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Original Article

Co-Infection of *Lophomonas blattarum* and *Pneumocystis jirovecii* in Patients with Respiratory Disorders in Northeastern Iran

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Abstract

Background: Respiratory infections caused by *Lophomonas blattarum* and *Pneumocystis jirovecii* are significant threats, especially to immunocompromised patients. Both pathogens are associated with severe pneumonia and are often underdiagnosed due to the challenges in identifying them accurately, particularly in co-infections. We aimed to evaluate the prevalence and clinical impact of co-infections with *L. blattarum* and *P. jirovecii* in patients with respiratory symptoms.

Methods: This cross-sectional study involved 111 patients admitted to the Pulmonary Ward of Imam Reza Hospital in Mashhad, Iran in 2023. Bronchoalveolar lavage (BAL) samples were collected from all patients and analyzed microscopically and molecularly. PCR amplification targeting *L. blattarum* and *P. jirovecii* was performed, with subsequent sequencing for molecular identification. The presence of *Pneumocystis* was identified using a 346-bp PCR band.

Results: Of the 111 patients, *Lophomonas* was detected in 48 patients (43.2%), and *Pneumocystis* in 47 patients (42.3%). Co-infections were identified in 26 patients (23.6%). Both infections were more common in males, though the difference between genders was not statistically significant. The highest prevalence was observed in patients over 60 years, with 18% and 19.8% infection rates for *Lophomonas* and *Pneumocystis*, respectively. Co-infection rates were significantly higher in older patients and in males (P= 0.028).

Conclusion: The study demonstrates a significant prevalence of co-infections with *L. blattarum* and *P. jirovecii* in patients with respiratory conditions, particularly in the elderly. The findings underscore the need for comprehensive diagnostic strategies, including molecular and microscopic approaches, to accurately diagnose and manage these co-infections in high-risk populations.

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Introduction

Respiratory infections are a significant cause of morbidity and mortality, particularly in immunocompromised patients or those with pre-existing pulmonary conditions (1). Among the myriad of pathogens responsible for these infections, *Lophomonas* and *P. jirovecii* are of particular concern due to their capacity to cause severe pneumonia, especially in patients with weakened immune systems (1,2).

Lophomonas blattarum, a protozoan traditionally associated with respiratory infections in immunocompromised individuals, has recently gained attention for its role in co-infections alongside other opportunistic pathogens (1). While initially considered a rare or incidental finding, emerging studies suggest that *Lophomonas* infections may be underdiagnosed due to the challenges in accurately identifying the organism under microscopy (2). The presence of *Lophomonas* in respiratory specimens has been increasingly reported in patients with chronic respiratory disorders, raising concerns about its pathogenic potential and the need for heightened clinical awareness (3).

On the other hand, *P. jirovecii* is a well-known opportunistic pathogen, particularly in patients with HIV/AIDS, cancer, or those undergoing immunosuppressive therapies (4). *Pneumocystis* pneumonia (PCP) remains a major cause of respiratory failure in these populations, with mortality rates ranging from 10% to 20% even with treatment (5). The diagnosis of PCP often relies on a combination of clinical, radiological, and microbiological findings, with molecular techniques offering increased sensitivity (6). However, the coexistence of PCP with other respiratory pathogens complicates diagnosis and treatment, leading to poorer outcomes (7)

Co-infections with *Lophomonas* and *P. jirovecii* present a unique challenge to clinicians, particularly in regions where both pathogens are prevalent but under-recognized (8). The overlapping clinical presentations of these infections, including symptoms like fever, cough, and dyspnea, complicate the diagnostic process, often leading to delays in appropriate treatment. Studies have highlighted the importance of considering co-infections in patients with severe respiratory symptoms, particularly in high-risk groups (9,10).

We aimed to investigate the co-infection of *L. blattarum* and *P. jirovecii* in patients admitted with respiratory disorders to the pulmonary department of Imam Reza Hospital in Mashhad, Iran during 2023. By identifying the prevalence and clinical impact of these co-infections, we hope to provide insights into their role in exacerbating respiratory illnesses and to underscore the need for comprehensive diagnostic approaches in managing complex cases of pneumonia.

Methods

Ethical Considerations

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. It is important to note that the sampling was not carried out specifically for research purposes, but rather as part of the routine diagnostic procedures used in the clinical care and treatment of patients. All patient data were anonymized and coded to ensure confidentiality, and no additional interventions were performed outside the standard diagnostic process. The study protocol was reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences under approval code IR.MUMS.MEDICAL.REC.1399.316, in full compliance with institutional and national ethical guidelines for research involving human subjects.

Study Design

This is a cross-sectional study conducted on 111 patients with respiratory symptoms who

were admitted to the pulmonary ward of Imam Reza Hospital in Mashhad, Iran. The study aimed to identify the co-infection rates of *L. blattarum* and *P. jirovecii* using molecular techniques. Bronchoalveolar lavage (BAL) samples were collected from 111 patients exhibiting respiratory symptoms. These samples were sent to the parasitology laboratory for further examination. The BAL samples were initially centrifuged and directly examined for the presence of *Lophomonas* under a microscope for microscopic Examination.

Patients were selected based on specific criteria to ensure a focus on high-risk individuals susceptible to pulmonary infections. Inclusion criteria consisted of patients with persistent respiratory symptoms who were admitted to the pulmonary ward, particularly those with immunosuppressive conditions such as HIV/AIDS, HTLV-1 infection, diabetes, autoimmune diseases, organ transplantation, chemotherapy, or long-term corticosteroid use. Additionally, patients with chronic lung diseases, including chronic obstructive pulmonary disease (COPD), interstitial lung disease, and bronchiectasis, as well as those with severe or unresolved respiratory infections requiring BAL for diagnostic purposes**,** were included.

Patients were excluded if they had a confirmed alternative diagnosis unrelated to *L. blattarum* or *P. jirovecii*, provided insufficient or degraded BAL samples, or had incomplete medical records. Patients who declined participation were also excluded. These criteria were established to enhance the reliability of the findings and to target populations at the highest risk for these infections.

PCR for Detection of Lophomonas blattarum and Sequencing

Respiratory samples were processed by digesting with proteinase K at 56 °C to extract genomic DNA using the Mini QIAamp DNA kit (Qiagen, Basel, Switzerland) according to the manufacturer's instructions. The extracted DNA was stored at −20 °C until further PCR amplification. As a positive control for the extraction process, amplification of human β-globin was performed using the primers PCO4 (5'-TCACCGCAACTTCATCCACGTTCACC-3') and GH20 (5'-GAAGAGCCAAGGACAGGTAC-3'). Following a similar approach to the study conducted by Fakhar et al , a PCR assay was employed to detect *L. blattarum* using specific forward (F) and reverse (R) primers (11). The forward primer sequence was 5'-GAGAAGGCGCCTGAGAGAT-3', and the reverse primer sequence was 5'-ATGGGAGCAAACTCGCAGA-3'. The PCR reaction was prepared in a total volume of 25 µL, which included 12.5 µL of Master Mix (Fermentas, Inc.), 1 µL of each primer, 5 µL of extracted DNA, and 5.5 µL of distilled water. The PCR cycling conditions involved an initial denaturation step at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 57 °C for 1 minute, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 3 minutes. The amplified PCR products were separated by electrophoresis on a 1.5% agarose gel in TBE buffer to verify the presence of *Lophomonas* DNA. Subsequently, the PCR products were sent to Pishgam Company (Iran) for sequencing to confirm the identity of the 214 bp amplified bands as *Lophomonas*.

PCR Amplification for Detection of Pneumocystis jirovecii detection

Molecular identification of *P. jirovecii* was carried out by PCR targeting the mtLSU region using specific primers pAZ102-E (5'-GATGGCTGTTTCCAAGCCCA-3') and pAZ102-H (5'-GTGTACGTTGCAAAGTACTC-3'). The PCR products were subsequently analyzed through 1.5% agarose gel electrophoresis (Parstous, Mashhad, Iran) stained with Green viewer® Nucleic Acid Gel Stain (Parstous,

Mashhad, Iran) for visualization of a 370 bp band indicative of the presence of *P. jirovecii* in the sample.

To ensure the reliability of our PCR assays, we used positive and negative controls in all experiments. For *L. blattarum*, a positive control was obtained from previously confirmed clinical samples. For *P. jirovecii*, a validated positive control sample was used. Negative controls (no-template controls) were also included in each PCR run to rule out contamination.

Data Analysis

The data was analyzed using SPSS software (version 25) (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the data, including patient demographics. Inferential statistics, including independent *t*-tests, were used to compare continuous variables between groups, while the Chi-square test was applied for categorical variables. A significance level of 0.05 was considered for all statistical tests.

Results

Overall, 111 patients were included in the study, with 65 males (58.6%) and 46 females (41.4%). The mean age of the patients was 55.27 years (SD = 20.77), with ages ranging from 2 to 88 years.

Although the PCR successfully produced a band of approximately 214 bp for *L. blattarum*, suggesting correct amplification, the subsequent sequencing results were inconclusive, with sequences not aligning with *Lophomonas* in existing genetic databases (Fig. 1A). As a result, molecular identification could not be fully confirmed, so 48 positive direct microscopic examinations were relied upon for definitive detection of *L. blattarum* in our samples. Additionally, specific primers for *Pneumocystis* were used, resulting in a 370-bp band for 47 samples (Fig.1 B).

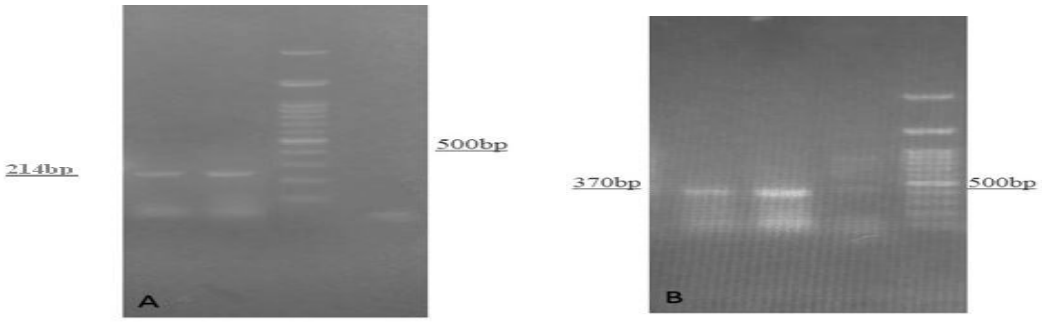

Fig. 1: PCR Amplification Results for *Lophomonas blattarum* and *Pneumocystis jirovecii*
A: PCR bands corresponding to *Lophomonas blattarum*-positive samples alongside the DNA marker and negative control.
B: PCR bands corresponding to *Pneumocystis jirovecii*-positive samples alongside the DNA marker and negative control

Lophomonas infection was detected in 48 patients (43.2%), with a higher prevalence in males (27%) compared to females (16.2%), though this difference was not statistically significant. Similarly, *Pneumocystis* infection was identified in 47 patients (42.3%), more frequent in males (27%) than females (15.3%), but this difference was also not statistically significant. Co-infection of *L.blattarum* and *P. jirovecii* was found in 26 patients (23.6%), showing a significant association between the two infections (P= 0.028). Both infections had the highest prevalence in patients over 60 years, with *Lophomonas* infection at 18% and

Pneumocystis infection at 19.8%. Co-infection rates were notably higher in patients over 60 years and in males.

The distribution of infections among patients with underlying diseases showed that 44.4% of COPD patients were positive for *L. blattarum* and *P. jirovecii*, with a co-infection rate of 37.0%. Among cancer patients, 46.3% tested positive for *Lophomonas* and *Pneumocystis*, while 17.1% had co-infection. In autoimmune disease patients, 42.3% were positive for *Lophomonas* and *Pneumocystis*, with a co-infection rate of 26.9%. Among transplant recipients, 37.5% had *Lophomonas* and *Pneumocystis*, and 25.0% had co-infection. No infections were detected in HIV or HTLV-1 patients. In diabetic patients, 33.3% tested positive for *Lophomonas* and *Pneumocystis*, with no cases of co-infection. The P-values indicate that none of these associations were statistically significant (Table 1).

Table 1: Prevalence of *Lophomonas blattarum*, *Pneumocystis jirovecii* Infections, and Co-infection in Patients Based on Gender, Age, and Underlying Diseases (n=111)

Variable	Total Patients n=111 (%)	Lophomonas Positive n=48(%)	*P*-value	Pneumocystis Positive n=47(%)	*P*-value	Co-infection n=26(%)	*P*-value
Gender							
Male	65 (58.6)	30 (27)	0.462	30 (27)	0.334	19 (17.1)	0.086
Female	46 (41.4)	18 (16.2)		17 (15.3)		7 (6.3)	
Age Group							
0-10	7 (6.3)	7 (6.3)	0.020	5 (4.5)	0.034	0	0.041
20-30	6 (5.4)	2 (1.8)		1 (0.9)		0	
30-40	12 (10.8)	7 (6.3)		6 (5.4)		2 (7.7)	
40-50	10 (9)	5 (4.5)		4 (3.6)		3 (11.5)	
50-60	21 (18.9)	7 (6.3)		9 (8.1)		6 (23.1)	
60+	55 (49.5)	20 (18)		22 (19.8)		15 (57.7)	
Underlying Disease							
COPD	27 (24.3)	12 (44.4)	0.590	12 (44.4)	0.847	10 (37.0)	0.354
Cancer	41 (36.9)	19 (46.3)		19 (46.3)		7 (17.1)	
Autoimmune diseases	26 (23.4)	11 (42.3)		11 (42.3)		7 (26.9)	
HIV	2 (1.8)	0 (0.0)		0 (0.0)		0 (0.0)	
Transplant recipients	8 (7.2)	3 (37.5)		3 (37.5)		2 (25.0)	
HTLV-1 infection	1 (0.9)	0 (0.0)		0 (0.0)		0 (0.0)	
Diabetes	6 (5.4)	2 (33.3)		2 (33.3)		0 (0.0)	

Discussion

The co-infection of *L. blattarum* and *P. jirovecii* is a growing concern in patients with compromised immune systems or chronic respiratory conditions. This study conducted on 111 patients at Imam Reza Hospital contributes valuable data to the limited literature on the subject, particularly from regions where such co-infections are understudied.

Several studies have underscored the increasing recognition of *Lophomonas* as an opportunistic pathogen, especially in patients with chronic lung diseases. For instance, Tajik Jalayeri et al highlighted that the protozoan may be underdiagnosed due to the difficulty in distinguishing it from other respiratory pathogens, particularly under microscopy (12). Similarly, Ghaisari et al pointed out that *Lophomonas* infections, though considered rare, are being reported with increasing frequency, especially in patients with immunosuppression (13).

In our study, *Lophomonas* was detected in 43.2% of patients, aligning with recent findings that suggest a higher prevalence of this organism than previously thought. Moreover, *P. jirovecii* infection, observed in 42.3% of patients, is consistent with the findings of Roux et al , who reported *Pneumocystis* as a leading cause of respiratory infections in immunocompromised individuals (14).

The co-infection rate of 23.6% in our study reflects the synergistic relationship between *Lophomonas* and *Pneumocystis*, which has been similarly noted by Thakkar et al in a case of interstitial lung disease complicated by dual infection (8). The combination of these pathogens likely exacerbates the clinical picture, contributing to more severe respiratory symptoms and complicating both diagnosis and treatment.

The findings of this study suggest that clinicians should maintain a high index of suspicion for co-infections in patients with severe respiratory conditions, particularly in endemic regions. The overlapping clinical symptoms of *Lophomonas* and *Pneumocystis* including fever, cough, and dyspnea necessitate comprehensive diagnostic approaches that include molecular methods for accurate identification. Brown et al emphasized the role of PCR in detecting *P. jirovecii*, especially in cases where traditional diagnostic methods fall short (15).

The study highlights the challenges in detecting *L. blattarum* using molecular techniques. While specific primers were employed, sequencing results did not confirm the presence of *Lophomonas*, making direct microscopy the primary method for identification. This issue aligns with concerns raised by Mevara et al, who reported that PCR primers for *Lophomonas* detection may lack specificity, potentially amplifying other protozoa such as *Pentatrichomonas hominis* and *Tetratrichomonas spp.*. These findings suggest that current PCR methods may not reliably distinguish *Lophomonas* from morphologically similar organisms, emphasizing the need for more specific primers and refined molecular techniques to enhance diagnostic accuracy (16). To address this limitation, future studies should consider using optimized primer sets, nested PCR, or qPCR to improve molecular detection. Additionally, complementary approaches such as immunostaining and in situ hybridization may further enhance specificity and provide more reliable identification of *Lophomonas*.

Our study also revealed significant associations between age and infection rates, with the highest prevalence in patients over 60 years. This finding is consistent with the well-documented vulnerability of older adults to respiratory infections due to immune senescence and the higher likelihood of chronic comorbidities (17).

Patients with underlying diseases, particularly those with compromised immune systems or chronic pulmonary conditions, exhibited notable infection rates with *L. blattarum* and *P. jirovecii*. In this study, COPD, cancer, and autoimmune diseases were the most prevalent comorbidities among infected patients. COPD patients had the highest co-infection rate, which may be attributed to structural lung damage, impaired mucociliary clearance, and chronic inflammation, creating a favorable environment for pathogen colonization (18,19). Similarly, immunosuppressive conditions such as cancer and autoimmune diseases were associated with a higher prevalence of both infections, likely due to weakened immune responses or immunosuppressive therapies (20). Although transplant recipients and

diabetic patients also showed notable infection rates, no significant correlation was found between these conditions and co-infection (21). The absence of infection in HIV and HTLV-1 patients may be due to the small sample size, limiting interpretation. These findings highlight the importance of considering underlying conditions in diagnosing and managing *Lophomonas* and *Pneumocystis* infections, emphasizing the need for targeted screening and preventive measures in high-risk populations.

The possible synergistic relationship between *L. blattarum* and *P. jirovecii* may be explained through several mechanisms (1,22). *Lophomonas* infections could modulate local immune responses, increasing lung susceptibility to secondary infections such as *P. jirovecii*. Additionally, the disruption of pulmonary homeostasis caused by *Lophomonas* may alter lung microbiota and compromise epithelial integrity, creating a more favorable environment for *Pneumocystis* colonization and persistence (16). Furthermore, both pathogens trigger strong inflammatory responses, which can exacerbate lung tissue damage and facilitate co-infection (8). These interactions suggest a complex interplay between the two organisms, underscoring the need for further experimental models and immunological studies to clarify their impact on disease progression and patient outcomes.

Given the high co-infection rate observed, it is plausible that one pathogen may act as a facilitator for the other in immunocompromised individuals or those with chronic lung diseases. This potential synergistic relationship could contribute to more severe clinical outcomes, highlighting the importance of further investigation into the pathophysiological interactions between these two pathogens.

Conclusion

This study highlights the importance of considering co-infections with *L. blattarum* and *P. jirovecii* in patients with severe respiratory symptoms, particularly in high-risk groups such as the elderly and immunocompromised. The significant prevalence of co-infection underscores the need for comprehensive diagnostic strategies, including molecular testing, to ensure timely and effective treatment. Further research is needed to understand the mechanisms underlying the interaction between these pathogens and to develop targeted interventions that can improve patient outcomes.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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