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## CASE REPORT

# Metagenomic Next-Generation Sequencing for the Diagnosis of Trichomonas Vaginalis-Associated Empyema: a Case Report and Literature Review

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## ABSTRACT

***Background:*** **This report describes a rare case of empyema caused by Trichomonas vaginalis co-infected with Streptococcus agalactiae and Streptococcus pyogenes, aiming to explore the role of T. vaginalis in the development of empyema, its diagnostic methods, and treatment strategies.**
***Methods:*** **The patient was a 46-year-old male who presented with cough, sputum production, and shortness of breath. The diagnosis was made using chest CT, routine pleural fluid analysis, bacterial culture, and metagenomic next-generation sequencing (mNGS). Pleural fluid examination revealed numerous motile Trichomonas organisms, and bacterial culture identified Streptococcus agalactiae and Streptococcus pyogenes as the pathogens. Further mNGS confirmed these bacteria as the causative agents, with 39 Trichomonas sequences detected, including 32 sequences specific to T. vaginalis.**
***Results:*** **The patient received combination antimicrobial therapy and underwent chest tube drainage and thoracoscopic empyema debridement. Post-treatment, the patient's condition significantly improved. A literature review revealed that while Trichomonas tenax is a common pathogen in empyema, T. vaginalis is an extremely rare cause. T. vaginalis infection may be associated with bacterial co-infections, immunosuppression, or poor hygiene.**
***Conclusions:*** **This case is the first report of T. vaginalis induced empyema, expanding the understanding of Trichomonas infections. Although such infections are rare, they should be considered in high-risk patients. Further studies are needed to investigate the infection pathways of T. vaginalis and its mechanisms of bacterial synergy in disease pathogenesis to improve clinical diagnosis and treatment strategies.**
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**KEYWORDS**

Trichomonas vaginalis, empyema, metronidazole, metagenomic next-generation sequencing (mNGS)

## INTRODUCTION

Empyema is typically bacterial in origin; however, in susceptible individuals, it may also be caused by non-bacterial microorganisms or mixed infections. Among these, empyema due to Trichomonas co-infection is extremely rare. Humans are hosts to three different species of Trichomonas: Trichomonas vaginalis, Trichomonas

hominis, and Trichomonas tenax [1]. These species can be distinguished through morphological, epidemiological, serological, culture, and PCR-based techniques [2,3]. We report a case of empyema in a male patient co-infected with Trichomonas vaginalis, Streptococcus agalactiae, and Streptococcus pyogenes.

## CASE PRESENTATION

A 46-year-old male from Yunnan Province, China, unmarried and without children, presented to our hospital on April 8, 2025, with a 10-day history of "cough, sputum production, and shortness of breath accompanied by poor appetite". The symptoms began insidiously without any apparent trigger, starting with paroxysmal coughing and the production of white sputum, followed by shortness of breath, initially occurring during physical activity but progressively worsening to include rest-induced discomfort. The patient also experienced poor appetite, nausea, and vomiting. He denied chest pain, fever, chills, abdominal pain, diarrhea, urinary frequency, urgency, headache, or dizziness. On April 6, he visited a local hospital in Zhaotong, Yunnan. After receiving intravenous treatment at the local hospital, there was no significant improvement (details unclear). Seeking further diagnosis and treatment, he was transferred to our hospital's emergency department and was admitted to the respiratory intensive care unit with a diagnosis of "pulmonary infection and respiratory failure".

April 8, 2025, Emergency Department Admission: Laboratory Results: Complete Blood Count: White blood cell count 19.30 x $10^9$/L, Neutrophil percentage 82.7%. Renal Function: Blood urea nitrogen 17.99 mmol/L, C-reactive Protein: 353.9 mg/L. Procalcitonin (PCT): 4.55 ng/mL. Cardiac Ultrasound: Mild regurgitation of the mitral and tricuspid valves, pulmonary hypertension, normal left ventricular systolic function, minimal pericardial effusion, and tachycardia. Chest Imaging: Bilateral pleural effusion, with the deepest left costophrenic angle at approximately 15 mm. The right pleural cavity showed an irregular fluid collection with the maximum anterior-posterior diameter of 102 mm and superior-inferior diameter of 65 mm. Electrocardiogram (ECG): Sinus tachycardia, left ventricular high voltage, and early repolarization. Physical Examination: Left lung breath sounds slightly coarse with moist rales; no breath sounds were detected in the right lower lung, while breath sounds were diminished in the right upper lung. Treatment: The patient received intravenous Cefoperazone-Sulbactam, Metronidazole for infection management, chest closed drainage, and high-flow oxygen therapy. Pleural fluid analysis showed a significant Trichomonas infection (Figure 1). Bacterial culture of the pleural fluid revealed Streptococcus agalactiae and Streptococcus pyogenes. Pulmonary alveolar lavage fluid metagenomic sequencing (mNGS) revealed over 9,000 sequences of Streptococcus agalactiae and Streptococcus pyogenes, and 51 sequences of Pseudomonas aeruginosa. Urinalysis, urine culture, and stool tests were negative. On April 10, 2025, the patient's condition deteriorated, necessitating endotracheal intubation, mechanical ventilation, and administration of Meropenem, Linezolid, and Metronidazole. Additionally, mNGS was performed on pleural fluid, blood, and alveolar lavage samples.

April 13, 2025: Metagenomic next-generation sequencing (mNGS) of pleural fluid revealed 39 sequences of Trichomonas, including 32 sequences specific to Trichomonas vaginalis (Figure 2A). No Trichomonas was detected in blood or alveolar lavage fluid. April 15, 2025: The patient's condition showed improvement, and the endotracheal tube was removed. High-flow oxygen therapy was initiated, and antibiotics were switched to Piperacillin-Tazobactam 4.5 g every 8 hours and continued Metronidazole for infection control. A cardiothoracic surgery consultation recommended surgical intervention. April 17, 2025: The patient underwent video-assisted thoracoscopic surgery (VATS) under general anesthesia for empyema debridement and pleural fibroplate removal. Postoperative pathological examination is shown in Figure 2B/2C**.** Post-surgery, the patient continued with Piperacillin-Tazobactam and Metronidazole for infection treatment. April 25, 2025: The patient's condition further improved, with no significant cough, sputum production, fever, or shortness of breath. Oxygen therapy was no longer required. The patient was discharged and returned to a local hospital to continue the course of antimicrobial therapy.

## DISCUSSION

Trichomonas vaginalis is an important protozoan parasite primarily transmitted through vaginal intercourse. The clinical spectrum of Trichomonas infection in women is well-documented, ranging from vaginitis to asymptomatic carriage. Over 50% of women with T. vaginalis infection develop symptoms within six months. In men, infections are often asymptomatic, but symptomatic individuals typically experience urethritis [4]. To date, no reports have been found linking T. vaginalis to empyema. This study used various diagnostic methods, including pleural fluid routine examination and mNGS, to identify a significant presence of T. vaginalis in the pleural fluid, which was effectively treated with Metronidazole. The lower sequence count of T. vaginalis in the pleural fluid may be attributed to prior treatment with Metronidazole before mNGS was performed. The presence of Trichomonas in an infected host does not necessarily indicate it is the pathogenic factor; however, large quantities of T. vaginalis could worsen the overall condition and prolong the disease course. Most reported cases of pulmonary Trichomonas infections have been successfully treated with Metronidazole [1,5,6].

Trichomonas tenax is the most common Trichomonas species responsible for pulmonary infections and empy-

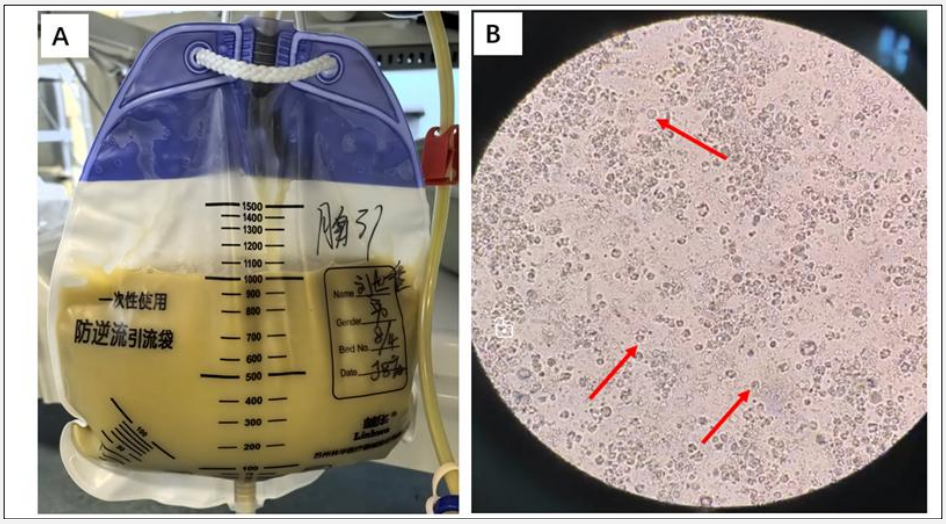
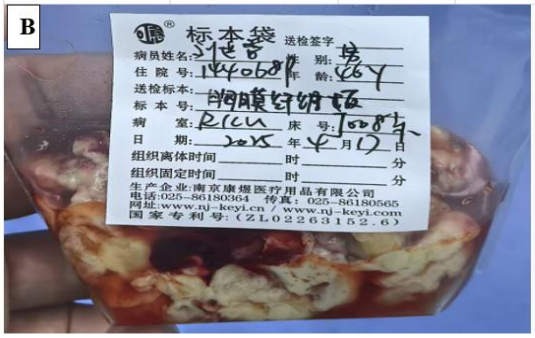



**Figure 1. A: Gross appearance of pleural fluid. B: Microscopic examination of pleural fluid, showing numerous motile Trichomonas organisms.**

A

| Gram Stain | Genus | Reads | Relative Abundance | Species | Reads | Confidence |
|---|---|---|---|---|---|---|
| G- | *Prevotella* | 46953 | 54.41% | *Prevotella oris* | 35255 | 99% |
| G+ | *Streptococcus* | 10185 | 11.80% | *Streptococcus constellatus* | 4078 | 99% |
| G- | *Fusobacterium* | 7224 | 8.37% | *Fusobacterium vincentii* | 5591 | 99% |
| G+ | *Parvimonas* | 5142 | 5.96% | *Parvimonas micra* | 5142 | 99% |
| G+ | *Peptostreptococcus* | 4793 | 5.55% | *Peptostreptococcus stomatis* | 4793 | 99% |
| Type | Genus | Reads | Relative Abundance | Species | Reads | Confidence |
| Parasite | *Trichomonas* | 39 | 100.00% | *Trichomonas vaginalis* | 32 | 56% |



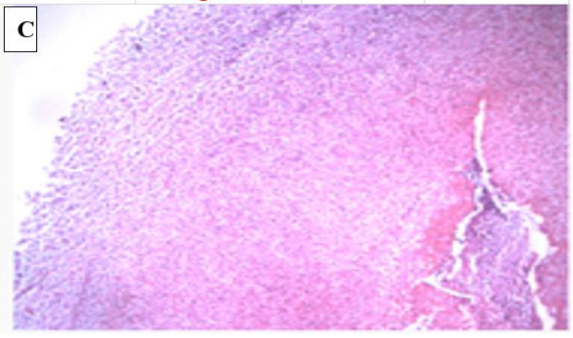



**Figure 2. A: Metagenomic Next-Generation Sequencing (mNGS) of pleural fluid detected Trichomonas vaginalis sequences. B: Gross appearance of pleural fibroplate. C: Pleural biopsy showing fibrinous exudative necrosis and abscess formation.**

ema [7]. All reported cases of Trichomonas-associated empyema have involved T. tenax infection [1,5,6]. T. tenax is typically found in the oral cavity and is associated with poor dental hygiene and oral health. Pulmonary Trichomonas infections usually result from the inhalation of T. tenax [5]. One study [8] suggested a significant association between T. tenax and bacterial infections, high pneumonia severity index (PSI) scores, nasogastric tube feeding, and pulmonary complications. Logistic regression analysis indicated strong correlations between T. tenax and these clinical factors in pneumonia patients. Research by Zih-Bin Hong et al. showed that T. tenax could trigger cytotoxicity in gingival cells, disrupt cell junctions, and induce the production of IL-6 (interleukin-6) in gingival and lung cell lines [7].

A limitation of this study is that the patient did not exhibit symptoms of urethritis, with negative urinalysis and urine cultures, and no abnormalities were found during the examination of the reproductive system. Furthermore, T. vaginalis was not detected in the pulmonary lavage fluid mNGS, leaving the source of T. vaginalis in the pleural fluid unclear. The exact route of entry into the pleural cavity remains undetermined.

Although Trichomonas-related empyema is exceedingly rare, it should be considered in high-risk patients, such as those with chronic lung disease, immunosuppression, or poor hygiene. When Trichomonas is detected in pleural fluid, it should not be dismissed as contamination but thoroughly investigated.

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**Declaration of Generative AI in Scientific Writing:**
In the preparation of this paper, artificial intelligence was solely utilized for grammatical error corrections, with no involvement in content creation, data analysis, or idea development.

**Declaration of Interest:**
The authors declare that they have no conflicts of interest.

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