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Application of metagenomic next-generation sequencing in the diagnosis and treatment of acute pneumonia caused by Tropheryma whipplei

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Abstract

Objective The treatment plan and process for acute pneumonia caused by Tropheryma whipplei have not been clearly defined. The study aimed to conduct a retrospective analysis of the treatment for patients with acute pneumonia, caused by Tropheryma whipplei, diagnosed through metagenomic next-generation sequencing (mNGS) of bronchoalveolar lavage fluid (BALF).

Methods All patients underwent routine blood examinations and chest CT scans. Electronic fiberoptic bronchoscopy was performed to collect BALF samples from the lesion subsegments. The BALF samples were subjected to mNGS analysis. During hospitalization, all patients were treated with imipenem-cilastatin combined with compound sulfamethoxazole (SMZ-TMP) tablets for anti-infection, and they took SMZ-TMP orally for 3 months after discharge and followed up.

Results We identified 7 cases where Tropheryma whipplei was the primary pathogen, with 3 of these cases having it as the sole detected pathogen. The clinical manifestations of acute Tropheryma whipplei pneumonia are atypical. Chest CT scans revealed that 3 cases had exudative lesions in both lungs, 4 cases had unilateral pulmonary exudative lesions, 3 cases had bilateral pulmonary nodules, 2 cases had interstitial changes, and 3 cases had pleural effusion. Following treatment, all follow-up cases showed no recurrence.

Conclusions The mNGS examination of bronchoalveolar lavage fluid can significantly improve the early diagnosis of acute pneumonia caused by Tropheryma whipplei. The treatment involving imipenem-cilastatin combined with SMZ-TMP, followed by oral SMZ-TMP for three months, is effective.

Keyword Tropheryma whipplei, Acute pneumonia, Bronchoalveolar lavage fluid, Metagenomic next generation sequencing

Introduction

Tropheryma whipplei (TW) is a rod-shaped Gram-positive pathogen, first described by American pathologist George Hoyt Whipple in 1907. The discovery of"silver-stained rod-shaped microorganisms"in the vacuoles of intestinal macrophages in affected patients was made by Whipple [1]. Subsequent 16S rRNA gene analysis revealed that Tropheryma whipplei belongs to the

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phylum Actinobacteria, class Actinobacteria, order Actinobacteria, family Cellulose Monomonase, and genus tropheryma. Acute pneumonia caused by Tropheryma whipplei is one of the pathogenic bacteria often overlooked in clinical practice. Due to the lack of convenient and effective detection methods, there are few relevant reports to date. However, with the application of mNGS in clinical diagnosis, acute pneumonia caused by Tropheryma whipplei has begun to attract the attention of clinicians. Nevertheless, its pathogenesis, therapeutic strategy, and treatment course remain to be elucidaed.

In this study, we analyzed the clinical manifestations, examinations, treatment plans, and outcomes to provide a reference for the clinical diagnosis and treatment of acute TW pneumonia.

Materials and methods

Case collection and exclusion criteria

Collect cases of pneumonia patients with a disease course of less than one month who underwent BALF-mNGS examination at our hospital from June 2021 to May 2024. Exclusion criteria: 1. Patients previously diagnosed with chronic obstructive pulmonary disease, pulmonary embolism, bronchiectasis, interstitial lung disease, or basic pulmonary diseases such as tuberculosis or lung cancer under treatment. 2. Patients lost to follow-up and those who actively withdrew. This study obtained informed consent from the patients and was approved by the Medical Ethics Committee of Nanjing Pukou People's Hospital (No.2023-SR- 009).

Examination

All patients underwent routine blood examinations, including biochemistry, blood gas analysis, lactate (Lac), procalcitonin (PCT), interleukin- 6 (IL- 6), etc., and chest CT. Electronic fiberoptic bronchoscopy was performed to collect BALF samples from the lesion susegments. The BALF samples were collected in sterile containers and transported in ice boxes. Within twenty-four hours, they were sent to Nanjing Dinfectome medical laboratory for mNGS sequencing using a high-throughput gene sequencer.

Treatment

All patients received empirical anti-infection, expectorant, cough suppressant, oxygen inhalation, fever reduction, and other symptomatic treatments. Upon diagnosis with TW as the primary pathogen, the patients were treated with intravenous infusion of imipenem-cilastatin at 1 g every 8 h, combined with oral SMZ-TMP, until clinical symptoms subsided. After discharge, the patients continued to take SMZ-TMP for three months. Those with fungal or viral infections were administered appropriate treatments.

Statistical analysis

The physiological data of the patients were expressed as mean ± standard deviation (SD), without the potential confounding effects of the standardized values across larger population norms that may not accurately reflect our small, single-center cohort (Table 2). The count data were expressed as frequency and composition ratio (Tables 1, 3, 4). Given that this study focused on retrospective and discovery aspects, the sample size was small (seven acute TW pneumoni patients), resulting in limited statistical power. GraphPad Prism 8.0 was utilized for the analyses.

Results

General clinical data

312 cases of acute pneumonia were examined using BALF-mNGS, among which 22 patients (7%) had TW in the BALF. However, only 7 cases were identified as the main pathogen by two respiratory specialists, of which 3 cases were the only detected pathogen. The ages of the 7 patients ranged from 51 to 85 years (mean age: 66.29 ± 10.45 years), with 6 males, their BMI within the normal range (mean BMI: 22.97 ± 2.27 kg/m^2), and a disease course of 3 h to 25 days (mean disease course: 11.02 ± 9.19 days).

Clinical symptoms: fever (4/7), cough (7/7), scant phlegm (6/7), chest tightness (2/7), dyspnea (1/7), pleural inflammatory chest pain (1/7), hemoptysis (1/7), gastrointestinal symptoms including abdominal pain,

Table 1 The characteristics of patients with TW positive in BALF

Parameters	Total (Case%)
Age,median(range,years)	66.29(51-85)
BMI(mean±SD,kg/m^2)	22.97±2.27
Male	6(85.71%)
Clinical manifestations	
Cough	7(100%)
Expectoration	6(85.71%)
Fever	4(57.14%)
Haemoptysis	1(14.29%)
Chest tightness	2(28.57%)
Chest pain	1(14.29%)
Dyspnea	1(14.29%)
Anorexia	4(57.14%)
Weakness	5(71.43%)
Gastrointestinal symptoms	2(28.57%)

Table 2 Laboratory examination of patients with TW positive in BALF

Characteristics (Normal value range)	Total (n=7, mean±SD)
White blood cell (3.5-9.5x10^9/L)	8.87±4.67
Neutrophils (1.8-6.3x10^9/L)	6.76±4.24
Lymphocyt (1.1-3.2x10^9/L)	1.46±0.46
RBC (4.3-5.8x10^12/L)	4.32±0.17
HGB (130-175) g/L	130.86±14.46
PLT (125-350x10^9/L)	249.57±112.17
CRP (0-10) mg/L	49.26±117.24
PCT (0-0.046) ng/ml	0.29±0.47
IL-6(0-7)pg/ml	11.61±6.92
ALT (9-50)U/L	24.16±10.13
AST (15-40) U/L	30.83±16.63
BUN (3.6-9.5) mmol/L	6.03±1.06
CRE (55-111) mmol/L	83.61±20.01
Total protein (60-85) g/L	70.47±5.33
ALB(30-55) g/L	39.81±4.46
GLB (25-35) g/L	30.66±2.77
PO2(80-105) mmHg	82.14±11.65
Lac (0.36-1.25) mmol/L	0.92±0.21
LDH (120-246)U/L	285.96±236.79

RBC red blood cell, *HGB* Hemoglobin, *PLT* platelet count, *CRP* C-reactive protein, *PCT* procalcitonin, *IL-6* Human interleukin-6, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *BUN* Blood Urea Nitrogen, *CRE* creatinine, *ALB* albumin, *GLB* globulin, *PO2* Partial Pressure of Oxygen, *Lac* Lactate, *LDH* lactate dehydrogenase

Table 3 Radiological manifestations of patients with TW positive in BALF

Imaging	Total (Case%)
bilateral pulmonary exudation	3(42.86%)
bilateral pulmonary nodules	2(28.57%)
unilateral pulmonary nodule	1(14.29%)
bilateral pleural effusion	1(14.29%)
unilateral pulmonary exudation	4(57.14%)
interstitial changes	2(28.57%)
bilateral pulmonary nodules	1(14.29%)
bilateral pleural effusion	2(28.57%)

distension, or vomiting (2/7), loss of appetite (4/7), anemia (3/7), no neurological symptoms or arthralgia (Table 1).

Laboratory characteristics

The peripheral blood white blood cell and neutrophil counts were significantly elevated in the three patients with TW detected only in BALF, whereas they remained normal in the four patients with mixed infections.

Table 4 Pathogen spectrum of patients with TW positive in BALF by mNGS

Pathogen	Total (Case%)
Troperyma Whipplei	7(100%)
Haemophilus parainfluenzae	4(57.14%)
Streptococcus pneumoniae	3(42.86%)
Klebsiella aerogenes	2(28.57%)
Pseudomonas aeruginosa	2(28.57%)
human cytomegalovirus	2(28.57%
Candida parapsilosis	1(14.29%)
Aspergillus flavus	1(14.29%)
Mycobacterium abscesses	1(14.29%)

Among the three patients, one case of type I respiratory failure exhibited significantly increased CRP and PCT levels, while the other cases had normal CRP levels and their PCT levels were either normal or not significantly elevated. Out of the seven patients, six cases had elevated IL- 6 levels, three cases had low hemoglobin levels, and all BALF cultures were negative. Additionally, there was one case of type I respiratory failure and one case of hypoxemia (Table 2).

Electronic fiber bronchoscopy revealed airway inflammation in all seven patients. One patient experienced hemoptysis with a small amount of bronchial bleeding. Tissue biopsies from six patients indicated infiltration of lymphocytes, plasma cells, and neutrophils, with no cancer cells detected. Additionally, two patients presented with interstitial edema, while one patient did not undergo tissue biopsy.

Radiological manifestations

Among the seven patients, there were three cases of bilateral pulmonary exudative lesions, including two cases of bilateral pulmonary nodules, one case of a unilateral pulmonary nodule, and one case of bilateral pleural effusion. Among the four cases of unilateral pulmonary exudative lesions, there were two cases of interstitial changes, one case of bilateral pulmonary nodules, and two cases of bilateral pleural effusion (Table 3).

BALF-mNGS

The number of sequences where TW was the only detected pathogen in the 3 BALF samples were 30, 121, and 5624, respectively. The most commonly detected pathogens in four mixed infected cases were Haemophilus parainfluenzae (4/4).Streptococcus pneumoniae (3/4), Klebsiella aerogenes (2/4), Pseudomonas aeruginosa (2/4), human cytomegalovirus (2/4), Candida

parapsilosis (1/4), Aspergillus flavus (1/4), and Mycobacterium abscessus (1/4) (Table 4).

Treatment and follow-up

The seven patients were empirically treated with penicillin, cephalosporin, or quinolone antibiotics for common pathogens associated with community-acquired pneumonia. Upon identifying TW as the major pathogen, all patients were administered an intravenous infusion of 1 g every 8 h of imipenem-cilastatin, combined with oral SMZ-TMP. The duration of hospitalization ranged from 6 to 16 days, with a mean time of 10.86 ± 2.68 days.

All seven patients experienced clinical symptom relief during their hospital stay, and no significant recurrence was observed during the follow-up period after discharge, including other systemic symptoms such as gastrointestinal, arthritic, and neurological disorders. The follow-up period ranged from 7 months to 39.2 months, with four cases having a follow-up period exceeding 30 months, two cases declining to undergo chest CT review, and five cases showing significant lesion absorption on chest CT (Fig. 1).

Discussion

Acute pneumonia is a prevalent ailment in the field of respiratory medicine, and identifying the causative pathogens is crucial for effective treatment. Targeted therapy against the primary pathogens can shorten the duration of the illness, alleviate patient discomfort, prevent disease progression, and also minimize the risk of secondary drug resistance that often results from the use of broad-spectrum antibiotics. In recent years, the advent of mNGS has brought new hope for diagnosing pneumonia of unknown etiology and those caused by rare pathogens, through BALF mNGS detection. In this context, we introduce an acute pneumonia caused by the uncommon pathogen Tropheryma whipplei, aiming to present the clinical features, diagnostic procedures, and related treatment strategies.

Tropheryma whipplei was initially classified as a Gram-positive actinomycete and is known to cause chronic gastrointestinal symptoms [1–3], as well as seronegative arthritis or arthralgia [4, 5]. In addition to these symptoms, TW can lead to systemic multiple organ infections, including endocarditis, heart valve inflammation, nervous system involvement, uveitis, lymphadenopathy, skin manifestations, and other related symptoms [6, 7]. In our study, the detection rate of TW was 7% among the 312 cases of BALF-mNGS we collected. None of the 7 cases of acute pneumonia with TW as the primary pathogen exhibited obvious immunodeficiency-related diseases. Research has revealed that TW lacks the biosynthetic pathway for essential amino acids [8, 9], and possesses a large number of genes involved in immune evasion strategies. This enables TW to rapidly undergo genetic variation in immune determinants, such as phase variation

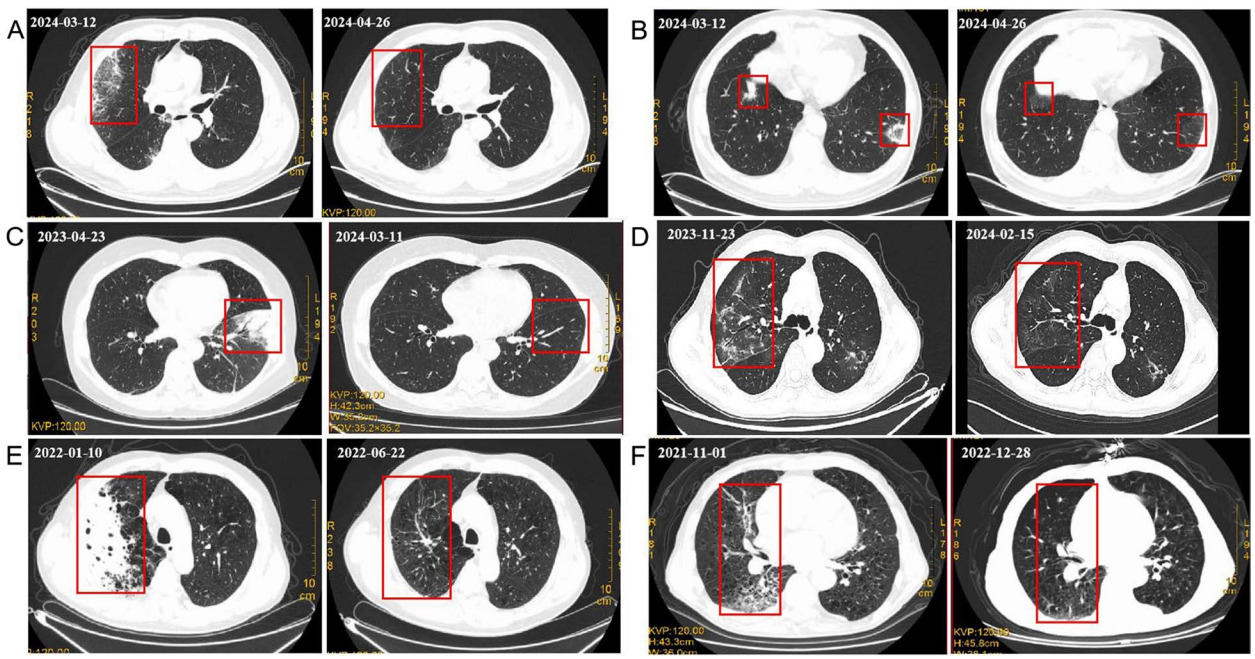

Fig. 1 Chest CT scan before and after treatment (Figures **A** and **B** show chest CT scans of the same patient. The left figures are before treatment and the right figures are after treatment. The rectangles indicate the focus.)

and homologous recombination, thereby facilitating its evasion of host immunity [8, 10]. In our study, the incidence of acute TW pneumonia was significantly higher in males than in females (6:1), with patients exhibiting a normal BMI. The clinical manifestations primarily include cough, scant phlegm, chest tightness, fever, hemoptysis, chest pain, and other atypical symptoms. Among the 7 cases, 2 presented with gastrointestinal symptoms, and none exhibited joint or neurological symptoms. In the 3 cases where TW was the sole detected pathogen, peripheral blood leukocytes and neutrophils were significantly increased, whereas the increase was less pronounced in the 4 cases of mixed infection. The main pathogens in mixed infections included Haemophilus parainfluenzae, Streptococcus pneumoniae, Klebsiella aerogenes, and Pseudomonas aeruginosa, among others.

Currently, the mechanism behind lung injury caused by TW remains unclear. The report indicates that TW can impair the degradation ability of macrophages, decrease cytokine secretion, and induce host cell apoptosis [11]. In our cases, chest CT scans primarily revealed pulmonary nodules, alveolar and interstitial exudation, and in some instances, a combination with pleural effusion. It has been reported that nodular-like lesions are the most common imaging manifestations of TW pneumonia, followed by interstitial changes and patchy infiltration, which may be accompanied by mediastinal lymph node enlargement, pleural thickening or adhesion, and pleural effusion, among others. In one case, cavitary lesions were observed [12]. The chest CT imaging findings of TW pneumonia may be related to TW's affinity for macrophages, as macrophages are the primary target cells of TW and there is a large number of macrophages in alveolar tissue, providing TW with a suitable environment for survival [3]. The macrophages in the lungs are predominantly distributed in the pulmonary interstitium, particularly around the lumen below the bronchioles and within the alveolar septa. Some of these macrophages migrate into the alveolar cavity, becoming alveolar macrophages.

Detecting TW in conventional culture is challenging, macrophage periodic acid Schiff (PAS) staining was initially utilized for intestinal biopsy tissue specimens [13]. A study indicated that simultaneous PCR detection of TW in feces and saliva is an effective screening method [14], but non-specific PCR results in higher false positives. Early and precise anti-infective treatment is crucial to reduce the mortality rate of pneumonia [15]. During the early stages of infection, at low pathogen concentrations, detection may be non-specific and inefficient, leading to adverse consequences without precisely targeted therapy or antibiotic overuse [16, 17]. Since the first reported clinical application of mNGS in 2014, it has shown promise in identifying multiple pathogens [18–20], particularly in the diagnosis of rare pathogen infections [21]. Additionally, the detection of pathogen resistance genes and virulence factors holds significant potential for predicting antibiotic resistance and guiding antibiotic use. Our 7 cases of acute TW pneumonia were all confirmed by BALF-mNGS, with 3 cases showing TW as the sole pathogen detected, and 7 cases yielding negative results in routine BALF culture. We also found that TW can be both a pathogenic bacterium and a colonized pathogen. We collected 312 cases, with 15 considering TW as a colonized pathogen. The study revealed that TW is widely colonized in immunocompromised patients, such as those with HIV, and the incidence of lung colonization in asymptomatic HIV-infected individuals is higher [22].

Acute TW pneumonia carries a high risk of respiratory failure and can escalate to severe pneumonia if not treated promptly. The antibiotics commonly used to treat TW include ceftriaxone, penicillin, meropenem, streptomycin, tetracycline, compound trimethoprim, and hydroxychloroquine. The recommended first-line treatment is an intravenous infusion of ceftriaxone 2 g daily or meropenem 3 g daily for 14 days, followed by a 12-month course of oral SMZ-TMP [23]. Previous studies have found that TW lacks the coding sequence for the trimethoprim target protein dihydrofolate reductase [24], and has several mutations in the folP gene, which encodes the sulfamethoxazole target dihydropteroate synthase [25], leading to TW's resistance to SMZ-TMP. Doxycycline 200 mg daily and hydroxychloroquine 600 mg daily were recommended as maintenance treatments for Whipple's disease, typically for at least 12 months, which may necessitate lifelong oral doxycycline treatment [26]. The lifelong oral administration of doxycycline also leads to resistance [27], and both doxycycline and hydroxychloroquine have adverse effects [28, 29]. The primary side effects of hydroxychloroquine are retinal toxicity, followed by cardiac toxicity and neuromuscular toxicity [26]. Doxycycline's side effects mainly include gastrointestinal and cutaneous symptoms [29]. In the study, seven patients with acute TW pneumonia were treated with intravenous infusions of imipenem-cilastatin at a dosage of 1 g every 8 h, in combination with oral SMZ-TMP during their hospital stay. Following discharge, they continued with oral SMZ-TMP for three months, and there were no recurrences observed during the follow-up period, including other systemic symptoms such as arthralgia, digestive system, and nervous system issues. Successful treatment of TW pneumonia with imipenem-cilastatin combined with oral SMZ-TMP has been reported [30]. However, one study found significant differences in the in vitro sensitivity of TW to imipenem-cilastatin and identified three different genotypes of TW:

the Twist strain was susceptible to imipenem-cilastatin, while the Endo2 and Slow strains were resistant to it [31]. We did not find TW resistant to imipenem-cilastatin, suggesting that the TW in this area may be the Twist strain. Reports of SMZ-TMP relapse are primarily associated with classic Whipple's disease (WD). WD typically presents as a chronic systemic disease, mainly affecting middle-aged men with symptoms such as diarrhea, weight loss, abdominal pain, and arthralgia [32, 33]. Meropenem combined with doxycycline [34], meropenem [35], or ceftriaxone [36] combined with SMZ-TMP have all been reported to successfully treat TW pneumonia; however, the subsequent antibiotic treatment plan and the risk of TW reinfection remain unclear and warrant further study.

In this study, we discovered that the short-course treatment regimen for acute pneumonia caused by TW has demonstrated a curative effect, with no recurrence observed during the follow-up period. Consequently, extending the duration of drug treatment and increasing the body's burden is not advised, due to the potential for recurrence. Furthermore, few patients persist in taking drugs with high side effects once chest CT scans indicate that the pneumonia has been fully absorbed. It is important to note that our study has significant limitations, such as a small sample size and diminished statistical power, as well as the retrospective study design that precludes the use of varied treatments. Additionally, a small cohort study may be subject to regional and strain differences, which could result in variations in treatment effect.

Conclusions

In summary, chest CT scans in clinical practice reveal pulmonary nodules, alveolar and interstitial exudation, and pleural effusion in patients with acute pneumonia, indicating the need to be vigilant about the possibility of acute pneumonia caused by TW. Currently, BALF-mNGS is anticipated to become an early, rapid, and accurate detection method. However, its high cost limits its widespread clinical application. The short-course treatment regimen of imipenem-cilastatin combined with SMZ-TMP has demonstrated clinical cure and is thus considered worthy of promotion.

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None.

Authors' contributions
(1) Yinping Huo, Chao Wu, Dawen Ma, conceiving and designing the study; (2) Yinping Huo, Chao Wu, Dawen Ma, collecting the data; (3) Yinping Huo, Chao Wu, Dawen Ma, analyzing and interpreting the data; (4) Yinping Huo, Chao Wu, writing the manuscript; (5) Yinping Huo, Chao Wu, Dawen Ma, providing critical revisions that are important for the intellectual content; (6) Yinping Huo, Chao Wu, Dawen Ma, approving the final version of the manuscript.

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the declaration of Helsinki. This study was approved by the Medical Ethics Committee of Nanjing Pukou People's Hospital (No.2023-SR- 009). This study obtained informed consent from the patients.

Clinical trial number
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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