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Experience of implementing metagenomic next-generation sequencing in patients with suspected pulmonary infection in clinical practice

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Pulmonary infections remain a leading cause of infectious disease-related hospitalizations. Metagenomic next-generation sequencing (mNGS) has emerged as a promising diagnostic tool for identifying pathogens responsible for pulmonary infections. However, implementing mNGS in clinical practice presents several challenges. We conducted a retrospective analysis of 97 patients with suspected pulmonary infections who were admitted to our hospital and underwent mNGS alongside conventional microbiologic tests (CMT) over the past three years. We compared the diagnostic efficacy of mNGS versus CMT and assessed the clinical applications and challenges associated with mNGS in managing pulmonary infections. mNGS detected pathogens in 63.9% of cases, outperforming CMT (27.8%) and showing notable improvements in identifying *Mycobacterium*, fungal species, and rare pathogens. Antibiotic regimens were adjusted for 77.4% of patients with positive mNGS results, with clinical improvement observed in 93.5%. Of the 138 microbial strains initially identified by mNGS as possible pathogens, 65 (47.1%) were reclassified as colonizing organisms upon further clinical evaluation, including bacteria and fungi commonly associated with pulmonary infections. Notably, one patient was diagnosed with aspiration pneumonia due to oral anaerobes, which mNGS had categorized as normal microbial flora. In conclusion, mNGS serves as a valuable diagnostic approach for pulmonary infections, enhancing etiologic precision and informing patient management. Nevertheless, a comprehensive clinical interpretation of mNGS-identified microorganisms is essential to achieve accurate diagnosis.

Keywords Metagenomic next-generation sequencing, Etiological diagnosis, Clinical utility, Result interpretation, Treatment impact

Pulmonary infections remain a leading cause of hospitalization worldwide, with recent studies highlighting an increasing incidence of pneumonia among Chinese adults, particularly in those aged ≥ 60 years and individuals with underlying health conditions[1]. Accurate identification of the causative pathogens of pulmonary infections poses significant challenges given the diversity of pathogens, sample heterogeneity, and limitations associated with conventional microbiologic testing (CMT)[2]. Notably, a large proportion (62%) of community-acquired pneumonia cases lack an etiological diagnosis[3].

Metagenomic next-generation sequencing (mNGS) has emerged as a culture-independent, unbiased, and hypothesis-free approach that shows substantial promise in clinical applications, particularly for the diagnosis of respiratory tract infections, central nervous system infections, and bloodstream infections[4]. While existing studies on mNGS primarily emphasize its methodological attributes, such as sensitivity and specificity[5,6], clinical adoption by physicians necessitates additional insights, including the therapeutic benefits of mNGS, as well as guidance on interpreting bioinformatic-derived results[7]. Therefore, the field of clinical metagenomics for infectious diseases is increasingly focusing on the practical aspects of its implementation[8].

In this study, we retrospectively analyzed data from 97 patients admitted to our hospital with suspected pulmonary infections who had been prescribed mNGS over the past three years. Our objective was to evaluate

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the advantages and limitations of mNGS from a clinical perspective, particularly in terms of its applicability and interpretative challenges faced by physicians.

Materials and methods

Patient enrollment and data collection

In 2021, mNGS was integrated into clinical practice at our hospital and designated as a first-line diagnostic tool for urgent pathogen identification in life-threatening cases, or as a second-line approach in instances where CMT yielded negative results or was insufficient to fully explain patient symptoms. This retrospective study consecutively enrolled patients with suspected pulmonary infections who met the following criteria: (i) age ≥ 18 years; (ii) indication for bronchoalveolar lavage (etiological diagnosis or differential diagnosis of bronchopulmonary diseases, suspected infection with specific or mixed pathogens, or endoscopic treatment of airway obstruction, mucous sputum and sputum plugs), with bronchoalveolar lavage fluid (BALF) samples collected[9,10]; and (iii) availability of complete demographic and clinical data. Patient data were extracted from electronic medical records, including age, sex, underlying medical conditions, results of biochemical tests, clinical presentations, and antibiotic administration histories.

This study was conducted in compliance with ethical standards, receiving approval from the Medical Ethics Committee of Shenzhen Longgang Central Hospital (approval number: 2023ECPJ052). All procedures adhered to the relevant guidelines and regulations of this ethics committee. The requirement for informed consent was waived by the Medical Ethics Committee of Shenzhen Longgang Central Hospital.

Bronchoalveolar lavage and BALF collection

Bronchoscopy and bronchoalveolar lavage were performed by experienced bronchoscopists according to standard procedures. Briefly, patients' nasal or oral cavities were first irrigated with normal saline, and then patients were sedated with intravenous midazolam or dexmedetomidine. Local anesthesia with 2% lidocaine was applied sequentially during the examination. Bronchoalveolar lavage was performed when the tip of the bronchoscope was embedded in the opening of the target lung segment or subsegmental bronchus with a total of approximately 100 mL (20 mL each time) of sterile normal saline in batches. The first 20 mL of BALF were discarded to avoid contamination and the remainder were collected for microbiologic testing.

Conventional microbiologic testing

CMT for patients with suspected infections included Gram staining, bacterial and fungal cultures, as well as additional diagnostic methods such as polymerase chain reaction (PCR), galactomannan antigen testing, serum (1,3)-β-D-glucan tests, and Xpert MTB/RIF for the detection of associated infections.

mNGS and bioinformatics pipeline

BALF samples underwent mNGS testing at Guangzhou Huayin Medical Laboratory. Specifically, 500 µL of BALF was initially homogenized using dithiothreitol, followed by the addition to a tube containing zirconia beads and lysis buffer. Microbial cells, including bacteria and fungi, were disrupted through 10 min of vortexing. Nucleic acid extraction was performed using the TIANamp Micro DNA Kit (Tiangen Biotech, China). DNA libraries were then prepared following the manufacturer's guidelines with the KAPA HyperPlus Kit (Roche). To control for potential contamination from reagents and laboratory, a no-template water control was processed concurrently in each run. Sequencing was performed in single-end 50 bp mode on an Illumina NextSeq 550Dx platform, achieving a sequencing depth of ≥ 20 million reads per sample.

The bioinformatics analysis pipeline entailed several stages: quality control of sequencing reads, trimming and filtering of low-quality reads, and alignment against an in-house clinically validated database. Microorganism detection was based on predefined threshold criteria[11,12]: (i) reads per million (RPM) were required to be at least 10 times higher than that in the no-template control for each microorganism, otherwise the microorganism was classified as a contaminant; (ii) for *Mycobacterium tuberculosis*, *Brucella*, and *Nocardia*, which present challenges in DNA extraction and low contamination risk, a stringently mapped read count of ≥ 1 was considered indicative, while for other bacteria and fungi, a minimum of 3 mapped reads was set to mitigate false-positive from low-level contamination; and (iii) a relative abundance threshold of ≥ 1% was used for normal microbial communities. The resulting mNGS report categorized detected microorganisms into two groups: potential pathogens and members of normal microbial communities.

Interpretation of mNGS results by physicians

The pathogenic potential of microorganisms identified through mNGS was further evaluated by physicians. Specifically, pulmonary pathogens, such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*, were considered significant only if their RPM or relative abundance exceeded that of normal microbial communities, except when culture results were positive for these organisms. Fungal infections were diagnosed by integrating immunological status, underlying comorbidities, serological markers, radiographic findings, culture results, direct microscopy, and histopathological analysis. For cases where no potential pathogens were identified, normal microbial communities were investigated, particularly in patients with suspected aspiration pneumonia or lung abscess. Following a comprehensive evaluation by two experienced physicians, microorganisms were reclassified as causative pathogens, colonizers, or components of normal microbial communities. Based on these classifications, patients were categorized into either the mNGS-positive group or mNGS-negative group.

Evaluation of clinical impact of mNGS

We evaluated the clinical impact of mNGS on antibiotic adjustment and patient outcomes. In cases where mNGS identified pathogens not covered by initial empirical therapy, antibiotic regimens were adjusted in the mNGS-positive cohort. Adjustments were categorized as follows: (i) based solely on mNGS (pathogens identified exclusively by mNGS), (ii) informed by both CMT and mNGS (pathogens identified by both modalities), and (iii) informed by mNGS in conjunction with clinical judgment (mNGS results partially aligned with clinical presentation). In the mNGS-negative cohort, adjustments were made for patients who showed no clinical improvement under empirical therapy. Clinical outcomes were classified as improved, unchanged, or deteriorated, based on clinical symptoms, biomarker levels, and radiographic findings.

Statistical analysis

Continuous data were presented as medians and interquartile range (IQR), while categorical data were summarized as frequencies and percentages. Statistical analysis was performed using IBM SPSS Statistics (Version 25.0).

Results

Baseline characteristics

This study enrolled 97 patients with clinically suspected pulmonary infections, with demographic and clinical data provided in Supplementary Table 1. Table 1 summarizes the cohort, which comprised 55 men and 42 women with a median age of 56 years (range: 18–90 years). Among these, 58 patients (59.8%) presented with one or more underlying conditions, including chronic respiratory disease (21/97, 21.6%), hypertension (17/97, 17.5%), diabetes mellitus (13/97, 13.4%), cardiovascular disease (9/97, 9.3%), tumor (9/97, 9.3%), chronic liver disease

Characteristic	*n* (%)
Gender	
Male	55 (56.7)
Female	42 (43.3)
Age, years	
18–30	9 (9.3)
31–50	26 (26.8)
51–70	43 (44.3)
>70	19 (19.6)
Underlying disease	
Chronic respiratory disease	21 (21.6)
Hypertension	17 (17.5)
Diabetes mellitus	13 (13.4)
Cardiovascular disease	9 (9.3)
Tumor	9 (9.3)
Chronic liver disease	4 (4.1)
Autoimmune disease	4 (4.1)
None	39 (40.2)
Clinical manifestation	
Cough	73 (75.2)
Expectoration	49 (50.5)
Fever	16 (16.5)
Shortness of breath	14 (14.4)
Hemoptysis	12 (12.4)
Chest pain	9 (9.3)
Asthma	8 (8.2)
Chest congestion	5 (5.1)
Dyspnea	2 (2.1)
Radiographic findings without overt symptoms	6 (6.2)
Duration from hospitalization to mNGS	
<2 days	30 (30.9)
2–7 days	48 (49.5)
>7 days	19 (19.6)
Days of antibiotics administration before mNGS, median (IQR)	3 (2–6)
Days of hospitalization, median (IQR)	13 (8.5–18)

Table 1. Patient demographics and clinical features.

(4/97, 4.1%), and autoimmune disease (4/97, 4.1%). All patients exhibited radiographic evidence indicative of pulmonary infection, with prevalent symptoms such as cough (75.2%), sputum production (61.0%), and fever (16.5%). Notably, 6 patients presented without overt symptoms.

The mean interval from hospital admission to mNGS analysis was 4.8 days, with 30.9% of patients receiving mNGS within 48 h. The median duration of antibiotic administration prior to mNGS testing was 3 days (IQR: 2–6), with only 2 patients not receiving antibiotics before the procedure. The median length of hospitalization for this cohort was 13 days (IQR: 8.5–18).

Pathogen spectrum identified by CMT and mNGS

In total, 75 distinct pathogen strains were identified among 63 patients (64.9%) using CMT and mNGS. As shown in Fig. 1, the most frequently detected pathogens included *Mycobacterium tuberculosis* complex (16/97, 16.5%), *Pseudomonas aeruginosa* (14/97, 14.4%), *Haemophilus influenzae* (6/97, 6.2%), *Mycobacterium avium complex* (6/97, 6.2%), and *Aspergillus fumigatus* (5/97, 5.1%). Additional common pathogens identified were *Mycobacteroides abscessus*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Cryptococcus neoformans* species complex, each present in 3 patients. One case of anaerobic infection was noted with *Prevotella melaninogenica* and *Porphyromonas somerae* (ID 74). Co-infections were identified in 11 patients (11.3%) (Supplementary Table 1).

Comparison of diagnostic yield between CMT and mNGS

The positive detection rates for CMT and mNGS were 27.8% and 63.9%, respectively (Supplementary Table 1). Of the 75 pathogen strains, 48 were uniquely identified by mNGS, 2 exclusively by CMT, and 25 by both methodologies (Fig. 2). mNGS failed to detect a single strain each of *Mycobacterium tuberculosis* complex and *Pseudomonas aeruginosa*, whereas CMT exhibited limitations in detecting various fungal species as well as *Mycobacterium*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.

Clinical impact evaluation of mNGS

As shown in Fig. 3, antibiotic regimens were modified in 48 (77.4%) patients within the mNGS-positive group. Adjustments were informed by mNGS alone in 33 cases, by combined CMT and mNGS findings in 11 cases, and by mNGS in conjunction with clinical judgment in 4 cases. In 14 mNGS-positive patients, antibiotic adjustments were deemed unnecessary as empirical therapy already targeted the identified pathogens. In the mNGS-negative group, antibiotic modifications were implemented in 9 (25.7%) patients, based on clinical judgment in 8 cases and CMT findings in 1 case.

Clinical improvement was observed in 58 (93.5%) of the mNGS-positive group and 31 (88.6%) of the mNGS-negative group. In the mNGS-positive group, 1 patient's clinical condition deteriorated despite no change in antibiotic therapy. Among the remaining 7 patients with no clinical change, 3 were mNGS-positive.

Colonized pathogens

Among the 138 potentially causative strains initially identified through mNGS, 65 strains (47.1%) were subsequently reclassified as colonizing organisms by clinicians. These comprised 42 strains isolated from 25 patients in the mNGS-positive cohort and 23 strains from 17 patients in the mNGS-negative cohort (Table 2).

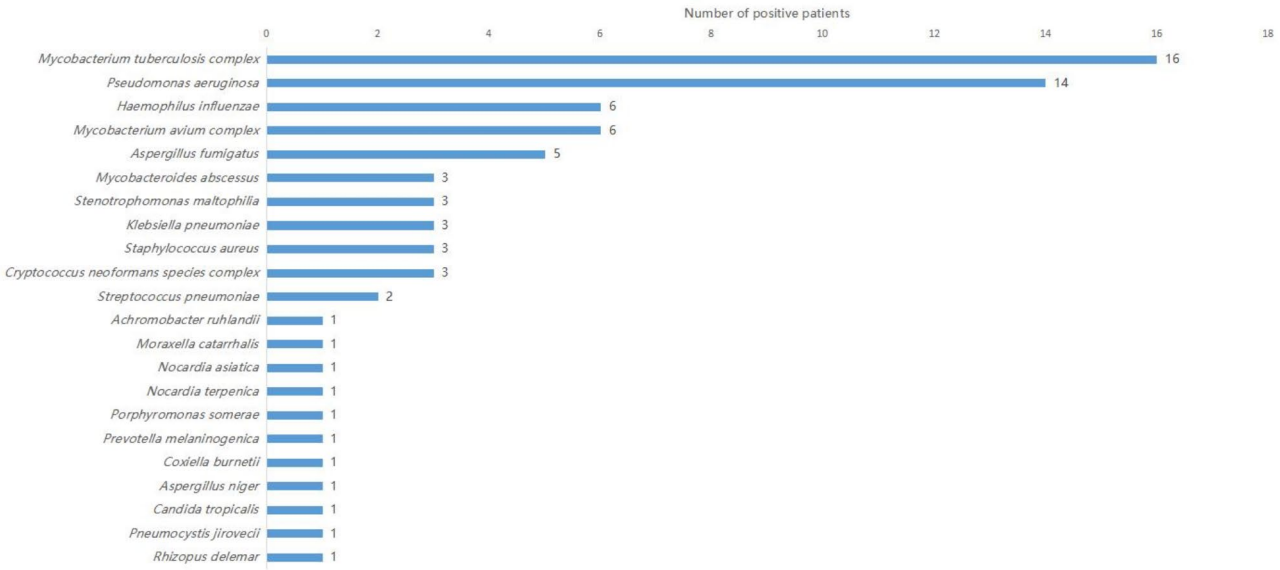

Fig. 1. Pathogens detected in 97 patients with suspected pulmonary infection using mNGS versus conventional microbiologic tests.

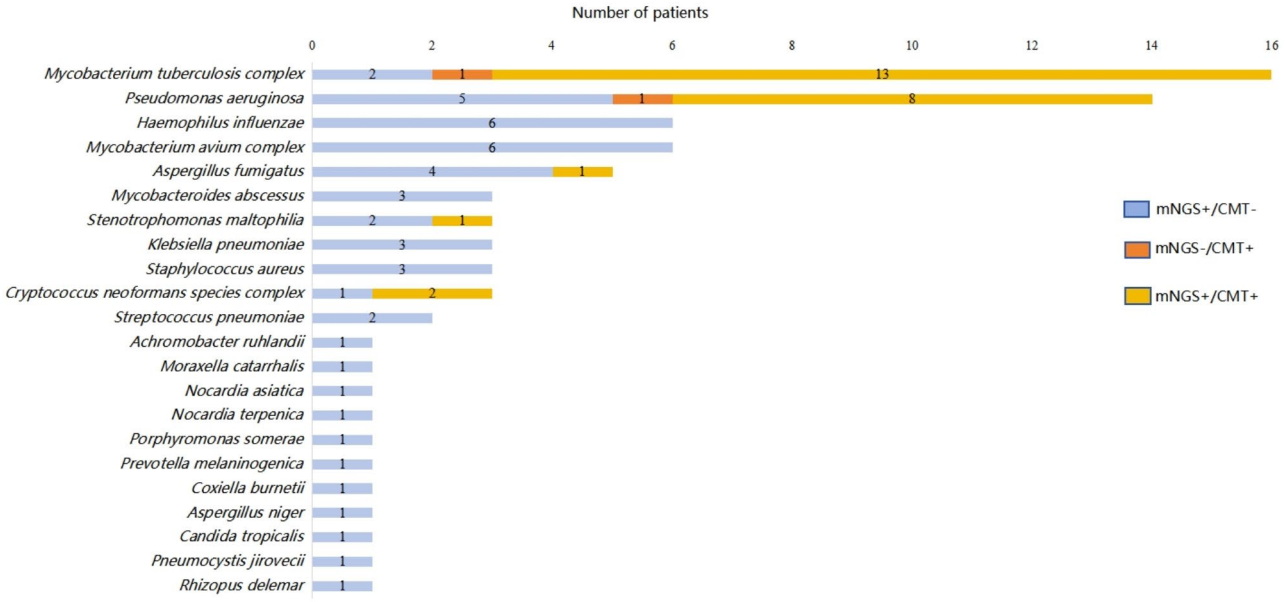

Fig. 2. Comparison of pathogens identified by mNGS and CMT.

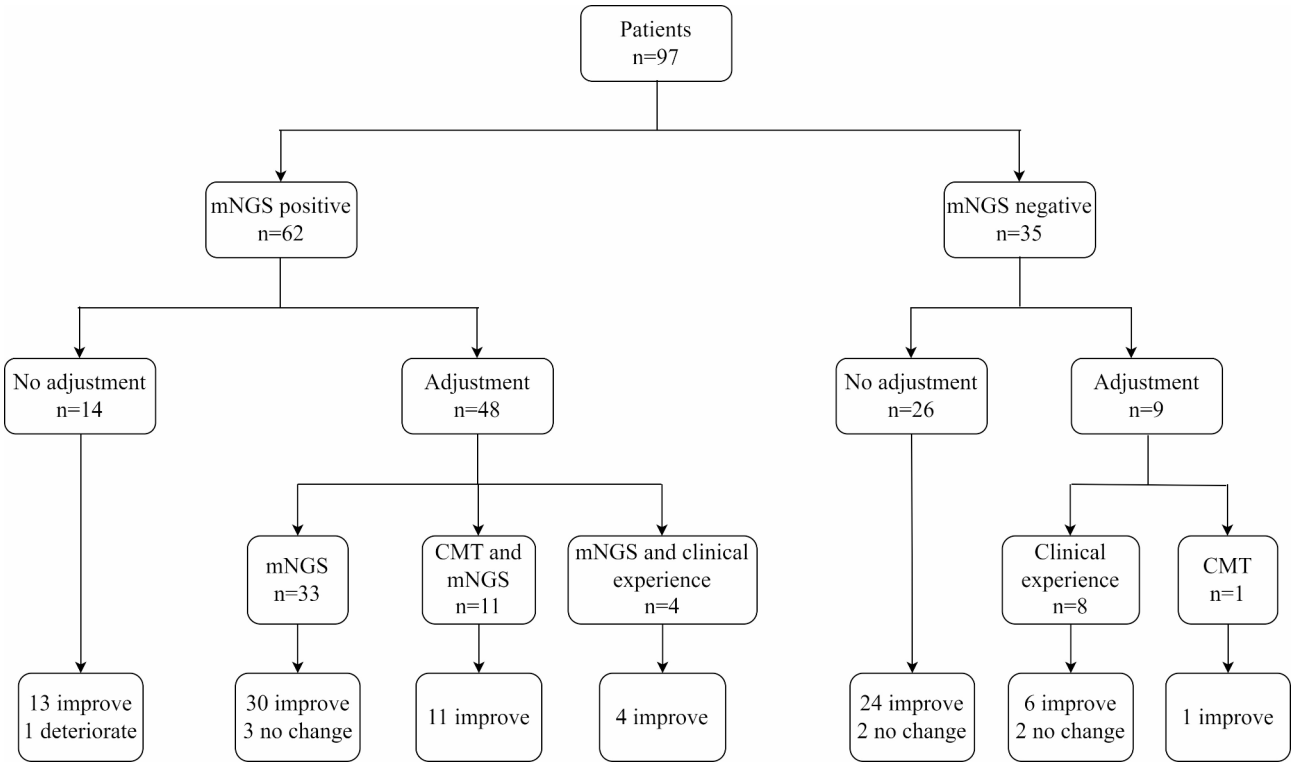

Fig. 3. Clinical impact of mNGS on antibiotic adjustments and patient outcomes.

The most frequently identified colonizers were *Haemophilus influenzae*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Additionally, non-tuberculous mycobacteria (NTM) and fungal strains were detected in select cases, including 4 NTM strains identified in 4 patients and 8 fungal strains found in 7 patients. Identified NTMs consisted of *Mycobacteroides chelonae* and *Mycolicibacterium wolinskyi*, while fungal species included *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Pneumocystis jirovecii*, *Trichosporon asahii*, *Fusarium fujikuroi* species complex, and *Candida albicans*. Notably, *Aspergillus flavus* and *Fusarium fujikuroi* species complex concurrently colonized a single patient within the mNGS-negative group.

Colonized pathogens	Number of strains	
	mNGS positive group	mNGS negative group
Bacteria		
Haemophilus influenzae	5	9
Klebsiella pneumoniae	5	1
Staphylococcus aureus	3	2
Moraxella catarrhalis	2	2
Enterobacter cloacae complex	3	0
Escherichia coli	3	0
Mycoplasma pneumoniae	2	1
Pseudomonas aeruginosa	1	2
Streptococcus pneumoniae	2	0
Acinetobacter baumannii	1	1
Acinetobacter pittii	1	0
Elizabethkingia anophelis	1	0
Enterococcus avium	1	0
Enterococcus faecium	1	0
Klebsiella aerogenes	1	0
Klebsiella quasipneumoniae	1	0
Ochrobactrum anthropi	1	0
Tropheryma whipplei	1	0
Legionella pneumophila	0	1
Mycobacteria		
Mycobacteroides chelonae	1	2
Mycolicibacterium wolinskyi	1	0
Fungi		
Aspergillus fumigatus	1	0
Aspergillus niger	1	0
Aspergillus flavus	0	1
Pneumocystis jirovecii	1	0
Trichosporon asahii	1	0
Fusarium fujikuroi species complex	0	1
Candida albicans	1	0

Table 2. Colonized pathogens in mNGS-positive and mNGS-negative groups.

Discussion

This study details our clinical implementation of mNGS in etiological diagnostics, specifically focusing on pulmonary infections. The incorporation of mNGS led to a causal diagnosis in 63.9% of patients with suspected pulmonary infections, significantly surpassing the 27.8% diagnostic rate achieved by CMT. These findings underscore the added diagnostic value of mNGS in routine workflows for identifying infectious pathogens in pulmonary disease, aligning with results from previous studies[4].

Despite the consistent decline in reported pulmonary tuberculosis incidence over the past decade, China remains a high-burden country, contributing approximately 7.1% of global cases[13]. *Mycobacterium tuberculosis* complex was the most frequently detected pathogen in our cohort, with a detection rate of 16.5%, consistent with similar findings from other mNGS studies[14,15]. The sensitivity of mNGS for tuberculosis detection is comparable to that of the Xpert MTB/RIF assay and traditional culture methods[16]. Additionally, mNGS facilitated the identification of mixed infections and NTM. Specifically, we identified two cases of co-infection with *Mycobacterium tuberculosis* complex and *Aspergillus*, as well as nine cases of NTM pneumonia. However, mNGS's lower detection rate for drug-resistant tuberculosis remains a limitation, potentially attributable to limited sequencing depth and pathogen abundance. Technological advancements in sequencing may enhance this limitation, and the WHO-endorsed targeted next-generation sequencing offers a promising alternative for rapid and comprehensive tuberculosis detection[17].

Beyond *Mycobacterium tuberculosis*, mNGS exhibited robust performance in identifying difficult-to-culture and rare pathogens. For instance, mNGS identified a case of Q fever (Patient ID 58, Supplementary Table 1), a zoonotic disease caused by the intracellular bacterium *Coxiella burnetii.*[18] Although seropositivity for Q fever is documented in humans and animals across China, clinical cases are likely underdiagnosed as a result of atypical presentations[19]. The patient, a 49-year-old male with a three-day history of unexplained fever, exhibited elevated C-reactive protein (94.46 mg/L) and leukocytosis (11.95×10^9/L) on admission, alongside bilateral lung inflammation on CT. Standard empirical therapy yielded no improvement, and cultures of blood and BALF were negative. mNGS analysis of BALF subsequently revealed 13 specific reads for *Coxiella burnetii*. A tick bite history

was subsequently confirmed, leading to a final diagnosis of Q fever and an adjustment in antibiotic therapy to doxycycline.

This study evaluates the clinical impact of mNGS on treatment decisions and clinical outcomes. Antibiotic regimens were adjusted in 77.4% of patients in the mNGS-positive group compared with 25.7% in the mNGS-negative group. Clinical improvement was observed in 93.5% of patients; however, no statistically significant difference was observed between the mNGS-positive and mNGS-negative groups ($p = 0.859$). Notably, clinical improvement was not observed in four patients within the mNGS-positive group, likely attributable to antibiotic resistance in the causative pathogens. Further well-designed prospective clinical trials are warranted to clarify the effects of mNGS on clinical management.

The detection of microorganisms in clinical samples by mNGS can reflect a variety of states, including normal microbial communities, transient colonization, contamination, or true infection[16]. This complexity complicates result interpretation, particularly in distinguishing colonization from infection. In the absence of a standardized diagnostic approach, physicians must integrate multiple factors to accurately interpret mNGS results. These factors may include RPM counts, relative abundance of microorganisms, CMT results, patient clinical characteristics, immune status, underlying diseases, and clinical experience[21–24]. In our patient cohort, 138 strains of microorganisms were initially reported by mNGS as potential pathogens, but 65 (47.1%) were ultimately classified as colonization by the treating physicians. These organisms included both common and rare pathogens typically associated with pulmonary infections, such as *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Aspergillus*, *Pneumocystis jirovecii*, and *Legionella pneumophila*. Bacterial isolates were primarily classified as colonizing agents, given low RPM counts or relative abundance and negative culture results. For fungal infections, clinical factors such as immune status, underlying diseases, serological findings, and radiographic evidence were prioritized. Particular attention was given to *Legionella pneumophila*, as its presence in the respiratory tract, even at low abundance, often warrants clinical significance. However, *Legionella* pneumonia was ruled out in Patient 33 (Supplementary Table 1), as supportive evidence was lacking, including epidemiological risk factors (e.g., exposure to contaminated water), clinical manifestations (e.g., chills, fever, or dyspnea), and laboratory findings (e.g., urinary antigen test). The source of *Legionella pneumophila* DNA in the BALF sample of this patient was not further investigated, but it may have originated from a non-viable organism or contamination. We strongly advise that physicians incorporate comprehensive clinical assessment beyond mNGS-detected pathogens to ensure accurate diagnoses and optimal patient management.

Oral anaerobic bacteria have been associated with aspiration pneumonia, potentially leading to complications such as lung abscess, necrotizing pneumonia, and empyema[25]. Despite their low prevalence in pneumonia cases, anaerobes present significant challenges in clinical microbiology due to their fragile, fastidious nature and slow growth, necessitating specialized culture systems. Consequently, these bacteria are infrequently isolated in routine microbiological laboratories. In this study, we identified a case of anaerobic infection (Patient ID 74, Supplementary Table 1). The patient, a 30-year-old man with no underlying conditions, presented with symptoms of cough and expectoration. Chest radiography revealed inflammatory consolidation in the middle and lower lobes of the right lung, as well as multiple small nodules in both lungs. mNGS analysis did not identify a definitive pathogen. However, high abundances of anaerobic bacteria, specifically *Prevotella melaninogenica* and *Porphyromonas somerae*, were detected but categorized as components of the normal microbial flora. Subsequent pathological examination revealed organizing pneumonia with granulomatous lesions in the middle lobe of the right lung, leading to a diagnosis of anaerobic infection. Given that oral anaerobic bacteria are often classified as normal flora in mNGS analyses, it is crucial for physicians to consider these organisms, particularly in cases where no clear pathogen is identified.

Our study has several limitations. First, as a retrospective analysis with a small sample size, our findings may be subject to data bias. Second, the positive culture rate in our patient cohort was relatively low, likely due to a high proportion of antibiotic administration prior to mNGS testing or selection bias, as only patients with indications for bronchoalveolar lavage were enrolled. Third, the absence of standardized criteria for interpreting mNGS results may have influenced the outcomes of our study. Lastly, we did not perform a cost-effectiveness analysis of mNGS implementation in clinical practice, an area that warrants further investigation.

Conclusion

This study presents our experience with implementing mNGS in clinical practice from a physician's perspective. mNGS offers a comprehensive and unbiased method for the etiological diagnosis of patients with suspected pulmonary infections. As the cost of mNGS ($300 to $500/sample) is still higher than that of conventional methods, we propose that mNGS may be particularly valuable in clinical settings for critically ill patients, cases with negative CMT results, and cases with rare or atypical infections. The clinical relevance of microorganisms identified through mNGS should be carefully evaluated, considering CMT results, clinical presentation, laboratory findings, and epidemiological data. Prospective, well-designed clinical trials are necessary to further validate the clinical utility of mNGS. Additionally, standardization of both methodological approaches and value-based outcomes—such as conventional metrics for diagnosing infectious diseases—will be crucial for enhancing the operational value of mNGS in clinical practice.

Data availability

The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA020408) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

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Author contributions

Y.L. and Y.S. contributed to the conception and design of the research. Y.L. drafted the manuscript, and Y.S. provided revisions. B.C. and S.C. conducted data investigation and analysis. All authors have read and approved the final version of the manuscript.

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Declarations

Ethics approval and consent to participate

This study was conducted in compliance with ethical standards, receiving approval from the Medical Ethics Committee of Shenzhen Longgang Central Hospital (approval number: 2023ECPJ052). All procedures adhered to the relevant guidelines and regulations of this ethics committee. The requirement for informed consent was waived by the Medical Ethics Committee of Shenzhen Longgang Central Hospital.

Competing interests
The authors declare no competing interests.

Additional information
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