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Legionella co-infection in HIV-associated pneumonia

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ABSTRACT

Due to poor diagnostics and increased co-infections, HIV-associated *Legionella* infections are underreported. We aimed to retrospectively determine the frequency of *Legionella* infections in bronchoalveolar lavage (BAL) from HIV-associated pneumonia patients hospitalized in Medellin, Colombia, between February 2007 and April 2014. Although culture was negative, 17 BAL (36%) were positive for *Legionella* by quantitative polymerase chain reaction, most of which were in the *Mycobacterium tuberculosis* or *Pneumocystis jirovecii* co-infected patients, and included *L. anisa* ($n = 6$), *L. bozemanae* ($n = 4$), *L. pneumophila* ($n = 3$), and *L. micdadei* ($n = 2$). All *L. bozemanae* and *L. micdadei* associated with *Pneumocystis*, while all *L. pneumophila* associated with *M. tuberculosis*. *Legionella* probable cases had more complications and higher mortality rates ($P = 0.02$) and were rarely administered empirical anti-*Legionella* therapy while in hospital. Clinicians should be aware of the possible presence of *Legionella* in HIV and *M. tuberculosis* or *P. jirovecii* co-infected patients.

1. Introduction

Pneumonia remains the most common cause of hospital admissions among human immunodeficiency virus (HIV)–infected patients, causing up to 90 cases/1000 person-years (Benito et al., 2012; Thompson et al., 2012; Van Gaalen et al., 2016). Despite successful combination antiretroviral therapy, bacterial pneumonia, the most frequent HIV-associated pulmonary infection, remains an important cause of morbidity and mortality among those with HIV.

Typically, HIV-associated pneumonias are due to *Pneumocystis jirovecii* or bacteria such as *Streptococcus pneumoniae, Haemophilus influenza, Staphylococcus aureus*, or *Mycobacterium tuberculosis*. However, in many cases, the infectious culprit is not identified, and patients are treated empirically (Benito et al., 2001, 2012). As such, alternative pneumonia-causing agents, such as *Legionella* spp., are estimated to be underreported (Benito et al., 2012; Murdoch, 2003).

Legionella spp. cause a severe and potentially fatal pneumonia infection that accounts for up to 10% of all bacterial pneumonias in the general population, with higher rates among immunosuppressed individuals (Basnayake and Waterer, 2015; Franzin et al., 2002; Head et al., 2017; Nara et al., 2004; Pedro-Botet et al., 2003; Rodero et al., 1995; Weiss et al., 2017). In fact, in a 2015 outbreak of *Legionella* in New York City, immunocompromised individuals had increased susceptibility to *Legionella*, highlighted by the fact that majority of the individuals who contracted *Legionella* had HIV, alcoholism, or diabetes (Weiss et al., 2017).

Diagnosing HIV-associated *Legionella* pneumonia requires a high degree of suspicion and is reliant on urinary antigen testing, culture, and nucleic acid amplification techniques (Sandkovsky et al., 2008). However, urinary antigen testing is only specific for *Legionella pneumophila* serogroup 1 (the serogroup responsible for majority of the *Legionella* infections in North America), making diagnosis of other serogroups or species problematic. Moreover, culture requires specialized media and can be lengthy, limiting its usefulness in a clinical setting (Head et al., 2017). Consequently, nucleic acid amplification techniques are increasingly becoming the preferential method for *Legionella* detection, albeit they are not yet readily available. The lack of available diagnostic tools coupled with the fact that HIV patients are often co-infected with multiple pathogens may perhaps be the reason for the misunderstanding

Abbreviations: BAL, bronchoalveolar lavage; BCYE, buffered charcoal yeast extract; GPT, glutamate pyruvate transaminase; HIV, human immunodeficiency virus; HRZE, isoniazid, rifampin, pyrazinamide, and ethambutol; IQR, interquartile range; PCR, polymerase chain reaction; PSI, pneumonia severity index; qPCR, real-time PCR; SD, standard deviation; SG1, serogroup 1; TMS, trimethhoprim/sulfamethoxazole.

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and underrepresentation of *Legionella* in HIV. Consequently, this study aimed to determine the frequency of *Legionella* infections in an HIV and pneumonia cohort.

2. Materials and methods

2.1. Ethics and consent

Approvals for this single-center study were received by the Ethics Committees from the University of Manitoba (Winnipeg, Canada) and the Universidad de Antioquia (Medellín, Colombia). Sample shipment was approved by the Ministerio de Salud y Protección Social de Colombia. Written informed consent was obtained from all study participants.

2.2. Study design, population, and samples

A retrospective cohort study was conducted on samples from HIV and pneumonia co-infected patients hospitalized at Hospital Universitario San Vicente Fundación in Medellín, Colombia, between February 2007 and April 2014. All patients with both HIV and pneumonia at hospital admission that underwent BAL collection during the study period were eligible for this analysis. Patients were excluded if they had: 1) any other underlying lung conditions or immunodeficiencies; 2) recently been admitted to the hospital; or 3) recent antibiotic use.

As per the in-hospital protocol, patient BAL samples underwent routine cultures (bacterial, mycobacterial, and fungal) and staining (Wright, Gram, Toluidine Blue O, and Ziehl–Neelsen). *P. jirovecii* diagnosis was confirmed in hospital using direct fluorescent antibody, Toluidine Blue O stain, and an in-house polymerase chain reaction (PCR) assay. Patient characteristics (past medical history, habits, symptoms, physical examination) as well as laboratory and microbiology results, and treatment were collected from hospital patient clinical charts. Aliquots of BAL were stored at −80 °C and shipped to Winnipeg, Canada, for the study analysis.

2.3. Microorganisms of interest

Legionella spp. of interest included *L. pneumophila, L. anisa*, *L. bozemanae*, and *L. micdadei*. An *L. pneumophila* serogroup 1 (SG1) ATCC 33153 strain, provided by the National Microbiology Laboratory (Winnipeg, Canada), and a clinical isolate of each *Legionella* spp. of interest, provided by the Health Science Centre (Winnipeg, Canada), served as positive controls.

2.4. DNA extraction and real-time PCR

Bacterial DNA was extracted from *Legionella* positive controls and from patient BAL samples using a QIAamp DNA Microbiome Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA was aliquoted and stored at −20 °C until required.

BAL DNA and positive control DNA were screened for *Legionella* spp. using a singleplex real-time PCR (qPCR) assay, optimized by Benitez and Cross et al (Table 1) (Benitez and Winchell, 2013; Cross et al., 2016). Primer and probes (Integrated DNA Technologies, Iowa, USA) were specific for PAN *Legionella* (a portion of the genome that has both conserved and variable regions allowing for the detection of all members of the *Legionella* family), *L. anisa, L. bozemanae, L. micdadei*, *L. pneumophila*, and *L. pneumophila* SG1. Each qPCR contained 12.5 μL of PrimeTime Gene Expression Mastermix (Integrated DNA Technologies, Iowa, USA), 5 μL of template, 100 nM of probe, and either 500 nM or 150 nM of each primer (*L. pneumophila* and *Legionella* nonpneumophila, respectively) with PCR Grade Water (ThermoFisher Scientific, Massachusetts, USA) to a final reaction volume of 25 μL. TE buffer served as a nontemplate control.

2.5. Culture

BAL samples that tested positive for *Legionella* by PCR were plated for culture. Briefly, 100 μL of sample was plated onto buffered charcoal yeast extract (BCYE) agar plates and incubated at 35 °C with 5% CO_2 for a maximum of 14 days.

2.6. Criteria for a confirmed vs probable legionella case

In accordance with the European Union and the Centers for Disease Control and Prevention case definitions (CDC, 2005; Jarraud et al., 2013), a *Legionella* case was considered probable if it was positive by PCR only and confirmed if it was positive by culture.

2.7. Data analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences statistical software version 25. Patient characteristics such as CD4 cell counts, viral load, and days since symptom onset are reported as the median plus interquartile range (IQR), while age is reported as the mean ± standard deviation. Categorical variables were analyzed using the Pearson's Chi-square tests. Continuous variables were compared using the Mann–Whitney U-Test. Differences between groups were considered to be statistically significant if $P \leq 0.05$.

Table 1
Primer and probe sequences for the detection of *Legionella* species.

Primers	Nucleotide sequence (5′ to 3′)	Probe	Nucleotide sequence (5′ to 3′)	Bacterium	Specificity/ sensitivity[a]	Size (bp)	Target gene	Cycling conditions
LPF	TTG TCT TAT AGC ATT GGT GCC G	LPP	56-FAM/CGG AAG CAA TGG CTA AAG GCA TGC A/3BHQ_1	*L. pneumophila*	100%/25 fg	115	mip	95 °C, 600 s; 45 × [95 °C, 15 s; 55 °C, 15 s; 66 °C, 10 s]
LPR	CCA ATT GAG CGC CAC TCA TAG							
LP-1F	TGC CTC TGG CTT TGC AGT TA	LP-1P	HEX-TTT ATT ACT CCA CTC CAG CGA T-MGBNFQ	*L. pneumophila* SG1	100%/25 fg	70	wzm	
LP-1R	CAC ACA GGC ACA GCA GAA ACA							
PanLegF	GTA CTA ATT GGC TGA TTG TCT TG	PanLegP	56-FAM/CGC TAT RGT C + G CCA GG+ AAA/3IABkFQ	*Legionella* spp.	100%/10 fg	200	23S–5S intergenic spacer region	95 °C, 300 s; 45 × [95 °C, – 15 s; 60 °C, 60 s]
		LBP	ROX-TAC GCC CAT TCA TCA TGC AAA CCA GTT T-BHQ_2	*L. bozemanae*	100%/10 fg			
		LAP	5HEX/CTC AAC CTA/ZEN/CGC AGA ACT ACT TGA GG/3IABkFQ	*L. anisa*	100%/50 fg			
PanLegR	TTC ACT TCT GAG TTC GAG ATG G	LMP	Cy5-AGC TGA TTG GTT AAT AGC CCA ATCGG-BHQ_2	*L. micdadei*	100%/10 fg			

F = forward; R = reverse; MGBNFQ = Minor Groove Binder nonfluorescent quencher; BHQ = Black Hole Quencher.
[a] From Benitez and Winchell, 2013; Cross et al., 2016.

Multivariate regression analyses were performed to identify variables associated with *Legionella* infection. Categorical variables were added directly to the model, while continuous variables were coded and then used for analysis. Results are expressed as relative risk (RR) and confidence intervals (CIs) with *P* values.

3. Results

3.1. In-hospital patient characteristics

HIV and pneumonia patient demographics and clinical characteristics are listed in Table 2. Of the 47 HIV and pneumonia-infected BAL samples available for this study, majority were from males (80.9%, $n = 38$), nonsmokers (59.4%, $n = 28$), with advanced immunosuppression (CD4 of 36.5 cells/μL, IQR 16–101 cells/μL), and a viral load of 2.16 $\times 10^5$ copies/mL (IQR 6.15 $\times 10^4$–6.34 $\times 10^5$ copies/mL). Pneumonia etiologies at the time of hospital admission were *M. tuberculosis* (40.4%, $n = 19$), *P. jirovecii* (31.9%, $n = 15$), or other (27.7%, $n = 13$). At admission, none of the included patients had been diagnosed with *Legionella* pneumonia.

3.2. Legionella detection

Culturing on BCYE resulted in nondetection of *Legionella* spp. in all of the clinical BAL samples. However, moderate to low *Legionella* DNA concentrations were detected in 17 of the 47 BAL samples using qPCR (Table S1) and were considered to be probable for *Legionella* (CDC, 2005; Jarraud et al., 2013). Of the 17 qPCR-positive samples, majority ($n = 16$) were from *M. tuberculosis*– or *P. jirovecii*–infected patient samples (see Table 3). Indeed, 10/19 *M. tuberculosis*– and 6/15 *P. jirovecii*–infected BAL were positive for PAN *Legionella*, while only 1/13 samples was positive from the "other" pneumonia group. Of the probable *Legionellaceae* cases, 35% were *L. anisa* ($n = 6$), 24% *L. bozemanae* ($n = 4$), 17% *L. pneumophila* ($n = 3$), 12% *L. micdadei* ($n = 2$), 41% were uncharacterized ($n = 7$), and none were *L. pneumophila* SG1. Interestingly, all *L. bozemanae* and *L. micdadei* infections were in *P. jirovecii*–infected patient samples, while all *L. pneumophila* infections associated with *M. tuberculosis*.

Table 2
Demographic characteristics of patients with HIV-associated pneumonia ($n = 47$).[a]

Mean age (years) ± SD	35.6 ± 8.8
Sex (*n*, %)	
Male	38 (80.9)
Pneumonia (*n*, %)	
Pneumocystis jirovecii	15 (31.9)
Mycobacterium tuberculosis	19 (40.4)
Other[a]	13 (27.7)
Symptoms (*n*, %)	
Cough	38 (80.9)
Fever	39 (83.0)
Dyspnea	18 (38.3)
X-ray (*n*, %)	
Normal	7 (14.9)
Abnormal	40 (85.1)
Smoker (*n*, %)	
Active	7 (15.1)
Previous	12 (25.5)
Non	28 (59.4)
CD4 cell count (median cells/μL, IQR)	36.5 (16–101)
HIV RNA viral load (median copies/mL, IQR)	215,500 (61,533–633,692)
Death (*n*, %)	15 (31.9)

IQR = interquartile range; SD = standard deviation.
[a] Virus, bacterial, *Mycobacterium* nontuberculosis, *Cryptococcus*, histoplasma, aspergillus.

3.3. Characteristics of Legionella co-infected patients

Detailed characteristics of the probable *Legionella* co-infected cases are listed in Table 4. Individuals with *Legionella* infection had a preponderance for longer hospital stays (29.6 ± 20.8 vs 21.7 ± 12.2 days) as well as significantly more intensive care unit admissions ($n = 9$ vs $n = 7$, $P = 0.04$) and higher mortality rates ($n = 9$ vs $n = 6$, $P = 0.02$) compared to *Legionella*-negative individuals. Surprisingly, only 1 of the *Legionella*-infected patients had received appropriate empirical antibiotic therapy with anti-*Legionella* activity (i.e., a fluoroquinolone or macrolide) while in hospital (see Table 3).

A regression was performed to ascertain the effects of *Legionella* infection, pneumonia co-infection, weight, and sex on the likelihood that participants would require ICU admission. In the adjusted multivariate model, *Legionella* infection associated with ICU admission even after adjusting for age and *M. tuberculosis* coinfection (RR 2.47, 95% CI 1.22–4.98, $P = 0.012$), indicating that *Legionella* and *M. tuberculosis* co-infected individuals were 2 times more likely to require ICU admission compared to *Legionella*-negative *M. tuberculosis*–infected individuals. Similar trends were also noted when *M. tuberculosis* was substituted for *P. jirovecii* infection.

4. Discussion

Our results demonstrate that *Legionella* spp. can frequently be found in the BAL of HIV and *M. tuberculosis* or *P. jirovecii* co-infected individuals. Moreover, we found that certain *Legionella* spp. seemed to associate with specific co-infecting partners (i.e., *L. pneumophila* with *M. tuberculosis* and *Legionella* nonpneumophila with *P. jirovecii*), a finding that has not yet been extensively studied thus far. Although the underlying mechanism behind these associations remains to be elucidated, a potential explanation may be found in the pathogenesis and virulence of these microbes. For instance, the immense destruction caused by *P. jirovecii* (and its corresponding immune response) may provide *Legionella* nonpneumophila spp. with access to growth factors and other host factors in the extracellular milieu which may otherwise be limited/absent during infection with other pathogens, like *M. tuberculosis* (Joshi and Swanson, 1999; Smith, 2003), a factor which may be less important for the more virulent *L. pneumophila* spp. and merits further investigation.

Contrary to expectations, this study did not find any *L. pneumophila* SG1 as a cause for the probable *Legionella* cases. This divergence from current literature may be due to the diagnostic methods themselves (i.e., using an unbiased nucleic acid amplification approach to detect PAN *Legionella*, whereas current diagnostics target *L. pneumophila* SG1) or to a difference in the global distribution of *Legionella*, which has previously been shown to vary (Gomez-Valero and Buchrieser, 2013; Qin et al., 2013; Yu et al., 2002). Regardless, studies looking at *Legionella* epidemiology in Colombia are warranted and may benefit from more unbiased or region-specific diagnostics.

Another important finding was that individuals with *Legionella* infection had a more severe clinical presentation than *Legionella*-negative individuals, illustrated by the increased need for intensive care admissions and higher mortality rates. Additionally, only 1 *Legionella*-probable case was administered appropriate anti-*Legionella* empirical antibiotic therapy, which may explain the increased complications and poor outcome of our patients. In accordance with the present results, previous studies have also demonstrated that *Legionella* pneumonia is more severe and has a worse evolution in patients with HIV (Franzin et al., 2002; Pedro-Botet et al., 2003; Sandkovsky et al., 2008). In contrast, a recent retrospective case–control study by Cillóniz et al (Cillóniz et al., 2018) found that *Legionella* pneumonia characteristics did not differ between HIV positive and negative individuals. However, as was highlighted in a letter by Head et al. (Head and Keynan, 2019), their study population consisted mainly of immune intact (high CD4 counts), virologically suppressed HIV patients who were

Table 3
Pneumonia risk class; treatment; and outcome for HIV and *Mycobacterium tuberculosis*, *Pneumocystis jirovecii*, or other (viral, fungal, or bacterial) co-infected patients with ($n = 17$) and without ($n = 30$) *Legionella* spp. infections.

	BAL	Sex	Age	Risk class[a]	Treatment	Outcome	*Legionella* coinfection
Mycobacterium tuberculosis	4	Male	49	II	TMS, Fluconazole, HR, Acyclovir	Alive	None detected
	6	Male	32	III	TMS, HRZE, Ceftriaxone, Moxifloxacin	Alive	None detected
	12	Male	42	III	HRZE	Alive	None detected
	14	Male	23	I	TMS, Fluconazole, HRZE	Alive	None detected
	30	Male	46	I	HRZE	Alive	None detected
	33	Male	30	II	Fluconazole, Amphotericin B	Alive	None detected
	50	Male	41	III	TMS, HRZE	Death	None detected
	55	Male	36	II	ND	Death	None detected
	56	Female	53	IV	TMS, Fluconazole, HRZE, Piperacillin/Tazobactam, Ceftriaxone	Alive	None detected
	2	Male	41	II	Fluconazole, Ethambutol, **Moxifloxacin**, Amoxicillin/Clavulanate	Death	***Legionella* spp.**
	20	Female	20	II	ND	Death	***Legionella* spp.**
	58	Male	31	I	Fluconazole, HRZE	Alive	***Legionella* spp.**
	61	Male	42	IV	HRZE, Acyclovir, Piperacillin/Tazobactam	Death	***Legionella* spp.**
	64	Female	32	II	TMS, HRZE, Doxycycline	Alive	***Legionella* spp.**
	54	Male	36	II	HRZE	Death	***L. pneumophila***
	13	Male	45	III	TMS, HR, Meropenem, Metronidazole	Alive	***L. pneumophila, L. anisa***
	19	Female	48	III	Fluconazole, Acyclovir, Ceftriaxone	Death	***L. pneumophila, L. anisa***
	5	Female	24	II	Fluconazole, HRZE	Death	***L. anisa***
	68	Male	32	II	Fluconazole, HRZE, Ganciclovir	Alive	***L. anisa***
Pneumocystis jirovecii	35	Male	42	II	TMS, Fluconazole, Ganciclovir	Alive	None detected
	39	Male	28	II	TMS, Fluconazole, Acyclovir, Doxycycline	Death	None detected
	40	Male	32	III	TMS, Fluconazole, Acyclovir, Voriconazole	Death	None detected
	43	Male	32	II	TMS, Fluconazole, Ganciclovir	Alive	None detected
	44	Male	32	II	TMS, Fluconazole, Acyclovir	Alive	None detected
	53	Male	46	V	TMS, Fluconazole, Acyclovir	Alive	None detected
	66	Male	32	I	TMS, Ceftriaxone, Clindamycin/Pyrimethamine	Alive	None detected
	67	Male	35	I	TMS	Alive	None detected
	70	Male	45	III	TMS, Fluconazole, Acyclovir	Alive	None detected
	49	Male	30	I	Fluconazole, Albendazole	Death	***Legionella* spp.**
	52	Male	51	IV	TMS, Vancomycin, Meropenem	Death	***L. bozemanae***
	57	Male	21	I	TMS, Fluconazole, Acyclovir	Alive	***L. bozemanae***
	71	Female	27	II	Fluconazole, Acyclovir	Death	***L. bozemanae***
	36	Female	30	II	TMS, Clarithromycin, Ceftriaxone	Alive	***L. anisa, L. micdadei***
	41	Male	25	II	TMS, Fluconazole, Acyclovir, Metronidazole, Clindamycin-Primaquine	Alive	***L. anisa, L. bozemanae, L. micdadei***
Other	9	Male	44	I	Piperacillin/Tazobactam, Metronidazole	Alive	None detected
	11	Female	36	II	Fluconazole, Clarithromycin, Ceftriaxone	Alive	None detected
	15	Male	22	I	TMS, Ceftriaxone	Alive	None detected
	21	Male	44	II	Amphotericin B, Ceftriaxone, Fluconazole	Death	None detected
	22	Male	24	I	Ceftriaxone	Alive	None detected
	24	Male	41	III	Fluconazole, Aztreonam, HRZE	Alive	None detected
	31	Male	24	II	TMS, HRZE	Alive	None detected
	34	Female	34	I	Amphotericin B, Piperacillin/Tazobactam	Alive	None detected
	47	Male	39	III	TMS, Fluconazole, HRZE, Amphotericin B	Death	None detected
	48	Male	29	I	Ceftriaxone	Alive	None detected
	63	Male	50	II	Piperacillin/Tazobactam, Ceftazidime, Amphotericin B, Fluconazole	Alive	None detected
	69	Male	37	II	TMS, Fluconazole, Acyclovir, Albendazole	Alive	None detected
	10	Male	42	I	TMS, Fluconazole	Alive	***Legionella* spp.**

ND = no data available; TMS = trimethhoprim/sulfamethoxazole; HRZE = isoniazid (H), rifampin (R), pyrazinamide (Z), and ethambutol (E).
[a] Risk class based on pneumonia severity index: I: 0.1% mortality, outpatient treatment reasonable; II: 0.6–0.9% mortality, outpatient treatment reasonable, barring other factors affecting care; III: 0.9–2.8% mortality, outpatient or inpatient treatment, depending on clinical judgment; IV: 8.2–9.3% mortality, hospitalization recommended based on risk; V: 27.0–29.2% mortality, hospitalization recommended based on risk.

administered appropriate empiric antibiotic therapy, and thus, their findings are not generalizable to immunosuppressed HIV-infected patients like those seen in our study.

A key strength of this investigation lies in the fact that this study looks at *Legionella* spp. in Colombia and *Legionella* co-infections in HIV-associated pneumonias, both of which are subjects that have been understudied to date. However, our study is not without limitations. Firstly, all study participants were from Medellín, and thus, the representation of *Legionella* herein may not be truly representative of other geographical locales. Secondly, although we were able to identify *Legionella* using qPCR, despite several attempts, we were unable to confirm our results using culture. This may be due to the retrospective nature of our study, as lower isolation rates have been shown to occur with delayed time till culture or due to bacteria in the viable but not culturable state (Aurass et al., 2016; Jarraud et al., 2013; Mercante and Winchell, 2015). *Legionella* culture sensitivity varies, with over one third of laboratories having reported being unable to culture *Legionella*, and as such, the use of culture as a diagnostic method for *Legionella* has decreased significantly (to just 5%) in the United States (Mercante and Winchell, 2015). In addition, the reader should also bear in mind that this study is based on participants who had multiple simultaneous infections (i.e., HIV plus *M. tuberculosis*, *P. jirovecii* either with or without *Legionella* infection); therefore, although our study highlights a relationship between *Legionella* infection and disease severity, we cannot indicate a causative relationship. Lastly, since this was a pilot study, which limited the number of patient BAL samples to which we had access, further validation of these findings in a larger cohort is warranted. Nonetheless, due to potential microbiological synergy and to the potential implications on clinical outcomes, clinicians should be aware of the possible presence of *Legionella* in HIV-associated pneumonia.

Table 4
Characteristics of HIV and pneumonia patients with and without probable *Legionella* infections.

	Legionella negative ($n = 30$)	*Legionella* positive ($n = 17$)	*P* value
Demographics			
Age, mean (SD), years	36.7 (8.4)	33.9 (9.5)	0.282
Male sex,[a] *n* (%)	27 (90.0)	11 (64.7)	0.054
Weight, mean (SD), kg	53.5 (8.7)	46.3 (9.9)	**0.016**
Medical history			
Cigarette smoking history, *n* (%)	8 (26.7)	7 (41.2)	0.311
CD4 count, median (IQR), cells/μL	25 (12–92.8)	32.5 (16–90.5)	0.737
Signs and symptoms			
Cough, *n* (%)	23 (76.7)	15 (88.2)	0.333
Fever, *n* (%)	24 (80.0)	15 (88.2)	0.470
Hemoptysis, *n* (%)	5 (16.7)	1 (5.9)	0.396
Adenopathy, *n* (%)	15 (50.0)	8 (47.1)	0.846
Dyspnea, *n* (%)	13 (43.3)	5 (29.4)	0.345
Systolic blood pressure, mean (SD), mmHg	105.8 (20.9)	107.4 (21.8)	0.900
Heart rate > 120/min, *n* (%)	9 (30.0)	8 (47.1)	0.277
Respiratory rate, mean (SD)	23.8 (9.4)	21.8 (5.1)	0.859
Chest computed tomography			
Pleural effusion, *n* (%)	2 (6.7)	3 (17.6)	0.344
Pneumothorax, *n* (%)	2 (6.7)	1 (5.8)	0.522
Pulmonary infiltrates,[b] *n* (%)	26 (86.7)	14 (82.4)	0.536
Ground glass, *n* (%)	6 (20.0)	6 (35.3)	0.103
Laboratory data			
Sodium ≤130 mEq/L, *n* (%)	5 (16.7)	1 (5.9)	0.403
Potassium ≥4.5 mEq/L, *n* (%)	6 (20)	3 (17.6)	1.000
Creatine ≥1.2 mg/dl, *n* (%)	5 (16.7)	4 (23.5)	0.711
GPT > 40, *n* (%)	14 (46.7)	10 (58.8)	0.360
Complications and outcome			
ICU admission, *n* (%)	7 (23.3)	9 (52.9)	**0.042**
Intubation, *n* (%)	3 (10.0)	3 (17.6)	0.829
Mechanical ventilation, *n* (%)	2 (6.7)	2 (11.8)	0.613
Length of hospital stay, mean (SD)	21.72 (12.2)	29.6 (20.8)	0.565
Mortality, *n* (%)	6 (20)	9 (52.9)	**0.020**

GPT = glutamate pyruvate transaminase.
[a] Cohort is predominantly male with only 9 females.
[b] Alveolar, interstitial, multilobar, and nodular infiltrates.

In conclusion, the data gathered in this pilot study suggest that *Legionella* infections occur frequently in HIV and *M. tuberculosis* or *P. jirovecii* co-infected patients. Since *Legionella* spp. have not been described as commensals in humans nor have they been found in any healthy human microbiome studies to date, their detection is likely to be clinically significant. Furthermore, we found that individuals with probable *Legionella* infections had increased complications and higher mortality rates compared to *Legionella*-negative individuals, highlighting that appropriate empirical anti-*Legionella* therapy is crucial for positive patient outcomes, albeit, as was seen in our study, it is often not administered. Although disease severity and inflammation associated with *Legionella* remain to be determined, this study is an important step towards better understanding *Legionella* co-infections and merits further investigation. If studies support these preliminary findings, this could change the way pneumonia is managed in immunosuppressed HIV-infected individuals.

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Declarations of interest

None.

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