Peer Review File

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Review Comments

Thank you for allowing me to review the paper. The authors conducted a retrospective study of 50 patients with pulmonary infections, comparing the diagnostic performance of metagenomic next-generation sequencing (mNGS) with conventional culture. They found that mNGS achieved a significantly higher detection rate (72.0% vs. 12.0%) and was able to identify viruses not detectable by culture. The study highlights the potential value of mNGS in improving pathogen detection, which is a clear strength of this work. I would like to raise several points for improvement:

1. It is expected that mNGS would show higher sensitivity than culture. However, mNGS positivity does not necessarily indicate true infection, as false positives, colonization, or contamination may occur. Including clinical outcomes—such as whether mNGS results led to treatment modifications or whether the detected pathogens were clinically relevant—would considerably strengthen the clinical impact of this work.
Reply 1: Thank you for your helpful suggestion. We added a table showing the mNGS results that led to treatment modifications by reviewing case records. As shown in Table 5, 8 cases added antifungal therapy, 7 cases adjusted antibiotic treatment, 2 cases adjusted both antifungal and antibiotic treatment, and 1 case adjusted antifungal treatment. Furthermore, our review of sample 19's case revealed that after empirical antimicrobial therapy, the patient's pulmonary inflammation improved and blood inflammatory markers decreased significantly (the result was not shown). According to the patient's symptoms, the clinician considered the small amount of *Aspergillus* detected by mNGS to be colonization and did not provide antifungal treatment. Therefore, the patient's treatment must be determined and modified based on actual symptoms and relevant laboratory results.
Changes in the text: We have added data on treatment modifications resulting from mNGS results, as shown in Table 5. See Page 2, line 52-55, Page 5, line 166-175, and the Table revise. All modified sections are highlighted in red.

2. Regarding viruses detected by mNGS, were they assessed for pathogenicity? Some may represent latent infections or viral reactivation, and their detection does not always imply causation of disease.
Reply 2: We thank the reviewer for the comment. We discussed the limitations of mNGS in line 228-235. For all microorganisms detected in samples using mNGS technology, it is difficult to determine which are true pathogens and to distinguish colonization from infection. Furthermore, mNGS cannot distinguish whether the nucleic acids detected are from live bacteria, dead bacteria, or free nucleic acid fragments. During the testing process, the laboratory environment, reagents, or

equipment may be polluted with nucleic acids, which may affect the mNGS test results. Therefore, mNGS cannot definitively prove the relationship between the pathogen and disease progression.

3. The inclusion criteria and patient characteristics, such as underlying conditions or use of immunosuppressive therapy, should be clearly described to help readers understand the study population and potential biases.

Reply 3: We thank the reviewer for the professional suggestions. In this study, we retrieved cases from the department of respiratory medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Patients diagnosed with pulmonary infection by clinicians based on symptoms, signs, and relevant auxiliary examinations between September 20 and November 20, 2024, were initially screened. Subsequently, fifty patients who received both BALF culture and mNGS were enrolled. Within the entire study cohort, thirty-four patients (68.0%) were male and sixteen (32.0%) were female, and the average age of patients was 62.9 years.

Changes in the text: We have modified our text as advised see Page 3, line 97-104. All modified sections are highlighted in red.