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Research article

Prospective observational study of *Trichomonas tenax* infection in patients with pneumonia

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ABSTRACT

Trichomonas tenax, an oral commensal parasite commonly found in the human mouth, is associated with periodontitis and poor oral hygiene. However, it has also been identified in the bronchoalveolar lavage fluid (BALF) of individuals with lung diseases. Notably, significant quantities of *T. tenax* have been isolated following bronchoscopy in cases of empyema and acute respiratory distress syndrome (ARDS). Furthermore, research has demonstrated its ability to induce inflammation in pulmonary epithelial cells. To comprehend the potential role of *T. tenax* in pneumonia, it is crucial to elucidate the relationship between the parasite and the disease. We investigated the clinical factors associated with *T. tenax* infection in patients with pneumonia. Employing nested polymerase chain reactions, we amplified nucleic acids from BALF and analyzed the relationships between *T. tenax* and various clinical factors. Our data revealed a significant association between *T. tenax* and bacterial infections, high pneumonia severity index (PSI) scores, nasogastric tube feeding, and pulmonary complications. Logistic regression analyses also showed strong associations between *T. tenax* and these clinical factors in pneumonia patients.

Abbreviations: ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; ICU, intensive care unit; PSI, pneumonia severity index.

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These findings suggest that *T. tenax* infection in pneumonia is accompanied by bacterial infection and severe clinical manifestations.

1. Introduction

Trichomonas tenax is an anaerobic single-celled parasite that primarily inhabits the oral cavity of humans, especially periodontal pockets around the teeth and gums. This oral commensal belongs to the genus *Trichomonas*, which can cause infections in humans and animals. The prevalence of *T. tenax* in the oral cavity ranges from 4% to 53 % worldwide [1]. Research has found *T. tenax* to be more strongly associated with periodontitis than either gingivitis or healthy gums [2,3]. Although it is most commonly found in the oral cavity, it has also been detected in the respiratory tract, where it can cause opportunistic lung infection and further trigger pulmonary trichomoniasis. This most often occurs in patients with pre-existing pulmonary diseases [4,5]. Patients with high levels of *T. tenax* in the bronchoalveolar lavage fluid (BALF) experience pyopneumothorax, respiratory failure, and pulmonary eosinophilia [6,7]. An *in vitro* study found that *T. tenax* induces pro-inflammatory cytokine secretion after co-incubation with A549 and NCI–H292 cells [8]. It can also adhere to mammalian cell and provoke host cell apoptosis [9]. Therefore, it has potentially significant effects, which are similar to *T. vaginalis*, leading to cellular damage. However, most studies of pulmonary trichomoniasis are case reports and there is a lack of clinical research on the relationships between *T. tenax* and pulmonary symptoms, complications, clinical markers, and other lung diseases or the role of these relationships in lung infections.

In patients suffering from chest complaints and chronic pulmonary diseases, the total prevalence of pulmonary trichomoniasis is 8 % [10]. Duboucher et al. found that *T. tenax* is most frequently detected in the late stages of ARDS [11]. *T. tenax* in the BALF of *Trichomonas*-infected ARDS patients has been correlated with higher mortality rates [11]. Studies have also identified the presence of *T. tenax* in patients with chronic lung diseases such as bronchiectasis and lung cancer [1,12]. Therefore, it is essential to expand our understanding of the relationship between *T. tenax* and clinical factors in pneumonia. Duboucher et al. have found *T. tenax* co-infection rates are 60 % among *Pneumocystis* patients with pneumonia [13,14]. Moreover, *T. tenax* coinfections have been found in cases of reflux esophagitis and empyema with pulmonary pathogens such as *Neisseria lactamica* and *Streptococcus constellatus* in the BALF [5, 15]. In the present study, we investigate the clinical and epidemiological characteristics associated with *T. tenax* infection in patients with pneumonia. We also aimed to identify the pathogens associated with *T. tenax* in pneumonia BALF samples.

2. Materials and methods

2.1. Ethics statement

This study was conducted in accordance with the tenets of the 2013 revision of the Declaration of Helsinki and was approved by the Chia-Yi Christian Hospital Institutional Review Board (approval no: CYCH-IRB2022070). Patients received appropriate explanations and gave written informed consent to participation. The samples were collected per the Institutional Review Board's instructions.

2.2. Patients and study design

In this prospective case-control study, 61 BALF samples were obtained from consecutive patients with varying degrees of pneumonia treated at Ditmanson Medical Foundation Chia-Yi Christian Hospital between August 11, 2022, and August 2, 2023. We aimed to investigate the association between clinicopathological characteristics and *T. tenax* in pneumonia. Our criteria for inclusion were that patients must be adults ($\geq$20 years); independently agree to a bronchoscopy examination; give written informed consent to study participation; receive rapid antigen test COVID-19 screening before their bronchoscopy; and have clinical or radiological evidence of pneumonia, traceable medical histories, and accessible laboratory data. We excluded patients younger than 20, those with immunosuppression (for example, due to acquired immunodeficiency syndrome or immunosuppressive medicine), and those who declined to participate. Clinical characteristics, radiological features, outcomes, and microorganism culture data were retrieved from patient records and confirmed at least 3 months after each patient's bronchoscopy. Pneumonia severity was determined by patient scores on the pneumonia severity index (PSI). This is the most rigorously studied assessment tool for adult community-acquired pneumonia [16]. All bronchoscopy procedures and BALF samples were performed and collected by a single physician (Y-T Lai) under standard sterile conditions to avoid possible sampling and performance biases.

2.3. BALF collection procedure

BALF specimens were acquired using fiberoptic bronchoscopy. This was performed via the nasal cavity to minimize oral contact and avoid possible contamination. Each patient received an instillation of normal saline, with a total instillation volume between 100 and 150 mL and retrieval volumes between 30 and 50 mL. The BALF was centrifuged at 400$\times g$ for 5 min. After centrifugation, the supernatant was decanted, and the pelletized sample was resuspended in 400 μl of phosphate-buffered saline for subsequent genomic deoxyribonucleic acid (DNA) extraction.

2.4. DNA extraction and nested polymerase chain reaction

To detect *T. tenax* by nested polymerase chain reaction (PCR) assay, we followed the procedure used in a previous study [17]. TRC1 and TRC2 primers were used to amplify the conserved region of 18 S ribosomal ribonucleic acid (rRNA) in trichomonads specifically [17]. Genomic DNA extraction from BALF sediment was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). To enhance signal strength, we increased the DNA volume to 2.0 μl in each round of PCR. Each round of nested PCR was carried out in a 20.0 μl reaction, comprising 2.0 μl of DNA template, 1.0 μl of 10 μM for each primer, 10.0 μl of Taq 2 × Master Mix Red (Ampliqon, Denmark), and ddH_2O. The PCR conditions included initial denaturation at 95 °C for 5 min, followed by 20 cycles of 30 s at 95 °C, 30 s at 53 °C, and 60 s at 72 °C, concluding with a final extension step at 72 °C for 7 min. Subsequently, second-round PCR was conducted to further amplify the 18 S rRNA gene. The reaction mixture contained 2.0 μl of DNA template derived from the product of the first-round PCR, 1.0 μl of 10 μM for each primer, 10.0 μl of Taq 2 × Master Mix Red (Ampliqon, Denmark), and ddH_2O. The conditions comprised an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 54 °C, and 60 s at 72 °C, with a final extension at 72 °C for 7 min.

2.5. Definition and microbiological evaluation

Pneumonia was diagnosed based on the presence of pulmonary infiltrates on chest radiography and symptoms and signs indicative of lower respiratory tract infection, including cough, chills, fever, sputum production, and dyspnea. All cases included in our study underwent chest computed tomography (CT) prior to bronchoscopy. High-resolution CT (HR-CT) is the gold standard for confirmation of bronchiectasis, which is characterized by irregular or untampered bronchial walls [18]. The CT images of our patients revealed the "tree-in-bud" pattern known as a manifestation of bronchiectasis [18]. The collected samples underwent thorough analyses, including evaluations for bacterial, viral, and tuberculosis infections. The bacteria species was defined as a concentration exceeding 10^4 CFU/mL in the BALF. To detect trichomonads, the product of the nested PCR was analyzed by 1.5 % agarose gel electrophoresis, and target bands at approximately 380 bp were isolated for subsequent sequencing using TRC2 primers. Sequence results were aligned using Genebank BLAST (Basic Local Alignment Search Tool) tools to identify the trichomonad species.

2.6. Statistical analysis

Categorical variables were described as count (%) Nonnormally distributed continuous variables were described as median (first quartile; third quartile) and normally distributed continuous variables as mean (standard deviation). Categorical variables were assessed using χ^2 or Fisher exact tests. Continuous variables were analyzed using either the student t-test or the nonparametric Mann-Whitney test, depending on the data distribution. Multivariate logistic regression analyses were conducted to identify the factors associated with a *T. tenax*-related pneumonia diagnosis. *P-values* <0.05 (two-tailed) were considered as statistically significant. All statistical analyses were performed using SAS, v. 9.4 (SAS Institute Inc, 2013, Cary, NC, USA) software.

3. Results

A cohort of 61 patients with pneumonia who underwent pulmonary bronchoscopy at our institution between August 11, 2022, and August 2, 2023, was enrolled in this study. Of these, 51 (83.6 %) were male and 10 (16.4 %) were female. The mean age of the patients

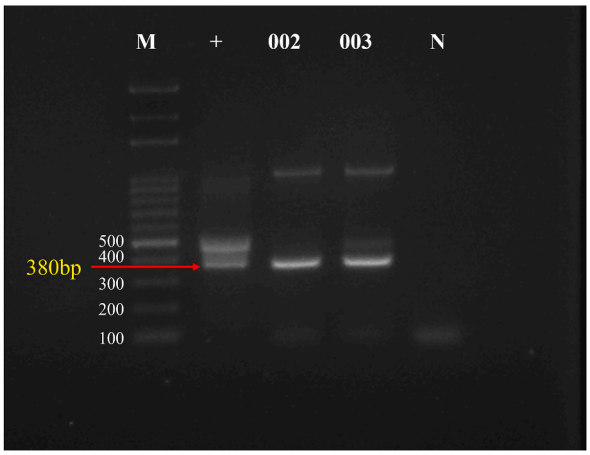

Fig. 1. Detection of *Trichomonas tenax* in bronchoalveolar lavage fluid (BALF) using agarose gel electrophoresis. The positive *T. tenax* results from BALF specimens 2 and 3 collected at Ditmanson Medical Foundation Chia-Yi Christian Hospital.
M (marker): DNA 100-bp molecular size markers; + (positive control): DNA of *Trichomonas tenax* strain Hs-4:NIH (ATCC_30207); N (negative control): ddH_2O. The arrow indicates the expected target product (380-bp).

is 69.8 ± 14.84 years. The results showed that the specific region of 18 S rRNA can be amplified through nested PCR (Fig. 1). A target band (380 base pairs) was sequenced to identify the trichomonad species in each infected patient. The incidence of positive *T. tenax* cases among our sample of patients with pneumonia was 34.4 % (21 of 61 cases) (Table 1). We utilized χ^2 or Fisher exact tests to analyze categorical variables. For continuous variables, we employed either the student t-test or the nonparametric Mann-Whitney test. Pathogenic bacteria were identified in the BALF samples. Given that *T. tenax* often co-exists with pathogenic bacteria, we investigated the association between *T. tenax* and pneumonia pathogens. Among the patients in our sample, 62.3 % (38 of 61) were infected with pathogenic bacteria. Notably, the patients who tested positive for *T. tenax* exhibited a significantly higher incidence of bacterial infection (47.5 % vs 90.5 %, $p < 0.001$).

We investigated the factors and comorbidities associated with the presence of *T. tenax* in pneumonia (Table 1). In individuals with pneumonia, there were no significant correlations between *T. tenax* infection and hospitalization, length of hospital stay, respirator use, intensive care unit (ICU) admission, and 30-day mortality. However, those with *T. tenax* exhibited significantly higher PSI scores (106.4 vs 132.4, $p = 0.044$) than those without. Moreover, patients infected with *T. tenax* experienced a significantly higher incidence of lung complications, such as ARDS, empyema, respiratory failure, sepsis, and effusion (53 % vs 81 %, $p = 0.029$). We found no significant association between *T. tenax* and either bronchiectasis or lung cancer. Lastly, the need for a nasogastric tube was also found to be significantly associated with *T. tenax* infection (33 % vs 71 %, $p = 0.004$).

To verify the variables significantly associated with *T. tenax* infection among patients with pneumonia, we conducted a multivariable analysis (Table 2). Before adjusting for sex and age, our analysis revealed strong associations with *T. tenax* and bacterial infection ($p = 0.004$), PSI risk class V ($p = 0.028$), nasogastric tube feeding ($p = 0.005$), and lung complications ($p = 0.035$). After these adjustments, high PSI scores ($p = 0.007$) and low serum albumin levels (<3.8 g/dL) ($p = 0.031$) were found to be closely correlated with *T. tenax*.

The prevalence of *T. tenax* among patients with bacterial infections was 50 % (19 of 38); whereas, among patients without bacterial infections, it was 8.7 % (2 of 23). In light of this finding, we explored the relationship between bacteria species and *T. tenax*. A total of

Table 1
Clinical and epidemiological characteristics of pneumonia in *Trichomonas Tenax* and Non *Trichomonas Tenax* cases.

Variable	Total	Non *Trichomonas tenax*	*Trichomonas tenax*	*P*-value
	N = 61	n = 40	n = 21	
Age	69.77 ± 14.96	70.68 ± 16.19	68.05 ± 12.46	0.519
<65	22(36.07)	13(32.50)	9(42.86)	0.424
≥65	39(63.93)	27(67.50)	12(57.14)	–
Sex				
Female	10(16.39)	7(17.50)	3(14.29)	>0.999
Male	51(83.61)	33(82.50)	18(85.71)	–
Bacterial infection	38(62.30)	19(47.50)	19(90.48)	<0.001
COVID-19	7(11.48)	5(12.50)	2(9.52)	>0.999
Tuberculosis	3(4.92)	3(7.50)	0(0.00)	–
Pneumonia Severity Index	115.31 ± 48.14	106.40 ± 42.43	132.40 ± 54.55	0.044
PSI Risk Class I ~ V				
1-3	19(31.15)	16(40.00)	3(14.29)	0.071
4	20(32.79)	13(32.50)	7(33.33)	–
5	22(36.07)	11(27.50)	11(52.38)	–
Ever smoker	20(32.79)	10(25.00)	10(47.62)	0.074
WBC	11072.44 ± 5576.05	11215.70 ± 5942.10	10799.50 ± 4930.00	0.784
Normal (3500–9900/μL)	29(47.54)	20(50.00)	9(42.86)	0.474
Abnormal (<3500 or >9900/μL)	30(49.18)	19(47.50)	12(57.14)	–
C-reactive proteins (0–0.3 mg/dL)[a]	10.04 ± 8.42	9.01 ± 7.92	11.99 ± 9.19	0.215
Serum albumin (3.8–5.3 g/dL)[b]	3.15 ± 0.68	3.30 ± 0.67	2.89 ± 063	0.074
Antibiotics or corticosteroid treatment (within one month)	37(60.66)	24(60.00)	13(61.90)	0.885
Hospitalization	53(86.89)	35(87.50)	18(85.71)	>0.999
Length of hospital stay	21.79(14.37)	21.57(14.74)	22.22(14.02)	0.790
respirator use	14(22.95)	11(27.50)	3(14.29)	0.342
ICU admission	15(24.59)	12(30.00)	3(14.29)	0.176
30-day mortality	9(14.75)	7(17.50)	2(9.52)	0.479
Hyperlipidemia	8(13.11)	3(7.50)	5(23.81)	0.110
High uric acid	6(9.84)	4(10.00)	2(9.52)	>0.999
Lung surgery history	1(1.64)	0(0.00)	1(4.76)	–
Bronchiectasis	19(31.15)	10(25)	9(42.86)	0.153
Lung cancer	24(39.34)	13(32.50)	11(52.38)	0.131
Electrotherapy(within six months)	12(19.67)	5(12.50)	7(33.33)	0.088
Chemotherapy(within six months)	12(19.67)	6(15.00)	6(28.57)	0.309
Nasogastric tube feeding	28(45.90)	13(32.50)	15(71.43)	0.004
Pulmonary complications[c]	38(62.30)	21(52.50)	17(80.95)	0.029

[a] Missing C-reactive protein data = 6.
[b] Missing serum albumin data = 23.
[c] Pulmonary complications: ARDS, empyema, respiratory failure, sepsis, effusionCI, confidence interval; COVID-19, coronavirus disease 2019; ICU, intensive care unit; PSI, pneumonia severity index; WBC, white blood count.

Table 2
Multivariate logistic regression analyses of factors associated with *Trichomonas tenax* in pneumonia.

Variable	Univariate			Multivariate		
	OR	95 % CI	*P*-value	OR	95 % CI	*P*-value
Bacterial infection	10.50	2.16–51.16	0.004	21.99	2.47–195.89	0.006
COVID-19	0.74	0.13–4.17	0.730	0.81	0.14–4.68	0.809
Tuberculosis	<0.001	<0.001->999.999	0.978	<0.001	<0.001->999.999	0.976
Pneumonia Severity Index	1.01	1.00–1.02	0.052	1.02	1.01–1.04	0.007
PSI risk class I ~ V						
1–3	–	–	–	–	–	–
4	2.87	0.62–13.37	0.179	5.80	0.95–35.52	0.057
5	5.33	1.20–23.65	0.028	19.01	2.39–151.46	0.005
Ever smoked	2.73	0.89–8.33	0.078	2.88	0.87–9.50	0.083
WBC						
Normal (3500–9900/μL)	–	–	–	–	–	–
Abnormal (<3500 or >9900/μL)	1.47	0.51–4.27	0.475	1.47	0.51–4.29	0.478
C-reactive proteins (0–0.3 mg/dL)[a]	1.04	0.98–1.12	0.212	1.04	0.97–1.12	0.242
Serum albumin (3.8–5.3 g/dL)[b]	0.36	0.12–1.14	0.082	0.23	0.06–0.88	0.031
Antibiotics or corticosteroid treatment (within one month)	1.08	0.37–3.20	0.885	1.39	0.41–4.76	0.598
Hospitalization	0.86	0.18–4.00	0.845	1.28	0.18–9.38	0.806
Length of hospital stay	1.01	0.97–1.04	0.786	1.02	0.97–1.06	0.480
Respirator use	0.44	0.11–1.79	0.252	0.47	0.10–2.21	0.341
ICU admission	0.39	0.10–1.57	0.185	0.41	0.09–1.82	0.242
30-day mortality	0.50	0.09–2.64	0.411	0.52	0.08–3.32	0.489
Hyperlipidemia	3.85	0.82–18.10	0.087	4.22	0.87–20.49	0.074
High uric acid	0.95	0.16–5.65	0.953	0.87	0.14–5.33	0.877
Lung surgery history	>999.999	<0.001->999.999	0.986	>999.999	<0.001->999.999	0.986
Bronchiectasis	2.25	0.73–6.91	0.157	2.35	0.74–7.48	0.148
Lung cancer	2.28	0.77–6.74	0.135	2.21	0.74–6.58	0.154
Electrotherapy (within six months)	3.50	0.95–12.90	0.060	3.37	0.89–12.85	0.075
Chemotherapy (within six months)	2.27	0.63–8.19	0.212	2.14	0.58–7.89	0.251
Nasogastric tube feeding	5.19	1.64–16.48	0.005	7.40	1.99–27.57	0.003
Pulmonary complications[c]	3.85	1.10–13.47	0.035	5.65	1.38–23.14	0.016

[a] Missing C-reactive protein data = 6.
[b] Missing serum albumin data = 23.
[c] Pulmonary complications: ARDS, empyema, respiratory failure, sepsis, effusionCI, confidence interval; COVID-19, coronavirus disease 2019; ICU, intensive care unit; PSI, pneumonia severity index; WBC, white blood count.

38 bacterial infection samples were included, evenly split between those testing positive for *T. tenax* and those testing negative. Some patients were found to have more than one type of bacteria. Although no significant correlations were found between the presence of *T. tenax* and any individual species of bacteria, the data revealed that the incidence of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Corynebacterium* spp. was higher in patients with *T. tenax* than those without it. Conversely, the incidence of *Streptococcus mitis/oralis* and *Acinetobacter baumannii* was higher in patients without *T. tenax* than those with it (Table 3).

Table 3
Etiology of 38 pneumonia cases with bacterial infections with and without *Trichomonas tenax*.

Bacterial species	*Trichomonas tenax* (+)	*Trichomonas tenax* (−)
	n = 19	n = 19
Pseudomonas aeruginosa	6 (31.6 %)	4 (21.1 %)
Klebsiella pneumoniae	6 (31.6 %)	4 (21.1 %)
Corynebacterium spp.	5 (26.3 %)	3 (15.8 %)
Prevotella spp.	3 (15.8 %)	1 (5.3 %)
Staphylococcus aureus	2 (10.5 %)	1 (5.3 %)
Streptococcus mitis/oralis	2 (10.5 %)	4 (21.1 %)
Stenotrophomonas maltophilia	2 (10.5 %)	2 (10.5 %)
Escherichia coli	2 (10.5 %)	1 (5.3 %)
Veillonella spp.	1 (5.3 %)	2 (10.5 %)
Neisseria spp.	1 (5.3 %)	1 (5.3 %)
Proteus mirabilis	1 (5.3 %)	0 (0)%
Staphylococcus haemolyticus	1 (5.3 %)	0 (0)%
Bacteroides pyogenes	1 (5.3 %)	0 (0)%
Providencia stuartii	1 (5.3 %)	0 (0)%
Acinetobacter baumannii	0 (0)%	3 (15.8 %)
Streptococcus salivarius/vestibularis	0 (0)%	1 (5.3 %)
Staphylococcus epidermidis	0 (0)%	1 (5.3 %)
Fusobacterium nucleatum	0 (0)%	1 (5.3 %)
Bacteroides fragilis	0 (0)%	1 (5.3 %)

4. Discussion

The main findings of this case-control study, conducted in Taiwan, were: (1) An incidence of *T. tenax* in pneumonia of 34.4 %; (2) Strong correlations between *T. tenax* in patients with pneumonia and bacterial infection, high PSI score, PSI risk class V, nasogastric tube feeding, and pulmonary complications. Our findings indicate that *T. tenax* is correlated with more severe clinical factors in pneumonia patients. These results suggest that, in pneumonia patients infected with *T. tenax,* clinicians should be vigilant for potential complications and bacterial infections.

Our study had three noteworthy limitations. First, the materials were collected from a tertiary hospital serving approximately 300,000 people. As such, the generalizability of our findings to other hospitals with different populations may be limited. Second, due to the one-year duration of the IRB approval, the sample size was restricted.

It should also be noted that the suitability of pneumonia patients for bronchoscopy is dependent on their overall health due to the more complicated specimen collection procedure compared to the sputum collection used in upper respiratory tract infections. Potential biases could also have arisen as a result of various confounding variables, such as the age and sex distribution in our sample. Nevertheless, logistic regression analyses revealed associations between *T. tenax* and bacterial infection, severe pneumonia, nasogastric tube feeding, and lung complications. Therefore, the risk of *T. tenax* appears to be higher in cases of bacterial pulmonary infection and more severe pneumonia. Given the limited sample size in this study, our investigation of the relationships between bacterial species and *T. tenax* was constrained. Further research with larger sample sizes is necessary to further explore the relationships between *T. tenax* and different pathogenic bacteria.

In this study, we have identified the prevalence of *T. tenax* in pneumonia and the clinical characteristics associated with *T. tenax* infection in pneumonia patients. Notably, the prevalence of *T. tenax* (34.4 %) in our study was considerably higher than that reported in a previous study of pneumonia patients (8.7 %) [17]. However, a study of patients with ARDS found a *T. tenax* rate of 30 %, which is similar to our findings [11].

Previous research suggests that *T. tenax* can elicit an inflammatory response of pulmonary epithelial cells [8]. The *T. tenax* parasite is capable of inducing the secretion of IL-6 in pulmonary cell lines, which may subsequently lead to downstream immune responses. Our findings demonstrate a correlation between *T. tenax* and clinical factors associated with more severe pneumonia. Thus, *T. tenax* may contribute to inflammation in pneumonia patients, leading to worsening of the pneumonia. Prior research has also shown that several proteins secreted by *T. vaginalis*, such as heat shock proteins and adhesin protein AP65, can induce the expression of IL-8 and COX-2 in vaginal epithelial cells [19]. Given that *T. tenax* belongs to the same genus as *T. vaginalis*, it is plausible that the proteins secreted by *T. tenax* may resemble those of *T. vaginalis*, potentially explaining its correlations with severe clinical factors.

Several previous case reports have isolated both anaerobic and/or aerobic bacteria in the BALF of patients with pleuropulmonary infections of *T. tenax* [1,5,20–22]. *P. aeruginosa* and *Klebsiella* spp. have both previously been isolated from the BALF of *T. tenax*-positive patients, which is consistent with the present findings [5]. In our study, the prevalence of *T. tenax* was significantly associated with bacterial infection in patients with pneumonia. However, determining the specific bacterial species linked to *T. tenax* was challenging due to the limited sample size. Therefore, the interaction between *T. tenax* and pulmonary pathogens requires further investigation.

A study similar to our own has shown a correlation between *T. tenax* and late-stage ARDS, at which point, there is immune cell infiltration and more severe outcomes [11]. They also observed that the density of *T. tenax* correlates with mortality rates, with higher densities increasing the mortality risk. Our findings align with such previous research as higher PSI scores and a PSI risk class of V were associated with the incidence of *T. tenax* in pneumonia. Additionally, we revealed a higher incidence of lung complications, such as ARDS and empyema, in pneumonia patients with *T. tenax* than those without, which was also in accordance with previous research [5,11]. Previous studies have shown that patients with severe PSI scores, risk class V, and hypoalbuminemia are frequently diagnosed with unstable vital signs, including hypoxemia [23,24]. We speculate that hypoxia might be the primary factor facilitating the development of *T. tenax* in pneumonia.

Our data indicated an association between *T. tenax* and pulmonary complications. As previously mentioned, several studies confirm that *T. tenax* is often detected in BALF samples alongside other pathogens such as *P. aeruginosa*. Among the *T. tenax*-positive patients in our sample, 90.5 % also had bacterial infections. In severe pulmonary infections, immune cell infiltration is commonly observed, potentially leading to conditions such as ARDS, respiratory failure, and empyema [25,26]. Therefore, we inferred that *T. tenax* may exacerbate bacterial pneumonia by triggering inflammation. However, the specific impact of *T. tenax* on pneumonia remains uncertain due to our limited sample size.

The use of a nasogastric feeding tube, which is used in patients who experience difficulty swallowing, has previously been identified as a risk factor for aspiration pneumonia [27–29]. Because of the dysfunction of esophageal sphincters and pharyngoglottal adduction reflex, such patients easily aspirate Gram-negative pathogens from pharyngeal secretions and develop aspiration pneumonia. Case reports have also identified *T. tenax* in pneumothorax patients with feeding tubes, supporting the association between *T. tenax* and nasogastric feeding tubes. Among patients with nasogastric tubes, dental diseases and poor oral hygiene are responsible for bacterial colonization of the oral flora and this may increase the likelihood of *T. tenax* migration from the oral cavity.

This study is the first to evaluate the relationship between *T. tenax* and clinical factors in pneumonia and to suggest the potential role of *T. tenax* in pulmonary infections. It is hoped that our findings will help to guide trichomonad risk assessment and therapy.

Ethics statement

In this prospective study, 61 BALF samples were prospectively obtained from consecutive patients treated for pneumonia at

Ditmanson Medical Foundation Chia-Yi Christian Hospital between August 11, 2022, and August 2, 2023. The study was conducted in accordance with the tenets of the 2013 revision of the Declaration of Helsinki and was approved by the Chia-Yi Christian Hospital Institutional Review Board (approval no: CYCH-IRB2022070). Patients received appropriate explanations and gave written informed consent to participation.

Data availability statement

Not applicable.

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CRediT authorship contribution statement

Zih-Bin Hong: Writing – original draft, Methodology, Investigation, Conceptualization. **Yu-Ting Lai:** Writing – original draft, Investigation, Conceptualization. **Chun-Hsien Chen:** Methodology. **Ching-Han Lai:** Formal analysis. **Yi-Jen Chen:** Writing – review & editing. **Chin-Wei Kuo:** Methodology. **Tzu-Yi Chan:** Methodology. **Pei-Chi Fang:** Methodology. **Chien-Chin Chen:** Writing – review & editing, Supervision, Formal analysis. **Wei-Chen Lin:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Wei-Chen Lin reports financial support was provided by National Science and Technology Council. Ching-Han Lai reports financial support was provided by National Cheng Kung University Hospital. Yu-Ting Lai reports financial support was provided by Ditmanson Medical Foundation Chiayi Christian Hospital Research Program. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33181.

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