization person-times to 54. In the 54 hospitalizations, BALF was collected from 17, blood from 15, CSF from 6, both BALF and blood from 6, both BALF and CSF from 2, for further detection. Therefore, specimens of 23 BALF, 23 blood and 8 CSF were subjected to standard culture and mNGS testing in a pairwise manner respectively.

Table 1
Characteristics of the patients in the current study.

Characteristics	**N = 36** **n (%), MD (LL, HL)**
Age	37.5 (26–59)
Male	30 (83.3%)
Weight (Kg)	68.5 (44-97.2)
WBC count (10^9/L)	5.7 (1.1–12.4)
Route of HIV infection	
sexual transmission	23 (63.9%)
intravenous drug addiction	1 (2.8%)
unknown	12 (33.3%)
CD4 + T cell count (/μl)	97.5 (2-549)
HIV viral load (cp/ml)	2.2E + 05 (undetectable-1.0E + 07)
HIV: human immunodeficiency virus.	

Table 2
Hospitalization person-times and number of samples

	Number of patients
Times of Hospitalization	
1	25
2	7
3	2
4	1
5	1
Sample Type	
BALF	17
Blood	15
CSF	6
BALF and Blood	6
Blood and CSF	2

mNGS: metagenomic next-generation sequencing; BALF□bronchoalveolar lavage fluid; CSF□cerebrospinal fluid.

3.2 Comparison of mNGS and Other Methods

The diagnostic performance of pathogens was compared between mNGS and traditional microbiological methodology. Among these 36 patients, the sensitivity for diagnosing infectious disease by mNGS of BALF (19/24, 79.2%), blood (28/32, 87.5%), CSF (9/9, 100%)were remarkably increased than culture of BALF (6/24, 25.0%), blood (6/28, 21.4%), CSF (2/9, 22.2%), and smear of BLAF (3/11, 27.3%), CSF (2/10, 20.0%) (Fig. 1). Although the total number of samples in each group is different, it can be seen that mNGS has obvious advantage in recognizing pathogen.

3.3 Comparison of mNGS and Culture Testing by Samples

The positivity rates of mNGS and culture tests of the BALF, blood, and CSF are illustrated in Fig. 2. Detection by mNGS and culture of BALF was compared in a pairwise manner, showing that the positivity rates of mNGS and culture of BALF were 21/23 (91.3%) and 5/23 (21.8%), respectively, with the statistical difference was significant ($P < 0.001$) (Fig. 1). As expected, mNGS increased the sensitivity rate by approximately 69.5% in comparison with that of culture. The similar situation goes for blood and CSF, suggesting the sensitivity of mNGS did not differ among sample types.

3.4 Comparison of mNGS and Culture Testing by Pathogens

HIV-infected patients are usually associated with multiple pathogens, which increased the difficulty of clinical diagnosis and treatment. Detections by mNGS and culture were compared in a pairwise manner, showing that the percentage of mNGS-positive samples was significantly higher than that of culture-positive samples in terms of virus (mNGS only; $P < 0.001$), including *Human herpesvirus*, (mNGS only; $P < 0.001$), which was the most commonly detected pathogen, *Alphatorquevirus* (mNGS only; $P < 0.001$), and *Polyoma virus* (mNGS only; $P < 0.05$). mNGS also showed an increased sensitivity in detecting fungus (odds ratio [OR], 1.1[95% confidence interval {CI}, 0.9–1.5]; $P < 0.05$) and parasite (mNGS only; $P < 0.01$), of which was *pneumocystis jiroveci* (mNGS only; $P < 0.01$) (Fig. 3). *Cryptococcus Neoformans* and *Cyanobacterium marneffei* were also observed to have a higher yield rate by mNGS than that by culture, although the difference was not significant due to the small sample size. There is no significant difference between mNGS and culture in the positivity rate of common bacteria, while in terms of MTB (OR, 1.7 [95% CI, 1.0 – 2.8]; $P < 0.05$), a higher positivity rate in mNGS can be seen. It's worth noting that *Coxiella burnetii* was culture negative and it was detectable in mNGS.

3.5 Diagnosis Assisted by mNGS for Patients without Identifiable Etiology by Conventional Testing

Among 54 samples, 38 (71.7%) were mNGS positive, while the comprehensive conventional method was inconclusive, including *Coxiella burnetii* and *Penicillium Chrysanthemum* in plasma, MTB and *Toxoplasma gondii* in CSF and *Listeria ivanovii* and *Haemophilus influenzae* in BALF. Based on mNGS diagnosis, seven (18.4%) identified microbes confirmed the clinical diagnosis while up to 27 (69.2%) modified the initial diagnosis. Three (7.9%) were discrepant with clinical diagnosis, whereas 1 (2.6%) pathogens were uncertainly associated with clinical diseases.

4. Discussion

This retrospective study evaluated the clinical relevance of mNGS for the investigation of co-infection in a cohort of 36 HIV-infected patients. In our study, we compared detection by mNGS and traditional detection methods and found mNGS to be advantageous in three aspects. Firstly, in general, mNGS showed a higher diagnostic efficiency of infectious disease than culture and smear, although the number of samples is varied. Secondly, in BALF, blood and CSF sample types, we found a remarkably higher sensitivity in detection by mNGS vs culture compared in a pairwise manner. More importantly, mNGS is noted for its superior feasibility in detecting virus, fungus, parasite and MTB in general. Also, mNGS has proved to be a better choice when detecting those special microbes such as *Coxiella burnetii*. In addition, we have identified that a considerable percentage of infection diagnoses were confirmed and modified according to mNGS.

The positivity rate of mNGS was consistent with the expectation, which have reported a

variety of sensitivities from 36% [17] to 100% [18]. And interestingly, our results indicated that in recognizing common bacteria (excluding MTB), the sensitivity of mNGS is not superior to that of culture,

which is consistent with a previous report that, comparing the results obtained by mNGS, a majority (74%) of bacterial pathogen is identified by standard culture in bacterium-associated pneumonia [19]. Therefore, we concluded that unlike other microbes like virus, fungus and parasite, mNGS might not have the significant advantage in identifying common bacteria. Although mNGS (3000 Ren Min Bi [RMB]) cost more than any other regular methodology, the low rate of positivity, long time consuming and lack of accuracy making pathogen screening of traditional techniques less cost-effective. In conclusion, mNGS could emerge as a promising technology for precision diagnosis and tailored therapy for HIV-infected patients with suspected infection.

For HIV-affected persons, a low CD4 + T-cell count exposes them to a higher risk of opportunistic infections and even worse, multiple infections may co-exist. However, conventional diagnostic assays lack breadth of detection and sensitivity, therefore unable to discover multiple infections, which make it ineffective in microbiological diagnosis. For example, 90–95% of blood cultures remain negative in immunocompromised patients suspected of infection, even in the cases where bacterial or fungal sepsis is likely, negative results are recorded for 50% of the blood samples [20]. A better microbiological test is urgently needed to break this situation. mNGS, with its non-targeted identification of microbes, through deep sequencing of biological samples, data mining, and identification of pathogen sequences in the absence of a priori assumption, constitutes a paradigm shift in microbiological [21, 22], which might contribute greatly to HIV-infected patients with suspected infection. To our knowledge, this is the first study to evaluate the contributions of mNGS in microbial testing in a cohort of HIV-infected patients.

Our research also has its limitations. As a small-scale cohort retrospective study of 36 patients, different samples from the same patient, including BALF, blood and CSF were treated as independent samples. Among these 36 patients, some of them have been hospitalized for several times due to different infectious diseases and each hospitalization was counted as an independent individual. Nevertheless, we believe that varied infectious diseases lead to different condition of patients, making each hospitalization analyzed independently practicable and having minimal disturbance to the final judgment. More prospective studies of mNGS testing with broader samples in real-life clinical practice remain to be documented. For HIV-infected patients with suspected infection, a vast amount of sequence data from a single clinical sample makes interpretation complicated. Can we refer to the conventional standardization? Whether the positive pathogen is causative pathogen or not. Whether treatment is needed. The added value of mNGS in clinical managements of these patients will have to be evaluated, and additional work to associate typologies of microbiota with patient status needed to be further studied. The progressive awareness of physicians to the patients' benefit will certainly help mNGS to become standard in the practice of conducting microbiological diagnosis in HIV-affected patients.

5. Conclusion

HIV-infected patients are are susceptible to various opportunistic infections due to their low immunity. mNGS can be an important tool to improve the diagnostic yield of pathogens to better guide clinical treatment.

Declarations

Ethical approval and Consent to participate

This study was approved by the institutional review board of the Fifth Affiliated Hospital of Sun Yat-sen University (Zhuhai, P.R. China). Waiver of consent was obtained given the observational nature of the project.

Consent for publication

Not applicable.

Availability of data and materials

Data are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Xi Liu and Jinyu Xia conceived the study. Jiehua Chen collected data, analyzed and interpreted clinical data. Guangying Deng, Ruihua Zhong, Hongqiong Zhu, Xinghua Li helped to collect data. Jiehua Chen and Xi Liu wrote the manuscript. All authors approved the final submitted version.

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References

1. UNAIDS. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf. Accessed February 12, 2019.
2. Limper AH. The changing spectrum of fungal infections in pulmonary and critical care practice: clinical approach to diagnosis. Proc Am Thorac Soc. 2010;7(3):163–8.
3. WHO. Laboratory Guidelines for Enumerating CD4 T Lymphocytes in the Context of HIV/AIDS; World Health Organisation, Regional Office for South-East Asia. India: New Delhi; 2007. p. 62.

4. Melkova Z, et al. Current views on HIV-1 latency, persistence, and cure. Folia Microbiol (Praha). 2017;62(1):73–87.
5. Wilson MR, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med. 2014;370(25):2408–17.
6. Gona P, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. JAMA. 2006;296(3):292–300.
7. Iroezindu MO. Disparities in the Magnitude of Human Immunodeficiency Virus-related Opportunistic Infections Between High and Low/Middle-income Countries: Is Highly Active Antiretroviral Therapy Changing the Trend? Ann Med Health Sci Res. 2016;6(1):4–18.
8. WHO., Guideline on When to Start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for Hiv. World Heal Organ [Internet]; 2015, September:78. Available from: http://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/..
9. Graf EH, et al. Unbiased Detection of Respiratory Viruses by Use of RNA Sequencing-Based Metagenomics: a Systematic Comparison to a Commercial PCR Panel. J Clin Microbiol. 2016;54(4):1000–7.
10. Fischer N, et al. Evaluation of Unbiased Next-Generation Sequencing of RNA (RNA-seq) as a Diagnostic Method in Influenza Virus-Positive Respiratory Samples. J Clin Microbiol. 2015;53(7):2238–50.
11. Alquezar-Planas DE, et al. Discovery of a divergent HPIV4 from respiratory secretions using second and third generation metagenomic sequencing. Sci Rep. 2013;3:2468.
12. Simner PJ, Miller S, Carroll KC. Understanding the Promises and Hurdles of Metagenomic Next-Generation Sequencing as a Diagnostic Tool for Infectious Diseases. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 2018. 66(5.
13.): p. 778–788.
14. Dietel M, et al. A 2015 update on predictive molecular pathology and its role in targeted cancer therapy: a review focussing on clinical relevance. Cancer Gene Ther. 2015;22(9):417–30.
15. Schlaberg R, et al. Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection. Arch Pathol Lab Med. 2017;141(6):776–86.
16. Miao Q, et al. Microbiological Diagnostic Performance of Metagenomic Next-generation Sequencing When Applied to Clinical Practice. Clin Infect Dis. 2018;67(suppl_2):S231–40.
17. Rossoff J, et al., Noninvasive Diagnosis of Infection Using Plasma Next-Generation Sequencing: A Single-Center Experience. Open Forum Infect Dis, 2019. 6(8).
18. Langelier C, et al. Metagenomic Sequencing Detects Respiratory Pathogens in Hematopoietic Cellular Transplant Patients. Am J Respir Crit Care Med. 2018;197(4):524–8.
19. Parize P, et al., Untargeted next-generation sequencing-based first-line diagnosis of infection in immunocompromised adults: a multicentre, blinded, prospective study. Clinical Microbiology and Infection, 2017. 23(8): p. 574.e1-574.e6.

20. Toma I, et al. Single-molecule long-read 16S sequencing to characterize the lung microbiome from mechanically ventilated patients with suspected pneumonia. J Clin Microbiol. 2014;52(11):3913–21.
21. Fenollar F, Raoult D. Molecular diagnosis of bloodstream infections caused by non-cultivable bacteria. Int J Antimicrob Agents. 2007;30(Suppl 1):S7–15.
22. Goldberg B, et al. Making the Leap from Research Laboratory to Clinic: Challenges and Opportunities for Next-Generation Sequencing in Infectious Disease Diagnostics. mBio. 2015;6(6):e01888-15.
23. Lecuit M, Eloit M. The potential of whole genome NGS for infectious disease diagnosis. Expert Rev Mol Diagn. 2015;15(12):1517–9.

Figures

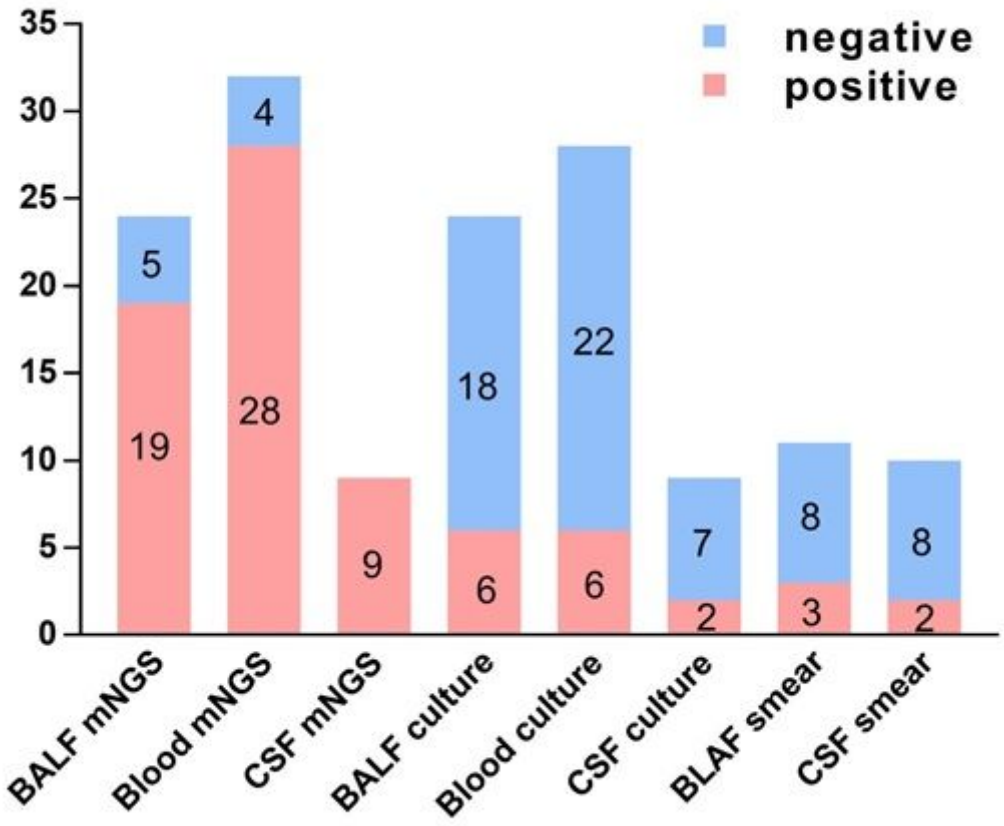

Figure 1

Positivity rate comparison among different detection methods. The number of positive samples (y-axis) for different detection methods (x-axis) is plotted against, showing that positivity rate was remarkably increased by metagenomic next-generation sequencing (mNGS) compared with culture and smear.

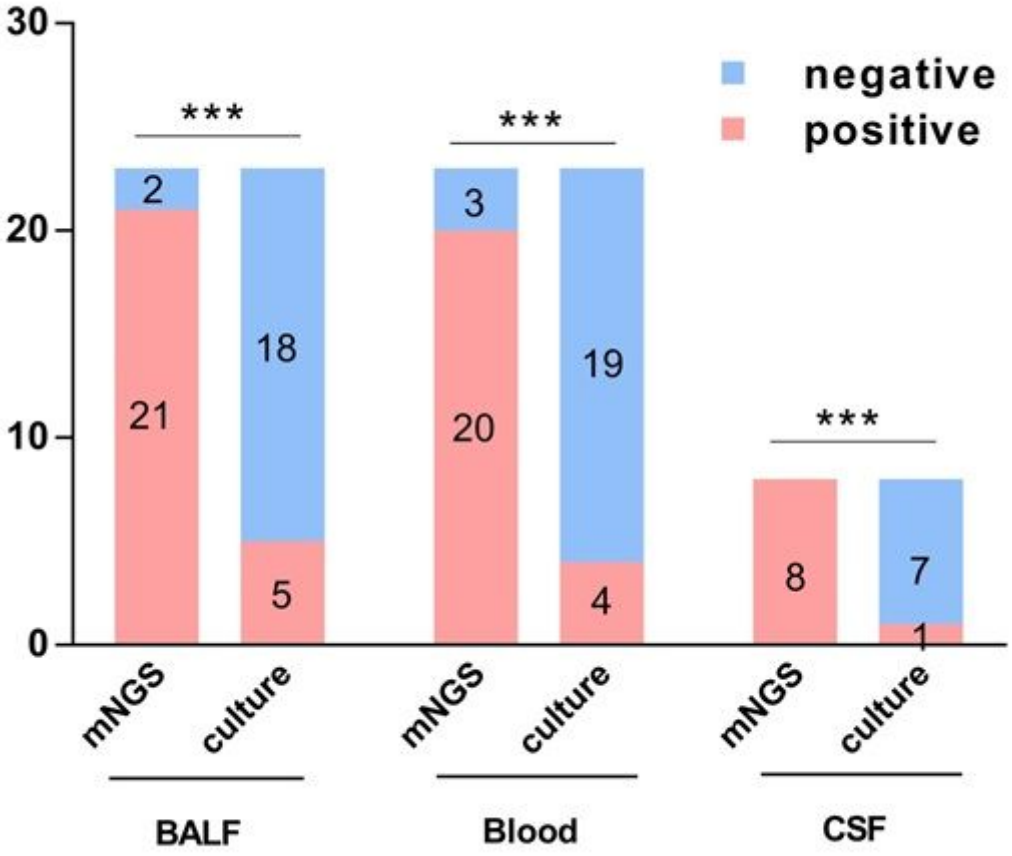

Figure 2

Positivity rate comparison between mNGS and culture in a pairwise manner. The sensitivity was increased by approximately 69.5% in mNGS in terms of bronchoalveolar lavage fluid (BALF) (91.3% vs 21.8%, P <0 .001), blood and cerebrospinal fluid (CSF) were similar situation. Interestingly, the overall positivity of mNGS and culture were unaffected by sample types.

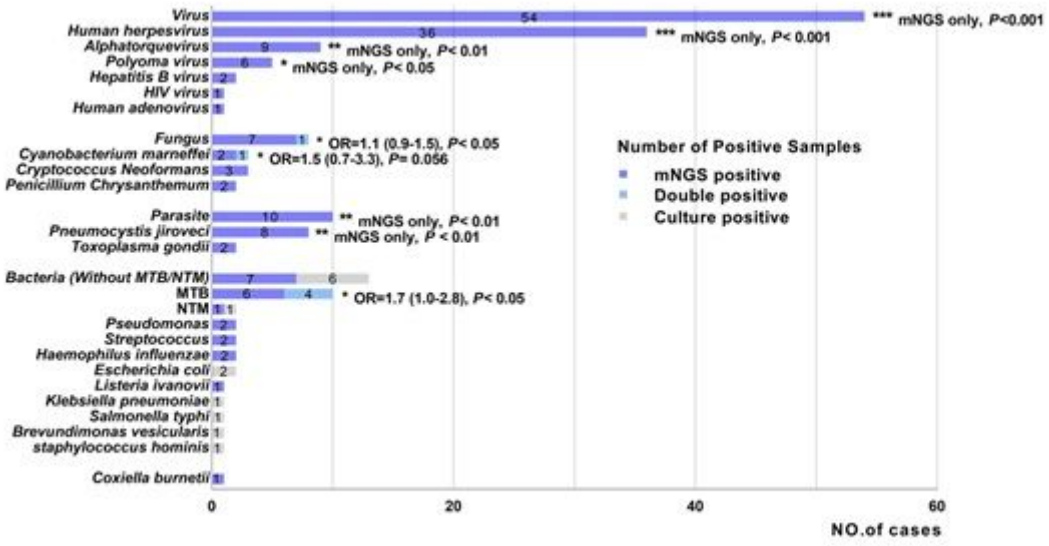

Figure 3

The overlap of positivity between mNGS and culture. By pairwise mNGS and culture testing, a total of 23 pathogens were detected and the corresponding frequencies are plotted in histograms. In general, compared to culture, mNGS was significantly more sensitive to detect fungi, virus, and parasite, but not bacteria (excluding MTB/ NTM). Besides, MTB demonstrated a higher positivity rate in mNGS in comparison to culture ($P < 0.05$). MTB: Mycobacterium tuberculosis; NTM: nontuberculous mycobacteria; OR: odds ratio.

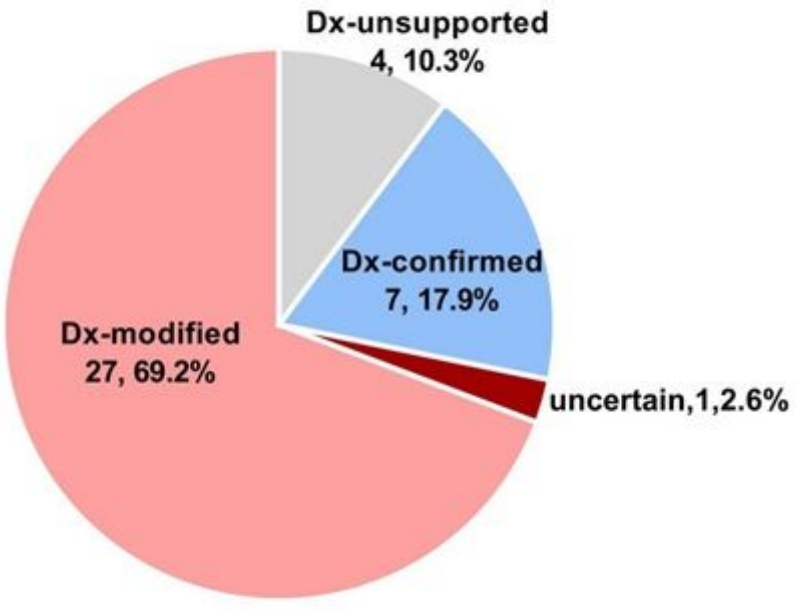

Figure 4

The concordance of Initial diagnosis and mNGS. Pie chart demonstrated that for those microbes identified only by metagenomic next-generation sequencing (mNGS), the primary empirical diagnosis was frequently confirmed by (17.9%) or modified (69.2%) according to mNGS, while only 10.3% was considered unreliable (diagnosis unsupported) and 2.6% uncertain. Dx, diagnosis.