International Journal of Infectious Diseases 165 (2026) 108457

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Clinical experience with metagenomic next-generation sequencing (mNGS) for the detection of *Tropheryma whipplei* in respiratory specimens: A multicenter retrospective observational study

Lingxiao Sun[1,#], Yimin Wang[2,#], Jian Fang[3], Zhenpeng Li[4], Yuyao Yin[1], Yifan Guo[1], Qi Wang[1], Hongbin Chen[1], Bin Cao[2], Hui Wang[1,*]

[1] *Department of Clinical Laboratory, Peking University People's Hospital, Beijing, China*
[2] *Department of Pulmonary and Critical Care Medicine, Center of Respiratory Medicine, China-Japan Friendship Hospital, Beijing, China*
[3] *Department of Pulmonary and Critical Care Medicine, The Quzhou Affiliated Hospital of Wenzhou Medical University, Quzhou People's Hospital, Quzhou, Zhejiang, China*
[4] *Department of Clinical Laboratory, Wan Bei General Hospital of Wanbei Coal power Group, Suzhou, Anhui Province, China*

ARTICLE INFO

Article history:
Received 2 November 2025
Revised 29 January 2026
Accepted 1 February 2026

Keywords:
Tropheryma whipplei
Pneumonia
Metagenomic next-generation sequencing (mNGS)
qPCR
Diagnosis
Treatment

ABSTRACT

Objectives: *Tropheryma whipplei* (*T. whipplei*) is the causative bacterium of Whipple's disease (WD), a chronic and systemic infectious condition that predominantly affects the gastrointestinal tract. Sporadic cases of *T. whipplei* pneumonia have been documented recently.

Methods: This multicenter retrospective observational study was conducted on patients with *T. whipplei* positive respiratory specimens admitted to Peking University People's Hospital and China-Japan Friendship Hospital, from Apr 2021 to Jul 2024. Metagenomic next-Generation sequencing (mNGS) was performed using the patient's bronchoalveolar lavage fluid (BALF), and the quantitative polymerase chain reaction (qPCR) of *T. whipplei* was also adopted. The clinical data of patients were systematically evaluated.

Results: Among 91 patients (aged 25-82, mean 57; 48% male), common symptoms included cough (60%), expectoration (48%), dyspnea (42%), and fever (30%). Notably, 22% were asymptomatic. Besides, 20 patients (22%) had a pre-existing condition of interstitial lung disease. Among all 91 patients, 14 were diagnosed with pneumonia, while the remaining 77 had bacterial colonization. Pneumonia cases showed higher *T. whipplei* mNGS reads than colonization ($P = 0.0298$). Samples testing positive for *T. whipplei* by qPCR exhibited significantly higher mNGS sequence reads compared to qPCR-negative samples ($P < 0.0001$). All pneumonia patients received antibiotics therapy tailored to their condition. One died from respiratory failure, while the remaining 13 recovered.

Conclusion: The application of mNGS on respiratory specimens stands as an exceptional diagnostic modality, proficient in identifying rare microbial infections, exemplified by those induced by *T. whipplei*. Future research should launch prospective trials to optimize regimens, assess outcomes, and track long—term survival precisely.

Introduction

Whipple's disease(WD) stands as an uncommon infectious ailment, with its prevalence estimated to range from 1 to 10 cases for every million people, and it predominantly impacts white men aged over 50 [1,2]. It primarily targets the joints (articular system), digestive tract (alimentary system), heart and blood vessels (cardiovascular system), and nervous system of those affected. Infection with *Tropheryma whipplei* (*T. whipplei*) can lead to chronic conditions, including systemic or classical WD or localized infections, as well as acute infections such as gastroenteritis and pneumonia, and may also result in individuals becoming asymptomatic carriers [3]. *T. whipplei* is ubiquitously present in the environment and transmitted by the fecal-oral or oral-oral route from a solely human pool [4].

* Corresponding author. Department of Clinical Laboratory, Peking University People's Hospital, Beijing 100044, China.
E-mail address: whuibj@163.com (H. Wang).
# These authors contributed equally to this work.

https://doi.org/10.1016/j.ijid.2026.108457

With advancements in molecular biology technology, nucleotide sequencing and PCR amplification techniques were employed in 1991 to initially identify the bacterial 16S ribosomal DNA in small-bowel biopsy samples taken from patients suffering from classic WD [5]. Later on, PCR technology has been utilized to detect the DNA of *T. whipplei* in a variety of samples, such as stool, saliva, urine, blood, cerebrospinal fluid, and bronchoalveolar lavage fluid (BALF) [6,7]. In 2011, *T. whipplei* was successfully cultured for the first time from the BALF of an elderly female patient who primarily exhibited symptoms of fever, night sweats, and respiratory distress. This breakthrough highlighted the emerging recognition of *T. whipplei* as a clinically significant respiratory pathogen [7]. In recent times, owing to the clinical application of mNGS, sporadic cases of *T. whipplei* pneumonia have been documented [3,8]. In this research, we conducted a retrospective analysis of *T. whipplei* identified through mNGS in BALF specimens collected from two hospitals located in Beijing.

Methods

Study design and data collection

This is a multicenter retrospective study conducted on patients with *T. whipplei* positive in BALF specimens admitted to Peking University People's Hospital and China-Japan Friendship Hospital, from Apr 2021 to Jul 2024. Epidemiological, demographic, clinical, laboratory, treatment, and outcome data were extracted from electronic medical records.

Patients

Inclusion criteria: (1) patients (≥18 years old) discharged from the hospital and detected with *T. whipplei* positive in BALF specimen sat Peking University People's Hospital and China-Japan Friendship Hospital, from Apr 2021 to Jul 2024; (2) complete required data; (3) all patients underwent metagenomic next-generation sequencing (mNGS); and (4) Two pulmonary physicians confirmed the diagnosis based on historical epidemiology, clinical manifestations, and laboratory tests.

mNGS

The 250 µl sample was used to extract nucleic acids using the MagPure Pathogen DNA/RNA Extraction Kit (Magen Biotechnology, Guangzhou, China). Jurkat cells (c101-b; IGE Biotechnology, Guangzhou, China) were used as negative controls (NCs) to detect contamination, and Jurkat cells spiked with *Bacillus subtilis* (Guangdong Microbial Culture Collection Center, Guangzhou, China) were used as positive controls (PCs).

Libraries were constructed for DNA samples using the KAPA DNA HyperPrep Kit (KK8504; Kapa Biosystems, Wilmington, MA, USA). Library concentrations were quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The libraries were pooled and sequenced using a NextSeq^{TM} 550Dx sequencer (Illumina) with a 75-bp single-end loading method to generate more than 20 million reads for each sample.

The FASTQ format data obtained from sequencing were subjected to Trimmomatic v0.40 [9] for high-quality sequencing data. Bowtie2 v2.4.3 [10] was used to compare the data with the human reference genome GRCh37 (hg19) to filter out human host sequences. The remaining sequences were aligned with a previously constructed reference database (including 24,000+ pathogens) [11] to identify the pathogens in the sample using Kraken2 v2.1.0 [12]. The specific parameters used with Kraken were the reference database, which was the PlusPF database (downloaded on 9/12/2022) containing refseq archaea, bacteria, viruses, fungi, and protozoa. The species-specific read number was normalized to reads per million (RPM). The RPM ratio is the ratio between the RPM values of the sample and the negative control.

Quantitative polymerase chain reaction (qPCR) detection

DNA was extracted from a 200 µl sample using a QIAamp® DNA Mini Kit (Qiagen, Germany), following the manufacturer's instructions, and stored at −80°C until processing for PCR. DNA was amplified via 2 qPCR targeting two different noncoding sequences that were repeated 7 times in the genome of *T. whipplei* [4]. The first *T. whipplei* PCR, which targeted a 105-bp repeated sequence of the bacterium, incorporated the primer pair TW27F (TGTTTTGTACTGCTTGTAACAGGATCT) and TW182R (TCCTGCTCTATCCCTCCTATCATC) and a Taqman probe 27F-182R (FAM-AGAGATACATTTGTGTTAGTTGTTACA-TAMRA) into the reaction mix. The primers and probe were synthesized by Tsingke (Beijing Tsingke Biotech Co., Ltd., Beijing, China). The qPCR was performed with an ABI 7500 instrument (Applied Biosystems, USA) using 20 µl reaction volumes consisting of: 1 µl of DNA, 10 µl of 2×AceQ Universal *U*+ Probe Master Mix V2 (Vazyme, Nanjing, China), 0.4 µl forward primer (10 µmol/l), 0.4 µl reverse primer (10 µmol/l), 0.2 µl probe (10 µmol/l), and 8 µl water. The amplification conditions were as follows: 95°C for 5 min, 40 thermocycles at 95°C for 15 s, and 60°C for 30 s. If the result of the first assay was positive, it was systematically confirmed by a second PCR assay, with a primer pair TW13F (TGAGTGATGGTATGTCTGAGAGATATGT) and TW163R (TCCATAACAAAGACAACAACCAATC) and Taqman probe 13F-163R (FAM-AGAAGAAGATGTTACGGGTTG-TAMRA) targeting a different DNA sequence; the same amplification conditions described above were used.

Ethics statement

This study was approved by the Ethics Committee of the Peking University People's Hospital (2025PHB001-001). Since the study was conducted retrospectively and did not involve any identifiable patient information in the manuscript, the requirement for obtaining consent was deemed unnecessary and was therefore waived.

Results

Patient characteristics associated with positive T. whipplei *detection*

From Apr 2021 to Jul 2024, a total of 91 bronchoalveolar lavage fluid specimens obtained from 91 distinct patients tested positive for *T. whipplei* via mNGS at two hospitals in Beijing. Specifically, 62 samples were collected from Peking University People's Hospital and 29 samples from China-Japan Friendship Hospital.

The patients' ages spanned from 25 to 82 years, with a mean age of 57 years. The gender distribution was equal, with a male-to-female ratio of 48% (44 males out of 91 patients). Among these patients, 20 (22%) had a pre-existing condition of interstitial lung disease, and 11 (12%) had bronchial asthma. Additionally, 15 patients (16%) were immunocompromised, and 27 patients (30%) had a history of allergies to drugs, pollen, or food (Table 1).

Notably, 20 patients (22%) were asymptomatic but exhibited abnormal lung imaging findings during routine physical examinations. The most prevalent clinical symptoms included cough (60%, $n = 55$), expectoration (48%, $n = 44$), dyspnea (42%, $n = 38$), and fever (30%, $n = 27$). Laboratory tests were conducted within 48 hours of hospital admission. The average white blood cell (WBC) count was 7.42×10^9/L, the average neutrophil count was 5.03×10^9/L, and the average erythrocyte mean corpuscular volume was 91 fl (Table 2).

Table 1
Demographic characteristics and basic diseases of patients with *T. whipplei* positive in lower respiratory tract specimens.

Parameters	Patients (n = 91)
Age (mean)	57
Male	44 (48%)
Basic diseases	
Hypertension	27 (30%)
Diabetes	19 (21%)
Interstitial lung disease	20 (22%)
Bronchial asthma	11 (12%)
Chronic bronchitis	6 (7%)
Bronchiectasis	6 (7%)
COPD	1 (1%)
Immunocompromised	15 (16%)
Allergies	27 (30%)
Smoking history	26 (29%)
Drinking history	19 (21%)

Table 2
Clinical features and laboratory examination of patients with *T. whipplei* positive in lower respiratory tract specimens.

Parameters	Patients (n = 91)
Clinical symptoms	
Asymptomatic	20 (22%)
Cough	55 (60%)
Expectoration	44 (48%)
Dypnea	38 (42%)
Fever	27 (30%)
Chest pain	10 (11%)
Hemoptysis	9 (10%)
Weight loss	5 (5%)
Anorexia	4 (4%)
Vomiting	3 (3%)
Abdominal pain	2 (2%)
Diarrhea	1 (1%)
Arthralgia	10 (11%)
Neurological	6 (7%)
Laboratory test results	
White blood cell count (3.5-9.5 × 10^9/L)	7.42
Neutrophil count (1.8-6.3 × 10^9/L)	5.03
Hemoglobin (male 130-175 g/L, female 115-150 g/L)	124
Erythrocyte mean corpuscular volume (82-100 fl)	91
Platelet count (125-350 × 10^12/L)	234
Albumin (40-55 g/L, n = 88)	39
Lactate dehydrogenase (109-245 U/L, n = 88)	204
CRP (0-10 mg/L, n = 81)	
CRP < 0.5	27 (33%)
0.5 ≤ CRP<10	35 (43%)
10 ≤ CRP<100	15 (19%)
CRP ≥ 100	4 (5%)
PCT (<0.05 ng/mL)(n = 51)	
PCT < 0.25	47 (92%)
0.25 ≤ PCT<2	2 (4%)
PCT ≥ 2	2 (4%)

Out of the 91 cases, 14 were diagnosed as pneumonia, while the remaining 77 cases were classified as *T. whipplei* colonization.

Results of mNGS for T. whipplei*-Positive samples*

The pathogen results of mNGS on samples that tested positive for *T. whipplei* are presented in Table 3. Among the cases, 39 (43%) identified *T. whipplei* as the sole pathogen. The most frequently co-detected pathogens alongside *T. whipplei* were Human gammaherpesvirus 4 (Epstein-Barr virus, EBV) (9%, n = 8) and *Klebsiella pneumoniae* (8%, n = 7). In addition, the *Mycobacterium tuberculosis* complex co-detected with *T. whipplei* was observed in 5 cases (5%), and *non-tuberculous mycobacteria* co-detected with *T. whipplei* were also found in 5 cases (5%).

Notably, the number of *T. whipplei* mNGS reads was significantly higher in pneumonia cases compared to those with colonization (P = 0.0298) (Figure 1). The coverage map illustrating *T. whipplei* reads for the 14 patients diagnosed with *T. whipplei* pneumonia is displayed in Figure S1.

Table 3
Pathogen detected by mNGS.

Pathogen	Cases (%) (n = 91)
T. whipplei as sole agent	39 (43%)
Klebsiella pneumoniae	7 (8%)
Mycobacterium tuberculosis complex	5 (5%)
Non-tuberculosis mycobacteria	5 (5%)
Acinetobacter baumannii	4 (4%)
Streptococcus pneumoniae	3 (3%)
Haemophilus influenzae	3 (3%)
Staphylococcus aureus	3 (3%)
Stenotrophomonas maltophilia	2 (2%)
Moraxella catarrhalis	1 (1%)
Staphylococcus cohnii	1 (1%)
Corynebacterium pseudodiphtheriticum	1 (1%)
Nocardia asiaca	1 (1%)
Mycoplasma pneumoniae	1 (1%)
Pneumocystis jirovecii	4 (4%)
Aspergillus fumigatus	3 (3%)
Aspergillus flavus	1 (1%)
Rhizomucor pusillus	1 (1%)
Cryptococcus neoformans	1 (1%)
Human gamma-herpes virus 4 (EBV)	8 (9%)
Human rhinovirus	4 (4%)
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	3 (3%)
Human beta-herpes virus 5 (CMV)	2 (2%)
Human herpesvirus 7 (HHV-7)	2 (2%)
Herpes Simplex Virus Type 1 (HSV-1)	1 (1%)
Respiratory syncytial virus (RSV)	1 (1%)
Parainfluenza virus	1 (1%)

Results of T. whipplei *qPCR Assays*

qPCR analysis for *T. whipplei* was conducted on 70 cases. Among these, 49 cases tested positive for *T. whipplei* by qPCR, while 21 cases were negative. Notably, the number of *T. whipplei* mNGS reads was significantly higher in samples that were qPCR-positive compared to those that were qPCR-negative (P < 0.0001) (Figure 2).

The results of mNGS and qPCR assays in patients diagnosed with *T. whipplei* pneumonia are summarized in Table 4. Of the 14 patients with *T. whipplei* pneumonia, qPCR analysis was performed on samples from 11 cases. Ten of these cases tested qPCR-positive,

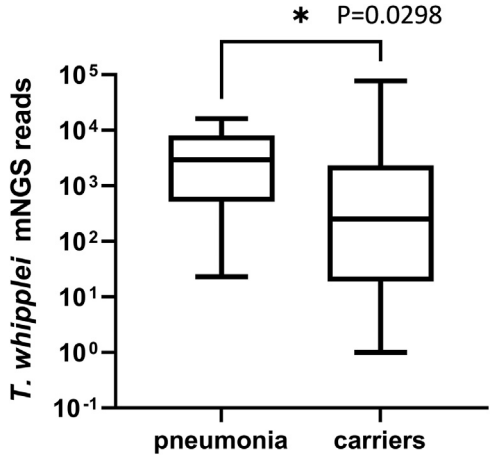

Figure 1. The *T. whipplei* mNGS reads of *T. whipplei* pneumonia and *T. whipplei* carriers.

Table 4
The results of mNGS and PCR in patients with *T. whipplei* pneumonia.

Case No	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sample collection	2022/4/2	2022/6/6	2022/7/1	2022/7/13	2022/9/7	2023/2/28	2023/7/31	2023/8/4	2023/10/10	2024/2/6	2024/2/29	2024/2/29	2024/3/27	2024/6/6
mNGS experiment	2022/4/7	2022/6/7	2022/7/4	2022/7/14	2022/9/7	2023/3/1	2023/7/31	2023/8/7	2023/10/11	2024/2/7	2024/2/29	2024/2/29	2024/3/28	2024/6/7
mNGS report	2022/4/8	2022/6/8	2022/7/5	2022/7/15	2022/9/8	2023/3/2	2023/8/1	2023/8/8	2023/10/12	2024/2/8	2024/3/1	2024/3/1	2024/3/29	2024/6/11
mNGS (reads)	639	148	7538	1894	11,754	4408	29	717	646	9579	16,040	3967	7379	23
Coverage length	115,285	10,638	369,767	121,160	477,765	252,820	1862	47,877	41,957	444,342	587,885	208,706	369,485	1724
Coverage percentage (%)	12.43	1.15	39.93	13.09	51.60	27.26	0.20	5.16	4.53	47.92	63.4	22.51	39.90	0.19
First PCR (Ct value)	NA	Undetermined	30.97	39.36	29.91	NA	NA	32.51	31.96	31.96	29.79	33.57	34.16	34.70
Second PCR (Ct value)	NA	Undetermined	31.20	39.73	29.58	NA	NA	32.24	31.88	32.02	28.89	32.87	32.92	34.59

Figure 2. The *T. whipplei* mNGS reads according to PCR results.

Table 5
Radiological manifestations of patients with TW positive in in lower respiratory tract specimens.

Imaging	Cases (%)(n = 91)
Nodules	49 (54%)
Patch	47 (52%)
Ground glass opacity	32 (35%)
Mass	22 (24%)
Pleural effusion	16 (18%)
Enlargement of mediastinal lymph nodes	14 (15%)
Cavity	5 (5%)

whereas Case 2 tested qPCR-negative. The PCR results of *T. whipplei* carriers were listed in Table S1.

Imaging characteristics of patients with T. whipplei *positive in lower respiratory tract specimens*

We conducted a comprehensive analysis of the radiological manifestations observed in patients with *T. whipplei*-positive lower respiratory tract specimens (Table 5). The most common imaging were nodules (54%, n = 49), patch (52%, n = 47), ground glass opacity (35%, n = 32), and mass (24%, n = 22). The representative imaging manifestations in patients with *T. whipplei* pneumonia are illustrated in Figure 3.

Characters of T. whipplei *infection/colonization pathology*

There were only two lung biopsies were done in all 14 *T. whipplei* infection specimens. The histopathological findings of the lung tissue in Case 10 reveal epithelioid granulomatous inflammation within the bronchial mucosa. The Gomori's methenamine silver (GMS) stain, acid-fast stain, and periodic acid-Schiff (PAS) stain all yielded negative results. The pulmonary histopathological findings of Case 12 are as follows: There is centrilobular deposition of carbon particles within the small airways, accompanied by a minor infiltration of lymphocytes. Sparse and scattered lymphocyte infiltrations are evident within the interstitial tissue, with focal areas of lymphocyte aggregation. Additionally, a small number of lymphocytes are aggregated within the alveolar lumens. Notably, the alveolar septa exhibit no signs of thickening. No malignant tumors or epithelioid granulomas are present in the examined tissue. Furthermore, PAS stain yields negative results. A total of 36 lung biopsies were performed on specimens colonized by *T. whipplei*. PAS staining was performed on seven of these specimens, and all exhibited negative results. Furthermore, among the remaining

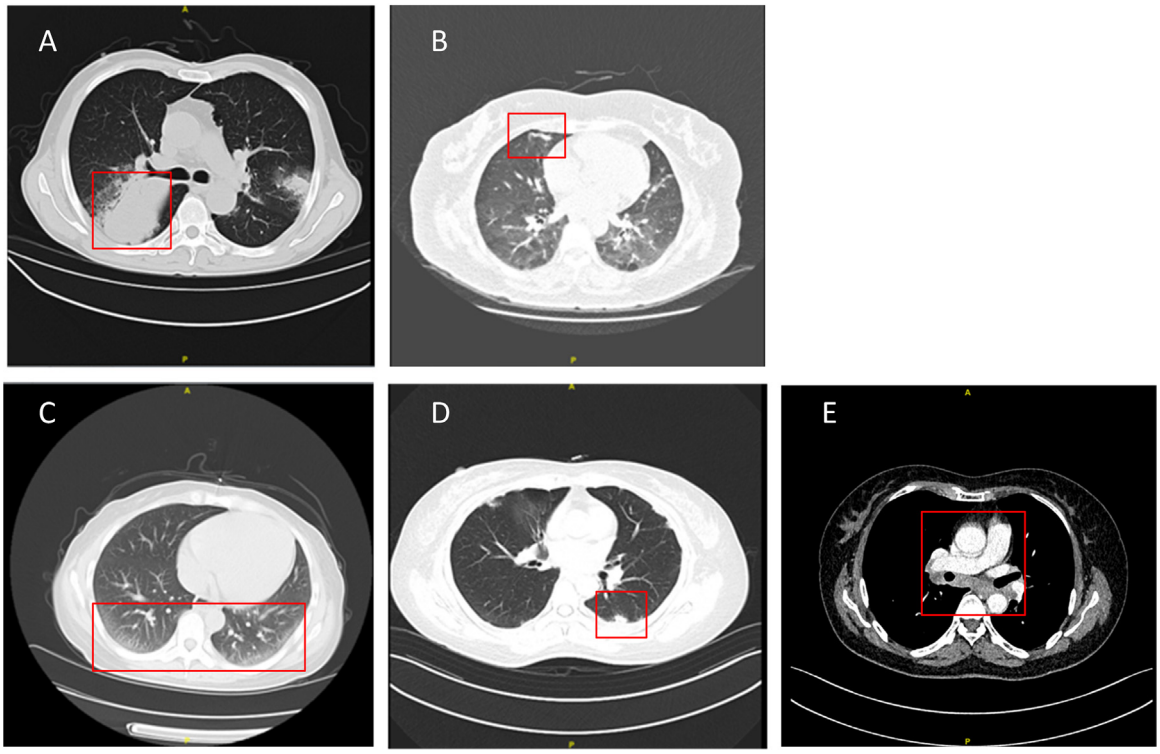

Figure 3. Imaging manifestations in *T. whipplei* pneumonia, including the mass (A), patchy (B), ground glass opacity (C), nodules (D) and enlargement of mediastinal lymph nodes (E).

29 specimens that were not subjected to PAS staining, pathological examination confirmed the presence of cancer in nine cases.

Treatment and prognosis

All 14 patients diagnosed with *T. whipplei* pneumonia received antibiotic therapy tailored to their condition. Specifically, Case 3 was treated with minocycline and hydroxychloroquine, Case 10 received amoxicillin and sulfamethoxazole, and Case 4 was administered piperacillin-tazobactam. The remaining patients were treated with cephalosporin-related antibiotics. Unfortunately, Case 5 succumbed to respiratory failure, while the other 13 patients were discharged in improved health.

Discussion

Pneumonia, as a common clinical condition, poses considerable difficulties in both diagnosis and treatment, especially when it arises from infections caused by rare or lesser-known pathogens [7]. After being infected with *T. whipplei*, most individuals either remain asymptomatic or experience a self-limiting infection due to the development of protective humoral and cellular immunity, whereas only a minority of cases progress to WD [13]. Common symptoms of WD encompass joint disorders or arthritis, unintended weight loss, abdominal discomfort, and diarrhea [14]. Pulmonary involvement represents a rare yet characteristic presentation of WD, with *T. whipplei* infection being identifiable in patients suffering from aspiration pneumonia, ventilator-associated pneumonia, as well as community-acquired pneumonia [15]. In our research, we documented a cumulative total of 91 cases where *T. whipplei* was positively identified in BALF specimens through the use of mNGS. To our knowledge, this study represents the largest series to date of *T. whipplei* detection in BALF specimens.

T. whipplei can exist in the saliva of asymptomatic carriers [16,17], and *T. whipplei* may co-cause aspiration pneumonia with other oral flora [15]. Furthermore, the prevalence of *T. whipplei* colonization in the lungs of healthy individuals can reach up to 26% [18]. Concurrently, it has been observed that the rate of *T. whipplei* lung colonization is notably higher among asymptomatic HIV-positive patients when compared to a control cohort [19].Although a large-scale study found no association between patients' immune status and the incidence of *T. whipplei* infection [20]. In our study, 15(16%) patients were immunocompromised, and 27 patients (30%) had a history of allergies to drugs, pollen, or food.

As documented in prior cases, *T. whipplei* can co-infect with *Pneumocystis jirovecii* [21]. Alternatively, it may solely induce pulmonary infection [22]. In other research studies, occasional reports have emerged regarding the co-infection or concurrent detection of *Mycobacterium tuberculosis* complex and *T. whipplei* [23]. In our study, we found that the *Mycobacterium tuberculosis* complex was detected together with *T. whipplei* in 5 (5%) cases. The reason may be that the incidence of tuberculosis in China is still high. In another study, Lin et al. [24] reported that their medical institution was designated for tuberculosis treatment, which contributed to a notably high co-detection rate of 14.3% for tuberculosis and *T. whipplei*. Besides, both the *Mycobacterium tuberculosis* complex and *T. whipplei* are classified under the phylum Actinobacteria. The *Mycobacterium tuberculosis* complex is a facultative intracellular bacterium, whereas *T. whipplei* is an obligate intracellular one. Infections caused by both of these bacteria have been closely linked to macrophages. In the context of classic WD pathogenesis, bacteria can induce macrophages within the duodenal mucosa to polarize into M2, or alternatively activated macrophages [25]. The compromised antigen-presenting capacity of both macrophages and dendritic cells further attenuates T-cell responsiveness [26]. Concurrently, elevated regulatory T-cell activity in both intestinal and systemic circulation exacerbates the immunosuppressive milieu [27].

In our study, a total of 39 patients (43%) were confirmed to have *T. whipplei* as the sole causative pathogen, whereas

the remaining 57% of specimens harbored other microorganisms. Among the 14 patients diagnosed with *T. whipplei* pneumonia, co-infections with additional pathogens were identified in merely 5 cases. Case 5 pertained to a 51-year-old male patient who presented with a history of cough and dyspnea and had been diagnosed with interstitial lung disease 3 years previously. He had undergone a one-year course of glucocorticoid therapy, which led to an alleviation of his symptoms. However, he experienced a relapse of cough and dyspnea 2 weeks prior to his current hospital admission. mNGS detected the presence of both *T. whipplei* and *Pneumocystis jirovecii*. The patient was subsequently treated with antibiotics, including moxifloxacin, ceftazidime, and sulfamethoxazole-trimethoprim (SMZ-TMP). Despite this therapeutic intervention, there was no improvement in the patient's dyspnea, and he ultimately succumbed to respiratory failure 1 month after hospitalization. Case 11 involved a 75-year-old male patient who presented with a two-week history of cough, expectoration, and fever (with a peak temperature of 39°C). He tested positive for the SARS-CoV-2 antigen, and was subsequently administered oral nirmatrelvir/ritonavir. The result of mNGS revealed the presence of both *T. whipplei* and SARS-CoV-2. Following treatment with compound SMZ-TMP combined with ceftriaxone, the patient showed clinical improvement and was discharged from the hospital. Excluding the two patients described in Case 5 and Case 11, the other three specimens demonstrated isolated *T. whipplei* infection, coexisting with the carriage of commensal normal flora.

We have analyzed the radiological manifestations of patients with *T. whipplei* positive in lower respiratory tract specimens. The imaging findings exhibited a wide range of manifestations, with pulmonary nodules being the predominant feature. This particular finding has also been documented in multiple prior case reports [24,28]. It is of paramount importance to emphasize that pneumonia resulting from *T. whipplei* infection represents an exceptionally elusive clinical entity, frequently subject to misdiagnosis or, in some instances, complete oversight. The definitive diagnosis of WD predominantly hinges on several diagnostic modalities, including PAS staining of duodenal/jejunal biopsy specimens, polymerase chain reaction (PCR) testing for *T. whipplei*, and immunohistochemical analysis of affected organs [29]. Nevertheless, obtaining duodenal or jejunal biopsy specimens or performing *T. whipplei* culture poses significant challenges in patients who do not exhibit gastrointestinal symptoms. In all *T. whipplei* pneumonia patients, duodenal biopsies were performed in three patients (Case 6, Case 9, Case 14). PAS staining of duodenal biopsy samples was performed in these three patients, and PAS staining was detected positive in Case 6 and Case 14. While the PCR of these two duodenal biopsy samples was negative. The diagnostic discordance between PAS staining and PCR analysis of duodenal biopsy specimens warrants further investigation. Of the 14 patients with *T. whipplei* pneumonia, qPCR analysis was performed on samples from 11 cases. Ten of these cases tested qPCR-positive, whereas Case 2 tested qPCR-negative. The reason may be that the sample of Case 2 was stored for a long time, and the DNA of *T. whipplei* was degraded.

T. whipplei, has proved particularly recalcitrant to cultivation. Over the course of numerous experimental attempts, a protracted period of nearly one century elapsed between the initial clinical description of the associated disease in 1907 and the landmark achievement in 2000, when this elusive microorganism was ultimately successfully and reproducibly cultured within the fibroblast cell line (HEL) [30]. In 2003, a synthetic medium (a specifically formulated axenic medium) enriched with amino acids and other essential nutrients that *T. whipplei* cannot synthesize on its own was, for the first time, successfully employed to culture *T. whipplei* without the need for host cells [31]. The cultivation of *T. whipplei* is significantly hindered by the substantial presence of commensal bacteria in saliva and stool specimens. These commensal bacteria tend to contaminate and overrun the bacterial cultures, thereby making it nearly unfeasible to reliably ascertain the presence of *T. whipplei* through the cultivation of patient samples [32].This study represents a retrospective investigation, and owing to the unavailability of specialized reagents essential for the cultivation of *T. whipplei*, we were unable to undertake *T. whipplei* culture. Instead, we exclusively employed PCR methodology to validate the presence of *T. whipplei* detected in mNGS results.

The pathological hallmarks of pneumonia caused by *T. whipplei* primarily manifest as alveolar and interstitial inflammation, accompanied by the distinctive presence of PAS-positive bacilli within macrophages. However, variations exist in the detailed descriptions of these pathological features across different studies. Shen et al. [33] have documented a case of acute pneumonia attributable to *T. whipplei* infection, in which the diagnosis of *T. whipplei* infection was facilitated by the integration of mNGS of alveolar lavage fluid with PAS staining of pathological specimens obtained from lung puncture biopsy. Chen et al. [34] documented a case involving an asymptomatic patient who was admitted to the hospital after a shadow was detected on his chest high-resolution computed tomography (HRCT) scan during a routine health checkup. The patient was subsequently treated with levofloxacin as an anti-infective agent. A transbronchial lung biopsy (TBLB) was performed, revealing disrupted alveolar septa, atrophied alveoli, scattered interstitial lymphocytes, and a small number of PAS-positive bacilli. mNGS of BALF identified *T. whipplei* as the sole causative pathogen. Despite these findings, the patient declined further treatment and was discharged. Upon follow-up 2 years later, the patient reported no respiratory symptoms. In another study centered on the detection of *T. whipplei* through mNGS in BALF, histopathological data of the lungs were accessible for 27 patients. Notably, the histopathological examination of one patient showed positive staining for both PAS and silver hexamine, which resulted in an initial diagnosis of cryptococcal infection. However, there was no description of PAS staining results related to *T. whipplei* [24]. Neither in cases of *T. whipplei* infection nor in those of *T. whipplei* colonization did our study identify any specimens with positive PAS staining. Antibiotic use, low bacterial load, or limited sampling sites may contribute to negative PAS staining results. In the future, more research and analysis are warranted to further elucidate the pathological characteristics of *T. whipplei* pneumonia.

A multitude of pharmaceutical remedies are readily available to treat *T. whipplei* infection. These pharmacological agents include, but are not limited to, penicillin, streptomycin, tetracycline, ceftriaxone, meropenem, trimethoprim, doxycycline, and hydroxychloroquine [35]. Current consensus recommends an initial 14-day course of parenteral antimicrobial therapy for WD, utilizing either ceftriaxone (2 g daily via intravenous infusion) or meropenem (1 g every 8 hours), followed by a prolonged 12-month oral regimen of sulfamethoxazole-trimethoprim to prevent relapse [32]. *In vitro* susceptibility testing indicates potential trimethoprim resistance in *T. whipplei* [13]. In such cases, SMZ-TMP may be substituted with doxycycline (100 mg twice daily) for the maintenance phase of WD therapy [36].

One notable limitation of this study lies in its relatively small patient cohort. Specifically, a mere 14 patients were diagnosed with *T. whipplei* pneumonia and subsequently treated with relevant antibiotics. Moreover, we encountered challenges in following up with patients who completed the full course of standardized treatment protocols, which hindered our ability to ascertain the most appropriate treatment regimen. Additionally, considering that this study adopts a retrospective design, there is an urgent necessity to initiate prospective cohort studies in the future. Such studies will empower us to meticulously monitor the treatment regimens employed for patients with *T. whipplei* pneumonia, precisely assess

their therapeutic outcomes, and comprehensively investigate their long-term survival prospects.

Conclusion

In conclusion, clinicians should approach cases of pneumonia with an unknown cause with great caution. The utilization of mNGS sequencing on respiratory specimens represents an outstanding diagnostic approach, capable of detecting rare microbial infections, such as those caused by *T. whipplei*. Implementing early mNGS sequencing of lower respiratory tract specimens can substantially aid patients suffering from pneumonia of unknown etiology by enabling prompt patient management and facilitating timely adjustments to their treatment plans.

Declaration of competing interest

The authors declare that they have no competing interests.

Funding

This study was supported by a grant from the National Natural Science Foundation of China (82302532/H2201).

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Peking University People's Hospital (2025PHB001-001). Because of the retrospective nature of this study, informed consent is not needed. The data used in this study was anonymized before its use.

Acknowledgments

The authors thank Yuanyuan Chen, Yue Wang, Ran Li, and Chunyu Liu for their assistance.

Authors' contributions

Study concept and design: LS, YW and HW. Acquisition of data: LS, YW, JF, ZL, YY, YG, QW and HC. Statistical analysis of data: LS and YW. Literature search: LS. Figures: LX. Drafting of the manuscript: LS. Critical revision of the manuscript for important intellectual content: BC and HW. Study supervision: HW. All authors read and approved the study.

Data availability

Data is available from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2026.108457.

References

[1] Fenollar F, Puechal X, Raoult D. Whipple's disease. *N Engl J Med* 2007;**356**(1):55–66. https://www.ncbi.nlm.nih.gov/pubmed/17202456.
[2] Boumaza A, Ben Azzouz E, Arrindell J, Lepidi H, Mezouar S, Desnues B. Whipple's disease and *Tropheryma whipplei* infections: from bench to bedside. *Lancet Infect Dis* 2022;**22**(10):e280–91. https://www.ncbi.nlm.nih.gov/pubmed/35427488.
[3] Fang Z, Liu Q, Tang W, Yu H, Zou M, Zhang H, et al. Experience in the diagnosis and treatment of pneumonia caused by infection with *Tropheryma whipplei*: a case series. *Heliyon* 2023;**9**(6):e17132. https://www.ncbi.nlm.nih.gov/pubmed/37484369.
[4] Fenollar F, Trani M, Davoust B, Salle B, Birg ML, Rolain JM, et al. Prevalence of asymptomatic *Tropheryma whipplei* carriage among humans and nonhuman primates. *J Infect Dis* 2008;**197**(6):880–7. https://www.ncbi.nlm.nih.gov/pubmed/18419351.
[5] Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* 1992;**327**(5):293–301. https://www.ncbi.nlm.nih.gov/pubmed/1377787.
[6] Schoniger-Hekele M, Petermann D, Weber B, Muller C. *Tropheryma whipplei* in the environment: survey of sewage plant influxes and sewage plant workers. *Appl Environ Microbiol* 2007;**73**(6):2033–5. https://www.ncbi.nlm.nih.gov/pubmed/17277223.
[7] Fenollar F, Ponge T, La Scola B, Lagier JC, Lefebvre M, Raoult D. First isolation of *Tropheryma whipplei* from bronchoalveolar fluid and clinical implications. *J Infect* 2012;**65**(3):275–8. https://www.ncbi.nlm.nih.gov/pubmed/22172770.
[8] Li W, Zhang Q, Xu Y, Zhang X, Huang Q, Su Z. Severe pneumonia in adults caused by *Tropheryma whipplei* and *Candida sp.* infection: a 2019 case series. *BMC Pulm Med* 2021;**21**(1):29. https://www.ncbi.nlm.nih.gov/pubmed/33451316.
[9] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;**30**(15):2114–20. https://www.ncbi.nlm.nih.gov/pubmed/24695404.
[10] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;**9**(4):357–9. https://www.ncbi.nlm.nih.gov/pubmed/22388286.
[11] Chen H, Zheng Y, Zhang X, Liu S, Yin Y, Guo Y, et al. Clinical evaluation of cell-free and cellular metagenomic next-generation sequencing of infected body fluids. *J Adv Res* 2024;**55**:119–29. https://www.ncbi.nlm.nih.gov/pubmed/36889461.
[12] Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol* 2019;**20**(1):257. https://www.ncbi.nlm.nih.gov/pubmed/31779668.
[13] Fenollar F, Perreal C, Raoult D. *Tropheryma whipplei* natural resistance to trimethoprim and sulphonamides *in vitro*. *Int J Antimicrob Agents* 2014;**43**(4):388–90. https://www.ncbi.nlm.nih.gov/pubmed/24613421.
[14] Duss FR, Jaton K, Vollenweider P, Troillet N, Greub G. Whipple disease: a 15-year retrospective study on 36 patients with positive polymerase chain reaction for *Tropheryma whipplei*. *Clin Microbiol Infect* 2021;**27**(6). 910 e919-910 e913 https://www.ncbi.nlm.nih.gov/pubmed/32896657 .
[15] Bousbia S, Papazian L, Auffray JP, Fenollar F, Martin C, Li W, et al. *Tropheryma whipplei* in patients with pneumonia. *Emerg Infect Dis* 2010;**16**(2):258–63. https://www.ncbi.nlm.nih.gov/pubmed/20113556.
[16] Grasman ME, Pettersson AM, Catsburg A, Koek AG, van Bodegraven AA, Savelkoul PH. *Tropheryma whipplei*, a potential commensal detected in individuals undergoing routine colonoscopy. *J Clin Microbiol* 2015;**53**(12):3919–21. https://www.ncbi.nlm.nih.gov/pubmed/26447117.
[17] Fenollar F, Laouira S, Lepidi H, Rolain JM, Raoult D. Value of *Tropheryma whipplei* quantitative polymerase chain reaction assay for the diagnosis of Whipple disease: usefulness of saliva and stool specimens for first-line screening. *Clin Infect Dis* 2008;**47**(5):659–67. https://www.ncbi.nlm.nih.gov/pubmed/18662136.
[18] Morris A, Beck JM, Schloss PD, Campbell TB, Crothers K, Curtis JL, et al. Comparison of the respiratory microbiome in healthy nonsmokers and smokers. *Am J Respir Crit Care Med* 2013;**187**(10):1067–75. https://www.ncbi.nlm.nih.gov/pubmed/23491408.
[19] Beck JM, Schloss PD, Venkataraman A, Twigg H 3rd, Jablonski KA, Bushman FD, et al. Multicenter comparison of lung and oral microbiomes of HIV-infected and HIV-uninfected individuals. *Am J Respir Crit Care Med* 2015;**192**(11):1335–44. https://www.ncbi.nlm.nih.gov/pubmed/26247840.
[20] Lagier JC, Papazian L, Fenollar F, Edouard S, Melenotte C, Laroumagne S, et al. *Tropheryma whipplei* DNA in bronchoalveolar lavage samples: a case control study. *Clin Microbiol Infect* 2016;**22**(10):875–9. https://www.ncbi.nlm.nih.gov/pubmed/27432769.
[21] Yan J, Zhang B, Zhang Z, Shi J, Liu S, Qi J, et al. Case report: *Tropheryma whipplei* hide in an AIDS patient with pneumocystis pneumonia. *Front Public Health* 2021;**9**:663093. https://www.ncbi.nlm.nih.gov/pubmed/34485213.
[22] Guo Y, Li L, Li Z, Sun L, Wang H. *Tropheryma whipplei* detection by nanopore sequencing in patients with interstitial lung disease. *Front Microbiol* 2021;**12**:760696. https://www.ncbi.nlm.nih.gov/pubmed/34912314.
[23] Zhu B, Tang J, Fang R, Fei X, Wang Q, Wang W, et al. Pulmonary coinfection of *Mycobacterium tuberculosis* and *Tropheryma whipplei*: a case report. *J Med Case Rep* 2021;**15**(1):359. https://www.ncbi.nlm.nih.gov/pubmed/34243811.
[24] Lin M, Wang K, Qiu L, Liang Y, Tu C, Chen M, et al. *Tropheryma whipplei* detection by metagenomic next-generation sequencing in bronchoalveolar lavage fluid: a cross-sectional study. *Front Cell Infect Microbiol* 2022;**12**:961297. https://www.ncbi.nlm.nih.gov/pubmed/36061864.
[25] Desnues B, Lepidi H, Raoult D, Mege JL. Whipple disease: intestinal infiltrating cells exhibit a transcriptional pattern of M2/alternatively activated macrophages. *J Infect Dis* 2005;**192**(9):1642–6. https://www.ncbi.nlm.nih.gov/pubmed/16206080.
[26] Moos V, Schmidt C, Geelhaar A, Kunkel D, Allers K, Schinnerling K, et al. Impaired immune functions of monocytes and macrophages in Whipple's disease. *Gastroenterology* 2010;**138**(1):210–20. https://www.ncbi.nlm.nih.gov/pubmed/19664628.
[27] Schinnerling K, Moos V, Geelhaar A, Allers K, Loddenkemper C, Friebel J, et al. Regulatory T cells in patients with Whipple's disease. *J Immunol* 2011;**187**(8):4061–7. https://www.ncbi.nlm.nih.gov/pubmed/21918190.

[28] Zhang WM, Xu L. Pulmonary parenchymal involvement caused by *Tropheryma whipplei*. *Open Med (Wars)* 2021;**16**(1):843–6. https://www.ncbi.nlm.nih.gov/pubmed/34131590.
[29] El-Abassi R, Soliman MY, Williams F, England JD. Whipple's disease. *J Neurol Sci* 2017;**377**:197–206. https://www.ncbi.nlm.nih.gov/pubmed/28477696.
[30] Raoult D, Birg ML, La Scola B, Fournier PE, Enea M, Lepidi H, et al. Cultivation of the bacillus of Whipple's disease. *N Engl J Med* 2000;**342**(9):620–5. https://www.ncbi.nlm.nih.gov/pubmed/10699161.
[31] Renesto P, Crapoulet N, Ogata H, La Scola B, Vestris G, Claverie JM, et al. Genome-based design of a cell-free culture medium for *Tropheryma whipplei*. *Lancet* 2003;**362**(9382):447–9. https://www.ncbi.nlm.nih.gov/pubmed/12927433.
[32] Dolmans RA, Boel CH, Lacle MM, Kusters JG. Clinical manifestations, treatment, and diagnosis of *Tropheryma whipplei* infections. *Clin Microbiol Rev* 2017;**30**(2):529–55. https://www.ncbi.nlm.nih.gov/pubmed/28298472.
[33] Shen Y, Cui SS, Teng XB, Han MF. Acute pneumonia due to *Tropheryma whipplei* diagnosed by metagenomic next-generation sequencing and pathology: a case report. *Heliyon* 2024;**10**(4):e26747. https://www.ncbi.nlm.nih.gov/pubmed/38434082.
[34] Chen Q, Gao B, Guo W, Liu H, Guo J. Clinical features of patients with *Tropheryma whipplei* detected in lower respiratory tract samples in China. *Infect Drug Resist* 2025;**18**:3439–48. https://www.ncbi.nlm.nih.gov/pubmed/40657278.
[35] Lozupone C, Cota-Gomez A, Palmer BE, Linderman DJ, Charlson ES, Sodergren E, et al. Widespread colonization of the lung by *Tropheryma whipplei* in HIV infection. *Am J Respir Crit Care Med* 2013;**187**(10):1110–17. https://www.ncbi.nlm.nih.gov/pubmed/23392441.
[36] Feurle GE, Moos V, Blaker H, Loddenkemper C, Moter A, Stroux A, et al. Intravenous ceftriaxone, followed by 12 or three months of oral treatment with trimethoprim-sulfamethoxazole in Whipple's disease. *J Infect* 2013;**66**(3):263–70. https://www.ncbi.nlm.nih.gov/pubmed/23291038.