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Validation of mNGS results using extensive lab and clinical data

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Abstract

Purpose Interpreting the results of metagenomic next-generation sequencing (mNGS) presents a significant challenge in both clinical and laboratory contexts.

Methods A retrospective analysis was conducted to validate mNGS findings, with a particular emphasis on *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, and *Pneumocystis jirovecii* as representative pathogens, examined from both clinical and laboratory perspectives.

Results Based on a comprehensive clinical analysis, the mNGS demonstrated detection accuracies for *M. tuberculosis*, *M. pneumoniae*, and *P. jirovecii* of 87.0% (60 out of 69; 95% confidence interval [CI], 77.04%–92.99%), 97.6% (81 out of 83; 95% CI, 91.63%–99.34%), and 78.9% (45 out of 57; 95% CI, 66.72%–87.53%), respectively. Conversely, when incorporating laboratory confirmation from a variety of detection methodologies, the accuracy rates for mNGS in identifying *M. tuberculosis*, *M. pneumoniae*, and *P. jirovecii* were 92.7% (51 out of 55; 95% CI, 82.74%–97.14%), 82.3% (51 out of 62; 95% CI, 70.96%–89.80%), and 83.9% (26 out of 31; 95% CI, 67.36%–92.91%), respectively. Additionally, our analysis revealed no statistically significant difference in read counts and relative abundances between mNGS results deemed clinically as false positives and those considered true positives ($P < 0.05$).

Conclusion In contemporary clinical practice, the detection of positive results from mNGS is notably high from both laboratory and clinical standpoints. Nonetheless, the interpretation of results with low read counts presents significant challenges for both clinical and laboratory environments under current conditions.

Keywords mNGS, Reads, Relative abundance, Laboratory, Clinical

Introduction

Interpreting the results of metagenomic next-generation sequencing (mNGS) experiments presents significant challenges for both clinical and laboratory teams due to their inherent complexity. Clinicians, when assessing positive mNGS results, integrate a wide array of information, including the patient's medical history, imaging findings, and current antibiotic treatment status, to determine the potential correlation between the detected pathogen and the disease [1, 2]. Conversely, from a laboratory perspective, the team endeavors to validate the positive mNGS findings through a variety of confirmatory testing methods [3]. In our laboratory, validation tests for *Mycobacterium tuberculosis* encompass culture and Gene Xpert MTB/RIF assays, while those for *Mycoplasma pneumoniae* involve targeted polymerase chain reaction (PCR) and IgM antibody detection. For *Pneumocystis jirovecii*, the validation employs a targeted PCR method. The positive results obtained from mNGS have been corroborated using various laboratory

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methods, thereby ensuring a high degree of credibility. However, from a laboratory standpoint, the task of individually verifying each positive mNGS result is substantial. In practical clinical settings, it is impractical to perform laboratory validation on all positive mNGS findings. Given that *M. tuberculosis*, *M. pneumoniae*, and *P. jirovecii* are clinically significant pathogens, our laboratory has developed multiple methodological validation experiments for these organisms. Consequently, selecting these three pathogens as representative models allows for a retrospective analysis of the potential clinical utility of mNGS positive results from both laboratory and clinical perspectives.

Currently, there is a paucity of studies that comprehensively validate mNGS results using a variety of laboratory methodologies. A study conducted by M.R. Wilson assessed the accuracy of mNGS results for encephalitis and meningitis by comparing them with several methodological approaches, including culture, targeted PCR, serology, Sanger sequencing, and pathological biopsy [3]. The findings revealed that in 19 patients, both mNGS and traditional techniques identified the same pathogen; however, 26 cases were detected by traditional methods but missed by mNGS, while 13 cases were identified solely by mNGS and subsequently validated by other methodologies [3]. Despite the study's rigorous design, validating mNGS results using multiple methodologies for the same sample remains challenging in real clinical practice. To date, most evaluations of mNGS have relied on comprehensive clinical judgment [4–8]. Research that assesses mNGS results from both laboratory and clinical perspectives remains limited.

Furthermore, the variability in the proportion of human genes within the sample, coupled with the uncertainty in nucleic acid extraction efficiency and variations in sequencing depth, results in the read counts detected by mNGS being an unstable metric [1, 9, 10]. Despite this instability, both the read counts and relative abundance serve as quantitative indicators. This prompts the consideration of their potential utility in aiding the interpretation of true or false positive mNGS results. However, research addressing this issue remains limited.

Methods

Study design

This study was a multi-center retrospective analysis utilizing data from all consecutive cases of *M. tuberculosis*, *M. pneumoniae*, and *P. jirovecii* identified via mNGS between October 12, 2021, and October 11, 2023, at Tongji Hospital, encompassing the Hankou, Guanggu, and Sino-French campuses. We assessed the accuracy of mNGS results through laboratory-based testing and a comprehensive clinical interpretation of the findings for *M. tuberculosis*, *P. jirovecii*, and *M. pneumoniae*. Additionally, we compared the differences in read counts and relative abundance between true positive and false positive results, as interpreted from the comprehensive clinical data.

mNGS workflow

We employed distinct processing methodologies tailored to various sample types. Cell-free DNA was detected in the serum derived from blood samples. Respiratory specimens, including bronchoalveolar lavage fluid (BALF) and sputum, underwent host cell removal via differential centrifugation. For cerebrospinal fluid samples, 600 µL was extracted and subjected to a cell wall disruption protocol. Pus samples, ranging from 300 to 500 µL, were liquefied by incubation in a 37 °C metal bath for a duration of 5 to 15 min. Post-liquefaction, 1 mL of the sample was centrifuged at 15,000 rpm for 5 min, after which the supernatant was discarded, and the precipitate was retained for subsequent analysis. Tissue samples, approximately the size of a green bean, were homogenized with 100 µL of PBS buffer using an automated grinder. Nucleic acid extraction was performed utilizing the column extraction method provided by Vision Medicals Co. Ltd. (Guangzhou, China). The subsequent purification of nucleic acids and library construction adhered to the standardized operating procedures outlined by the manufacturer. Library pooling was conducted using the Illumina Nextseq CN500 sequencer, executing 75 cycles of single-end sequencing (SE-75), which yielded approximately 20 million reads per library. The bioinformatics analysis involved several steps: filtering out sequencing adapters, low-quality sequences, low-complexity sequences, short sequences, and duplicate sequences; eliminating sequences of human origin by referencing the Human Genome database; and comparing pathogen base sequences against a comprehensive pathogen database.

Diagnostic criteria for tuberculosis

Clinical diagnosis of tuberculosis encompasses the following three criteria: (1) a history of contact with individuals diagnosed with tuberculosis; (2) the presence of characteristic symptoms indicative of tuberculosis infection, such as persistent cough and expectoration lasting more than two weeks, hemoptysis, night sweats, fatigue, intermittent or persistent low-grade fever in the afternoon, anorexia, and weight loss; (3) imaging findings that corroborate a tuberculosis diagnosis. Confirmed cases must not only satisfy these three criteria but also provide evidence of the pathogen or pathological changes. Laboratory investigations include the cultivation of *M. tuberculosis* and nucleic acid testing for the bacterium [11].

Diagnostic criteria for *M. pneumoniae* infection

The patient presents with the following clinical manifestations: 1) The fever is irregular or persists for an extended period, showing no significant improvement or recurrence following standard antibacterial treatment. 2) The respiratory symptoms are varied and complex, including intermittent cough, particularly paroxysmal or spasmodic cough, or a dry cough without phlegm. There is an increased respiratory rate, wheezing, and even difficulty in breathing. Physical examination reveals audible rales in the lungs. Imaging findings include unilateral or bilateral patchy shadows, interstitial changes, or lung consolidation. Laboratory tests indicate: 1) positive peripheral blood IgM antibodies; 2) a positive nucleic acid test for *M. pneumoniae* in throat swab or sputum; 3) positive detection of *M. pneumoniae* antigen in throat swabs or sputum samples; 4) a positive culture result for *M. pneumoniae*. A diagnosis can be confirmed by the concurrent presence of clinical manifestations, imaging findings, and at least one positive laboratory result [12].

Diagnostic criteria of *P. jirovecii* infection

Patients frequently present with the following risk factors: AIDS, cancer, iatrogenic immunosuppression due to organ transplantation, autoimmune and inflammatory diseases, and nephrotic syndrome, among others. Clinically, manifestations encompass pneumonia with a subacute onset, fever, dry cough, progressive dyspnea, and hypoxemia. Imaging studies typically reveal multiple diffuse interstitial changes bilaterally, including bilateral interstitial lesions or alveolar infiltration around the pulmonary hilum, as well as diffuse ground-glass opacities on both sides. Laboratory diagnostics include hexamine silver staining, PCR detection of *P. jirovecii* nucleic acid, the G-test, and lactate dehydrogenase assays [13]. Notably, the negative predictive value of the G-test and lactate dehydrogenase assay is relatively high.

Laboratory confirmation test

The laboratory validation techniques for *M. tuberculosis* comprised both *M. tuberculosis* culture technology and the GeneXpert MTB/RIF assay (Xpert, Cepheid, USA). The cultivation methods for *M. tuberculosis* included the solid medium approach utilizing LJ medium and the liquid medium approach using the MGIT 960 system (BD Company, USA). For *M. pneumoniae*, laboratory confirmation methods encompassed targeted PCR technology (Genetic Analyzer, 3500DX, ABI Japan) and IgM antibody detection via chemiluminescence immunoassay (i3000, Yahuilong, China). The laboratory validation method for *P. jirovecii* involved targeted PCR technology developed in-house. The upstream primer utilized in this study is designated as MGB-Pj-F, while the downstream primer is identified as MGB-Pj-R, and the probe employed is Pj FAM-MGB. The PCR conditions are delineated as follows: an initial denaturation phase at 95 °C for 5 min comprising a single cycle; a subsequent denaturation phase at 95 °C for 15 s over 45 cycles; followed by an annealing and extension phase at 60 °C for 45 s, also over 45 cycles. The experimental results demonstrated no cross-reactivity with a range of microorganisms and viruses, including *Candida albicans*, *Staphylococcus aureus*, *M. tuberculosis*, *M. pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum, Legionella pneumophila, Epstein-Barr virus, cytomegalovirus, herpes simplex virus, enterovirus, adenovirus, influenza A virus, influenza B virus,* and *respiratory syncytial virus.* The precision of the in-house developed assay is notably high, as evidenced by the repeated testing of positive reference samples at both high and low concentrations, conducted ten times. The intra-batch coefficient of variation (CV) for the cycle threshold (CT) values was consistently less than 5%.

Statistical analysis

Descriptive statistics were employed to analyze the population data. Mann–Whitney U tests were conducted on unpaired data to assess the results pertaining to both relevant and unrelated groups as determined by the clinical treatment team. This analysis included metrics such as the read counts and the relative abundance of pathogens. A p-value of ≤ 0.05 was established as the threshold for statistical significance. The statistical analyses were performed using SPSS version 21.0. Figures were generated using GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA, USA) and the online tool available at https://bioinfogp.cnb.csic.es/tools/venny/index.html.

Results

Demographic characteristics of the patients

Between October 12, 2021, and October 11, 2023, our center identified a total of 69 cases of *M. tuberculosis*, 57 cases of *P. jirovecii*, and 83 cases of *M. pneumoniae* using mNGS. Within the cohort of *M. tuberculosis* cases detected by mNGS, there were 43 male and 26 female patients, with ages ranging from 1 to 74 years and an average age of 49 years. The cases of *M. pneumoniae* detected by mNGS included 40 males and 43 females, aged from 3 months to 70 years, with an average age of 16 years. Among the *P. jirovecii* cases identified by mNGS, 36 were male and 21 were female, with ages spanning from 1 month to 80 years and an average age of 44 years (refer to Table 1). The primary sample type utilized for mNGS detection of *M. tuberculosis*, *M. pneumoniae*, and *P. jirovecii* was BALF (see Fig. 1).

Table 1 Demographic characteristics of the 209 patients

Characteristic	Mycobacterium Tuberculosis ($n=69$)	Mycoplasma pneumoniae ($n=83$)	Pneumocystis jiroveci ($n=57$)
Age			
Mean — yr	49.1	15.7	44.3
Distribution — no. (%)			
0-12yr	2 (2.9)	50 (60.2)	10 (17.5)
13-18yr	1 (1.4)	11 (13.3)	1 (1.8)
19-25yr	7 (10.1)	4 (4.8)	1 (1.8)
26-40yr	9 (13.1)	9 (10.9)	8 (14.0)
41-60yr	30 (43.5)	6 (7.2)	20 (35.1)
>60yr	20 (29.0)	3 (3.6)	17 (29.8)
Male sex — no. (%)	43 (62.3)	40 (48.2)	36 (63.2)

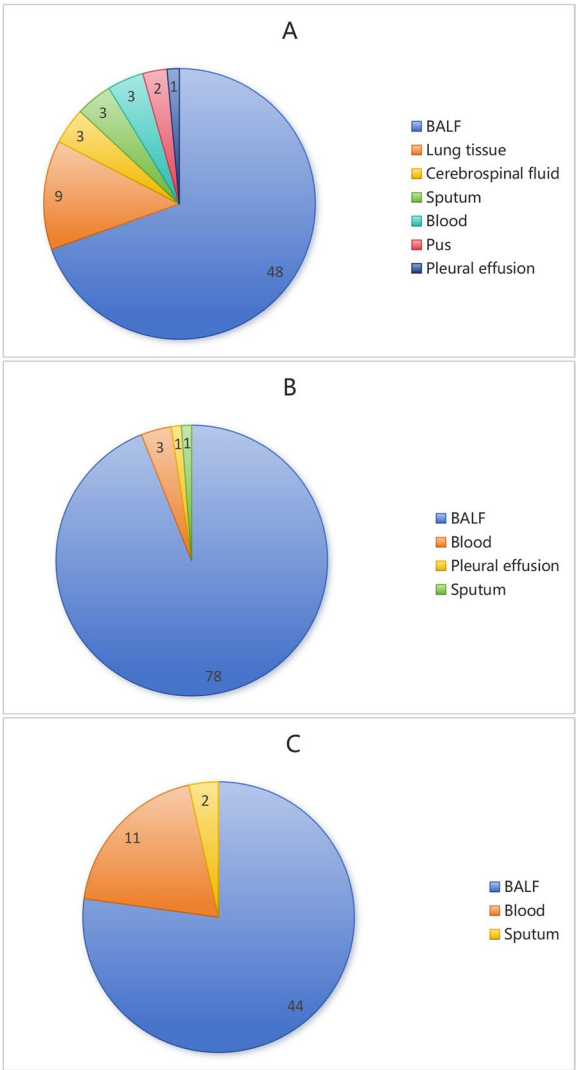

Fig. 1 The distribution of sample types for pathogens identified through mNGS: (**A**) *M. tuberculosis*, (**B**) *M. pneumoniae*, and (**C**) *P. jirovecii*

Validation of mNGS results from clinical and laboratory perspective

A total of 69 cases of *M. tuberculosis* were identified through mNGS, with 60 cases being clinically confirmed. Laboratory validation was performed on 55 of these samples, resulting in 51 positive tests. Similarly, 69 cases of *P. jirovecii* were detected via mNGS, with 45 cases clinically suspected to be disease-related. Targeted PCR validation was conducted on 31 samples, yielding 26 positive results. Furthermore, mNGS identified 83 cases of *M. pneumoniae*, with 81 cases clinically considered disease-related. Laboratory validation of 62 samples revealed 51 positive cases (Fig. 2). It is noteworthy that nearly all sample types exhibited both true positive and false positive cases (Fig. 3).

Comparison of read counts and relative abundance between true positive and false positive groups

The read counts and relative abundances of *M. tuberculosis* identified in nine mNGS cases, which were clinically deemed false positives, were compared to those considered true positives. The differences observed were not statistically significant ($P \geq 0.05$) (Fig. 4A, B). Similarly, the read counts and relative abundances of *P. jirovecii* detected in twelve mNGS cases, classified as false positives, were compared with true positive cases, and no significant differences were found ($P \geq 0.05$) (Fig. 4C, D). Furthermore, the read counts and relative abundances of *M. pneumoniae* identified in two mNGS cases, considered false positives, were compared with those classified as true positives, with no significant differences observed ($P \geq 0.05$) (Fig. 4E, F).

Verification of mNGS results from a laboratory perspective

In this study, 55 cases in which mNGS detected *M. tuberculosis* were concurrently subjected to tuberculosis

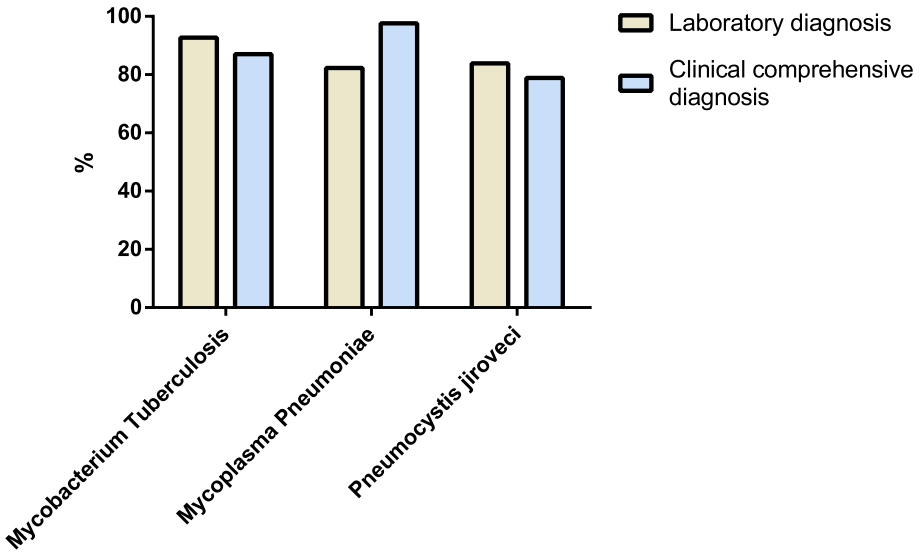

Fig. 2 The concordance between mNGS detection of *M. tuberculosis*, *M. pneumoniae*, and *P. jirovecii*, and the results from laboratory validation and comprehensive clinical assessment

culture and Gene Xpert MTB/RIF testing, with 51 cases yielding positive confirmations. Among 38 cases tested simultaneously using mNGS, culture, and Gene Xpert MTB/RIF, 27 cases demonstrated positive results across all three diagnostic methods (see Fig. 5A). Clinical evaluation identified three instances of false-positive mNGS detections for *M. tuberculosis*. Additionally, 62 cases of *M. pneumoniae* detected by mNGS were concurrently validated using targeted PCR or IgM assays, resulting in 51 positive confirmations. Of the 39 cases tested simultaneously with mNGS, PCR, and IgM, 18 cases were positive across all three methodologies (see Fig. 5B). mNGS identified four cases of *M. pneumoniae* exclusively; clinical evaluation confirmed consistency with clinical practice in three cases, while one case was deemed inconsistent. Furthermore, 31 cases in which mNGS detected *P. jirovecii* were subjected to targeted PCR validation, with 26 cases confirmed as positive.

Discussion

The evaluation of mNGS results involved two distinct dimensions: laboratory validation and clinical evaluation. Laboratory validation focused on confirming the actual presence of pathogens, whereas clinical evaluation assessed the association between the detected pathogen and infectious diseases. This study revealed discrepancies in the concordance rates between laboratory validation and comprehensive clinical evaluation of mNGS results, which varied across different pathogens. Specifically, the concordance rates for laboratory validation were 92.7% for *M. tuberculosis*, 82.3% for *M. pneumoniae*, and 83.9% for *P. jirovecii*. In contrast, the concordance rates for clinical comprehensive analysis were 87%, 97.6%, and 78.9%, respectively. For *M. tuberculosis* and *P. jirovecii*, the laboratory validation demonstrated higher consistency compared to clinical validation, whereas the opposite trend was observed for *M. pneumoniae*. A potential explanation could be that the targeted PCR validation for *M. tuberculosis* and *P. jirovecii* utilized the same sample, whereas the nucleic acid testing for *M. pneumoniae* employed throat swabs, and mNGS was conducted on BALF. It is plausible that the concentration and load of *M. pneumoniae* in BALF are greater than those in pharyngeal swabs.

Currently, there is a consensus in the field of infectious diseases that mNGS demonstrates superior performance in detecting *M. tuberculosis* compared to traditional culture methods. However, its superiority over Gene Xpert MTB/RIF is inconsistent across various studies. A retrospective analysis evaluating lung infections using mNGS and conventional techniques revealed that the positive detection rate of mNGS for tuberculosis was significantly higher than that of traditional methods (67.86% versus 17.86%, $P < 0.01$) [2]. Conversely, another study reported sensitivity rates of 44%, 42%, and 29% for mNGS, Gene Xpert MTB/RIF, and conventional methods, respectively, when compared to the clinical final diagnosis of all active tuberculosis cases [14]. In our study, mNGS identified *M. tuberculosis* in 38 cases, with 35 cases confirmed by either culture or Gene Xpert MTB/RIF. The three cases that were positive only by mNGS did not align with

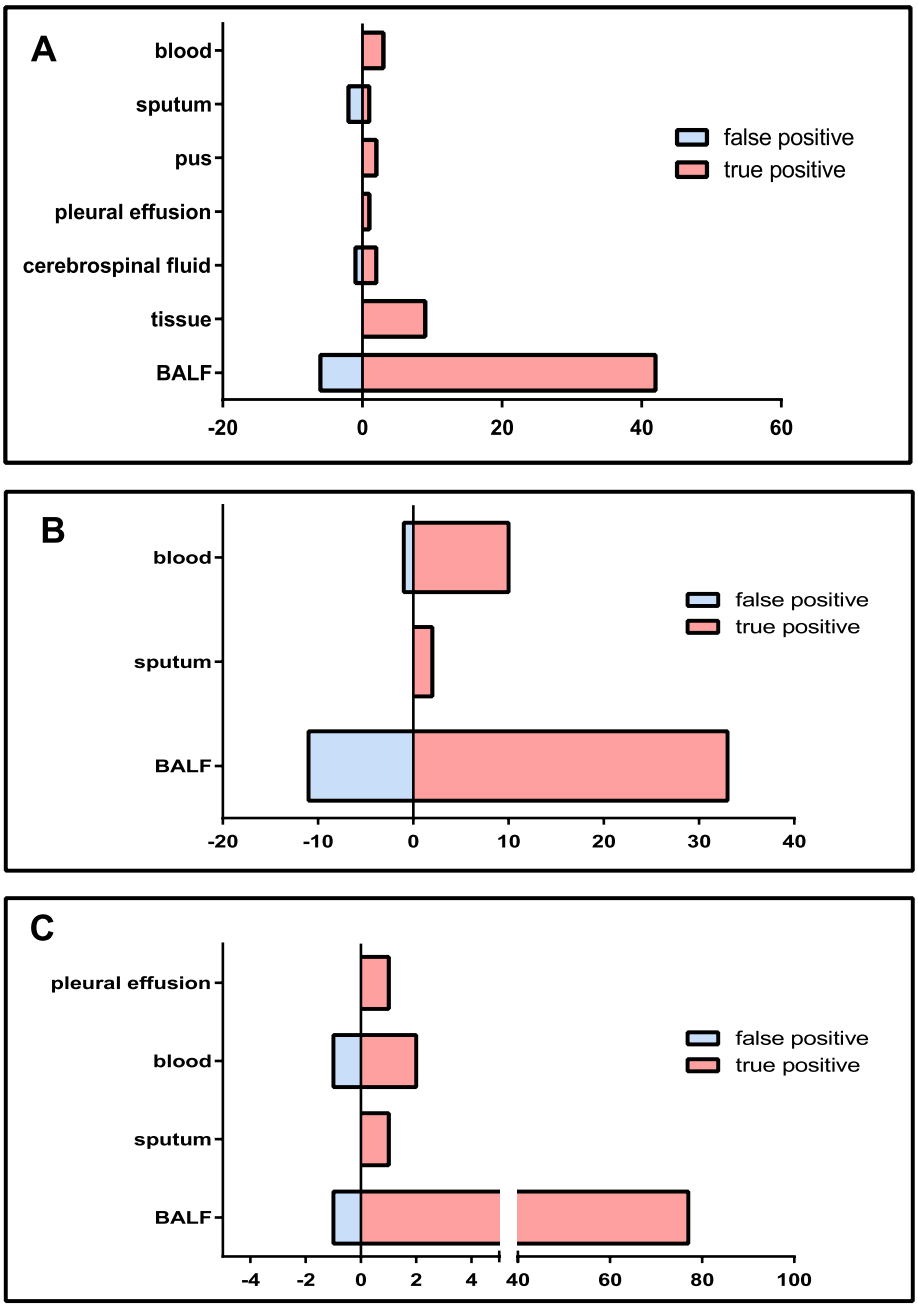

Fig. 3 The distribution of true and false positive cases for *M. tuberculosis*, *P. jirovecii*, and *M. pneumoniae* detected by mNGS across different sample types: (**A**) *M. tuberculosis*, (**B**) *P. jirovecii*, and (**C**) *M. pneumoniae*

comprehensive clinical assessments and were deemed false positives. Additionally, a study on the etiological diagnosis of suspected pulmonary tuberculosis cases found similar sensitivity rates for mNGS, Gene Xpert MTB/RIF, and culture, with values of 47.92%, 45.83%, and 46.81%, respectively [15]. A recent study assessing diagnostic methodologies for pulmonary tuberculosis reported diagnostic sensitivities of 78.95% for mNGS and 72.73% for Gene Xpert MTB/RIF, both markedly exceeding the 44.12% sensitivity of culture methods [16]. In the context of spinal tuberculosis, the diagnostic sensitivities were 39% for mNGS, 53.7% for Gene Xpert MTB/RIF, and 29.3% for culture [17]. For tuberculous meningitis, the sensitivities were 84.1% for mNGS, 73.9% for Gene Xpert MTB/RIF, and 25% for culture [18]. Current research indicates that mNGS

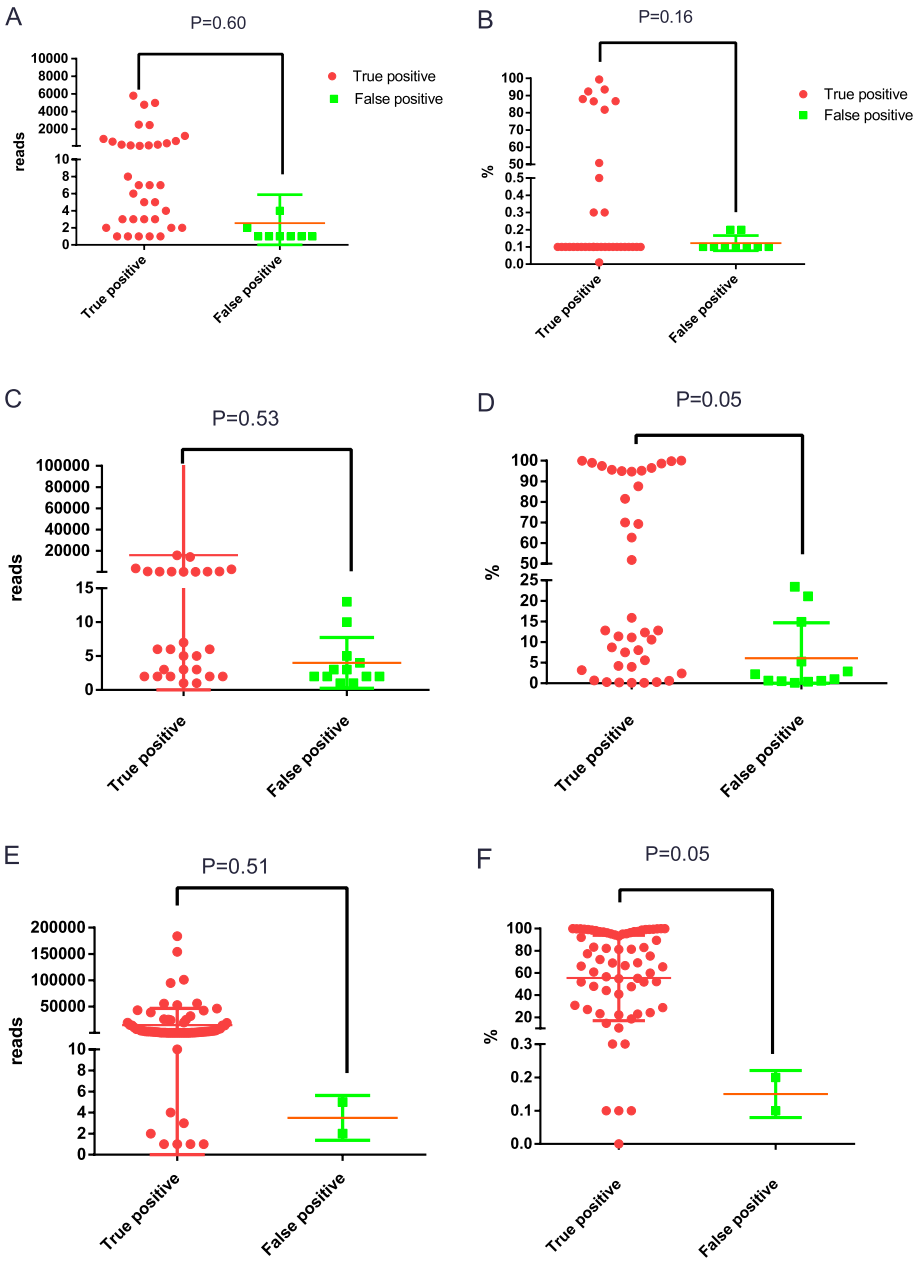

Fig. 4 The distribution of read counts and the relative abundance of *M. tuberculosis*, *M. pneumoniae*, and *P. jirovecii* as detected by mNGS in true positive and false positive cases is presented as follows: **A** illustrates the read counts distribution of *M. tuberculosis*; **B** depicts the relative abundance distribution of *M. tuberculosis*; **C** shows the read counts distribution of *M. pneumoniae;* **D** presents the relative abundance distribution of *M. pneumoniae*; **E** displays the read counts distribution of *P. jirovecii*; and **F** outlines the relative abundance distribution of *P. jirovecii*

exhibits diagnostic efficiency comparable to that of Gene Xpert MTB/RIF in identifying *M. tuberculosis*.

Traditional diagnostic methods for detecting *M. pneumoniae* have exhibited certain limitations. IgM antibodies may persist in the serum for extended periods, spanning weeks to years, thereby failing to accurately indicate recent infections [19]. Furthermore, there is a lack of consistency in detection outcomes across various serological test kits [20]. Although the PCR method demonstrates high diagnostic efficiency, it is unable to differentiate between colonization and active infection, and its concordance with IgM antibody results is suboptimal [21]. Consequently, the diagnosis of *M. pneumoniae* necessitates the integration of multiple diagnostic approaches. mNGS presents potential diagnostic advantages that may address the deficiencies of traditional

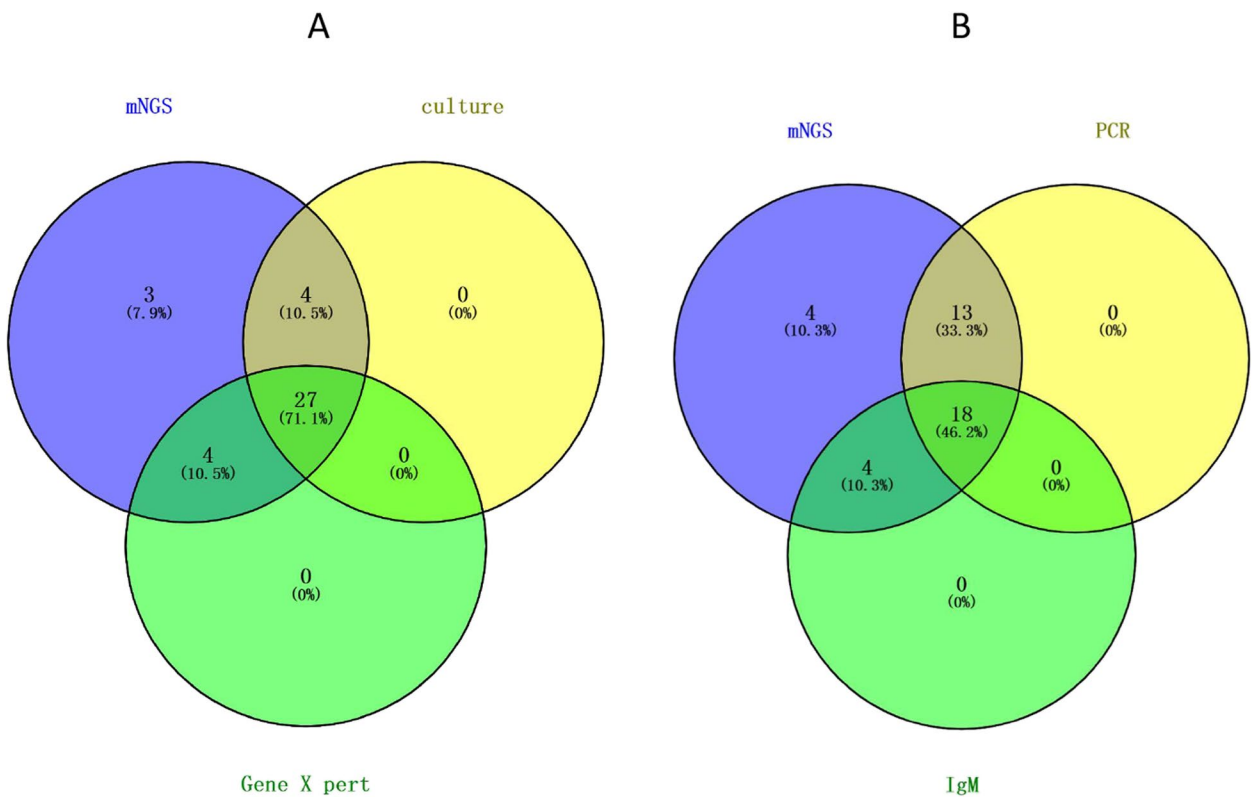

Fig. 5 The consistency between mNGS and traditional diagnostic methods is illustrated through Venn diagrams, with **A** depicting the overlap in active tuberculosis diagnostics and **B** illustrating the overlap in *M. pneumoniae* diagnostics

techniques. In detecting *M. pneumoniae*, mNGS exhibits significantly higher sensitivity compared to conventional methods (100.00% versus 7.14%, $P<0.01$) [2]. Our research findings corroborate this perspective, revealing a clinical judgment consistency rate of 97.6% for mNGS results, in contrast to a laboratory validation consistency rate of 82.3%. Thus, mNGS serves as a valuable adjunct to traditional diagnostic techniques, including targeted PCR and serological assays.

Traditional diagnostic methods for *P. jirovecii* primarily encompass Gomori methenamine silver staining (GMS), serum (1,3)-β-D-glucan (BDG) assays, and lactate dehydrogenase (LDH) detection. Previous literature indicates that the sensitivity of GMS detection ranges from 5.9% to 25%, while BDG demonstrates a sensitivity between 67.4% and 86.7%. In contrast, mNGS exhibits a sensitivity of 100% [22–24]. The diagnostic efficacy of mNGS is comparable to that of targeted PCR, with reported sensitivities of 97.4% and 94.3%, respectively [25]. The commercially available targeted PCR method for detecting *P. jirovecii* has been extensively adopted in clinical settings. A study evaluating the diagnostic performance of comprehensive evaluation and mNGS in immunocompromised patients with suspected pneumonia reported sensitivities of 93.3% and 100% for comprehensive evaluation and mNGS, respectively [2]. In our study, the concordance rates between clinical diagnosis and laboratory validation for the detection of *P. jirovecii* via mNGS were 78.9% and 83.9%, respectively. The lower concordance rate observed in clinical judgment compared to laboratory validation may be attributed to the colonization of *P. jirovecii* in the respiratory tract. On certain occasions, laboratory analyses identified the nucleic acid of *P. jirovecii* in BALF samples, whereas clinical assessment suggested that *P. jirovecii* was merely colonizing the respiratory tract and was not implicated in respiratory infections.

It is widely recognized that mNGS is not suitable for quantitative detection. Nonetheless, the potential use of read counts and relative abundance as quantitative indicators to assess result accuracy remains debatable. This study demonstrated that there were no statistically significant differences in read counts and relative abundance when distinguishing between true and false positive mNGS results for the detection of *M. tuberculosis*, *P. jirovecii*, and *M. pneumoniae* ($P<0.05$). Consequently, evaluating the authenticity of pathogens with low read counts presents considerable challenges for

both laboratory and clinical settings. The subsequent research indicates that the read counts play a significant role in assessing therapeutic efficacy and differentiating between infection and colonization [26]. One study demonstrated that, in central nervous system infections, the number of reads correlated with cerebrospinal fluid white blood cell counts, glucose ratio levels, and the progression of clinical disease [27]. Additionally, a study by Li Liu established that the optimal threshold value for distinguishing between infection and colonization by *P. jirovecii* was 14 reads, with a sensitivity and specificity of 83.3% and 95.7%, respectively, and a BDG level of 88.6 pg/ml, with a sensitivity and specificity of 79.2% and 92.9% [28]. The utility of read counts as an auxiliary diagnostic tool necessitates further exploration through the analysis of extensive clinical data.

Compared to conventional diagnostic methods, mNGS offers several advantages: (1) it can identify rare and uncommon pathogens that may fall outside the detection capabilities of traditional methods; (2) it does not require prior hypothesis generation, allowing for the simultaneous testing of nucleic acids from all pathogens, which is particularly beneficial in cases of multiple infections; and (3) it addresses the limitations of culture-based techniques, especially for pathogens that are challenging or impossible to cultivate. However, mNGS technology also presents certain drawbacks: (1) its detection process is longer than that of targeted PCR technology, typically requiring over 18 h compared to approximately 4 h for targeted PCR; (2) the cost of detection is significantly higher than that of traditional pathogen detection methods; and (3) positive results obtained through mNGS necessitate clinical validation, particularly when the read count is low, which complicates the differentiation between true positives and false positives.

This study is subject to several limitations. Firstly, it was conducted as a retrospective analysis rather than a prospective one. The use of different testing methods on varying samples, rather than a consistent sample set, may have influenced the sensitivity outcomes of these methods. Secondly, the mNGS technology employed has intrinsic limitations, such as the propensity to amplify background noise when the host read rate is low, potentially leading to false positive results. Lastly, the study's focus was restricted to evaluating mNGS with only three pathogens, which may have affected the overall assessment of mNGS's detection performance. Future research should aim to conduct prospective, comprehensive, and objective evaluations of mNGS to address these limitations.

Conclusions

The mNGS technology serves as an effective adjunct to conventional pathogen detection methods; however, the interpretation of its results necessitates careful consideration of both laboratory-specific testing parameters and comprehensive clinical information.

Abbreviations

mNGS	Metagenomic Next-Generation Sequencing
PCR	Polymerase chain reaction
BALF	Bronchoalveolar lavage fluid
GMS	Gomori methenamine silver staining
BDG	Serum (1,3)-β-D-glucan

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Authors' contributions

LT and ZZ wrote the main manuscript text and LT prepared figures 1-5. All authors reviewed the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This retrospective study complied with the Helsinki Declaration and was approved by the Ethics Committee of Tongji Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology. Based on a retrospective study design, the ethics committee approved exemption of informed consent for participating patients and all clinical information of patients was anonymous before analysis.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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