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Research article

Diagnostic Value of Metagenomic next-generation sequencing and X-pert in Bronchoalveolar lavage fluid for pneumonia in HIV-infected and HIV-uninfected patients

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ABSTRACT

Background: The pathogens causing unexplained pneumonia in both HIV-infected or HIV-unfected patients are likely to be complex. This retrospective study aimed to characterize the etiology of pneumonia in HIV-infected and HIV-uninfected patients using bronchoalveolar lavage fluid (BALF) analysis with metagenomic next-generation sequencing (mNGS) and X-pert MTB/RIF.

Methods: Between January 2022 and May2024, 141 HIV-infected and 104 HIV-uninfected patients admitted to Nanjing Second Hospital with pneumonia were included. BALF samples were collected and analyzed using mNGS to detect bacteria, fungi, viruses, tuberculosis (TB) and non-tuberculous mycobacteria (NTM), and X-pert for TB detection. Clinical data including CD4 T-cell counts, comorbidities, and ART status were collected and analyzed.

Results: HIV-uninfected patients were found to be older and exhibited a higher prevalence of comorbidities compared to HIV-infected patients. Despite higher median CD4 T-cell counts in HIV-uninfected individuals (412 cells/μL vs. 31 cells/μL in HIV-infected), TB detection rates using X-pert and mNGS were lower than anticipated, particularly in HIV-infected patients. Mixed-pathogen infections were significantly more prevalent in HIV-infected patients, especially those with lower CD4 T-cell counts. ART use showed variable impacts on pathogen diversity, with longer treatment durations associated with reduced infection complexity but persistent immunodeficiency in some cases.

In patients with pneumonia, whether HIV-infected or HIV-uninfected, pathogens often exhibit complexity, underscoring the critical role of timely mNGS and X-pert analysis of BALF for early pathogen detection.

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1. Introduction

Patients presenting with community-acquired pneumonia (CAP) typically do not require routine bronchoscopic alveolar lavage examination, metagenomic next-generation sequencing (mNGS) for comprehensive microbial detection, or X-pert MTB/RIF, a molecular test primarily used for detecting tuberculosis (TB) and rifampicin resistance, indicative of multidrug-resistant tuberculosis (MDR-TB) [1,2]. Diagnosis and treatment of CAP traditionally rely on clinical presentation, blood routine analyses, and chest computed tomography (CT) scans, facilitating effective selection of appropriate medications [3]. However, managing pneumonia becomes more complex in the presence of HIV infection, often involving multiple pathogens and complicating accurate pathogen identification solely through clinical and routine examinations [4]. Delayed treatment and increased mortality rates underscore the need for timely pathogen identification, which is critical for all pneumonia patients, regardless of HIV status.

Metagenomic next-generation sequencing (mNGS) demonstrates varying pathogen detection rates in sputum samples from both HIV-infected and HIV-uninfected individuals, influenced by potential colonization of oral cavity bacteria affecting diagnostic specificity [5]. Utilizing mNGS on bronchoalveolar lavage fluid (BALF) significantly enhances pathogen detection rates. Studies have reported mNGS sensitivity and specificity of 97.40 % and 85.12 %, respectively, for diagnosing Pneumocystis jiroveci pneumonia (PJP) [5], and sensitivity of 75.9 % for diagnosing pulmonary tuberculosis, surpassing X-pert's 60.3 % sensitivity [6]. Despite its advantages, mNGS may have limitations in detecting mycobacteria due to their thick cell walls, which the mNGS bacterial solution may not effectively penetrate [7–9]. Conversely, X-pert has demonstrated high accuracy (>90 %) in identifying culture-confirmed TB among severely ill HIV-infected patients [10,11].

Mycobacterial infections, particularly opportunistic in HIV-infected individuals with CD4 T-cell counts below 200 cells/uL, pose diagnostic challenges with conventional smear culture methods due to low positivity rates and prolonged culture times. X-pert's ability to rapidly detect tuberculosis genes has been pivotal in clinical settings. This study aims to analyze the diagnostic capabilities of mNGS and X-pert on BALF in identifying pulmonary infection pathogens in HIV-infected patients, explore diagnostic methodologies for pulmonary infections in this population, and compare pneumonia pathogens between HIV-infected and HIV-uninfected individuals.

2. Materials & methods

This retrospective study included HIV-infected or uninfected patients with pneumonia of unknown etiology admitted to Nanjing Second Hospital from January 2022 to May 2024. Patients meeting the following criteria were recruited: (1) Age ≥18 years; (2) Complete medical records available; (3) Confirmed diagnosis of pneumonia in HIV-infected or HIV-uninfected individuals; (4) Availability of mNGS and X-pert results from bronchoalveolar lavage fluid (BALF). Exclusion criteria were: (1) Age <18 years; (2) Incomplete medical records; (3) Patients without pneumonia; (4) Absence of mNGS and X-pert results from BALF.

2.1. Sample collection and etiological diagnosis

Bronchoscopy was performed by experienced physicians to obtain BALF, which was promptly refrigerated at 4–8 °C and processed within 24 h. Pathogen detection from BALF utilized metagenomic next-generation sequencing (mNGS) on the MGISEQ-2000 gene sequencer (MGI Tech Co., Ltd., Shenzhen, China), generating 30 million reads, and X-pert for TB on the GeneXpert Dx System (Cepheid, Sunnyvale, CA, USA).

Data collected included: age, sex, computed tomography (CT) findings, comorbidities, symptom onset and duration, and laboratory results such as CD4 T-cell count, CD8 T-cell count, CD4/CD8 ratio, white blood cell count, neutrophil percentage, hemoglobin concentration, and platelet count. The final diagnosis was determined by consensus among three senior clinicians.

2.2. Statistical analyses

Normally distributed continuous variables were reported as mean ± standard deviation (SD), while non-normally distributed variables were presented as median (25th, 75th percentiles). Group differences for normally distributed data were assessed using independent-samples t-tests, while the Mann-Whitney *U* test was employed for non-normally distributed data. The chi-square test evaluated significant associations between two categorical variables with adequate sample sizes (typically ≥5 expected counts per cell in a 2x2 table). Fisher's exact test was utilized for similar purposes when expected frequencies in any contingency table cell were <5. Statistical significance was defined as $P < 0.05$ (two-tailed). All statistical analyses were conducted using IBM SPSS version 26 (IBM SPSS, Armonk, NY, USA).

3. Results

3.1. Patient characteristics

Between January 2022 and May 2024, a total of 141 HIV-infected patients and 104 HIV-uninfected patients with pneumonia of unknown etiology, who underwent mNGS and X-pert analysis of BALF samples, were included in this study. The flowchart illustrating participant enrollment is presented in Fig. 1. Detailed clinical characteristics and laboratory findings of all patients are summarized in Table 1. HIV-uninfected patients had a significantly higher average age of 56.1 ± 17.4 years compared to HIV-infected patients, who had an average age of 43.4 ± 13.9 years ($P < 0.001$). Furthermore, HIV-uninfected patients exhibited higher CD4 T-cell counts (412

[250, 560] cells/μL) and CD4/CD8 ratios (0.900 [0.704, 1.441]) compared to HIV-infected patients, who had CD4 T-cell counts of 31 [8, 117] cells/μL and CD4/CD8 ratios of 0.056 [0.026, 0.166] (both $P < 0.001$). Chronic diseases such as heart disease, hepatopathy, chronic kidney disease, thyroid disease, old cerebral infarction, diabetes, hormone therapy, and systemic lupus erythematosus (SLE) were more prevalent among HIV-uninfected patients (Table 1).

3.2. Pathogens detection by mNGS and X-pert

The detection rates of single TB infection (6.73 %), single bacterial infection (24.04 %), bacterial + TB co-infection (3.85 %), and bacterial + fungal co-infection (22.12 %) were significantly higher in HIV-uninfected patients compared to HIV-infected patients (0 %, 4.26 %, 0 %, and 8.51 %, respectively) ($P = 0.002$, <0.001, 0.031, and 0.003, respectively). Conversely, HIV-infected patients exhibited significantly higher rates of bacterial + viral co-infection (7.80 %) and bacterial + fungal + viral co-infection (11.35 %) compared to HIV-uninfected patients (0 % for both) ($P = 0.003$ and < 0.001, respectively). In HIV-infected patients, TB detection rates were relatively low, while mixed infections, particularly bacterial + fungal co-infections, were more common. Combined use of mNGS and X-pert significantly reduced the missed diagnosis rate for TB compared to individual methods alone (Table 2).

To reduce the influence of age and gender on the results, we excluded the female group and reclassified the remaining data into two categories: the 18–64 years (non old age) group and the ≥65 years (old age) group. In the 18–64 years (non old age) group, the rates of single TB infection in HIV-uninfected individuals were 16.28 %, bacteria + TB infection was 9.3 %, and single pathogen infection was 51.16 %. These rates were significantly higher than those observed in the HIV-infected group (0 %, 0 %, and 20.31 %, respectively). Conversely, the infection rates for bacteria + fungi + viruses (10.94 %) and mixed pathogens (65.63 %) in HIV-uninfected group were significantly lower compared to those in HIV-infected patients (0 % and 41.86 %, respectively). In the ≥65 years (old age) group, the infection rates for bacteria + fungi + viruses and single fungal infections in HIV-infected individuals were both 25.00 %, significantly higher than the 0 % observed in the HIV-uninfected group. The infection rate for mixed pathogens was also higher in the HIV-infected group compared to the HIV-uninfected group, although this difference was not statistically significant. Conversely, the rates of single bacterial infections (0 %) and single pathogen infections (25 %) in the HIV-infected group were significantly lower than the 74.07 % observed in the HIV-uninfected group (Table 3).

3.3. Distribution of pathogenic microorganisms with different CD4 T-cell counts in HIV-infected group

The Consolidated Guidelines on HIV Prevention, Testing, Treatment, Service Delivery and Monitoring: Recommendations for a Public Health Approach from World Health Organization [12] and Chinese guidelines for diagnosis and treatment of HIV/AIDS (2024 edition) [13]indicate that AIDS patients with varying CD4 T-cell counts exhibit increased susceptibility to specific pathogens. For instance, when the CD4 cell count falls below 100 cells/μL, patients are at higher risk for infections with NTM, cytomegalovirus (CMV), Cryptococcus (a fungus), and Talaromyces marneffei (a fungus). Conversely, when the CD4 cell count drops below 200 cells/μL, the risk of infection with Pneumocystis jirovecii (PCP, a fungus) and Toxoplasma gondii (a parasite) increases. Accordingly, we have categorized the CD4 T-cell counts of our study subjects into three groups: 1–100 cells/μL, 101–200 cells/μL, and more than 200 cells/μL.

Patients with CD4 T-cell counts below 100 cells/μL showed a higher prevalence of bacterial + fungal co-infections, with a co-infection rate of bacterial, fungal, and viral pathogens reaching 16.33 %. Among patients with CD4 T-cell counts ranging from 101 to 200 cells/μL, bacterial + NTM co-infections were more prevalent. The study also revealed a increase in TB detection rates as CD4 T-cell counts decreased, with no detections in patients with CD4 T-cell counts above 200 cells/μL. Patients with CD4 T-cells in the 1–100 cells/μL range had a significantly higher proportion of mixed pathogen infections compared to those with CD4 T-cells in the 101–200

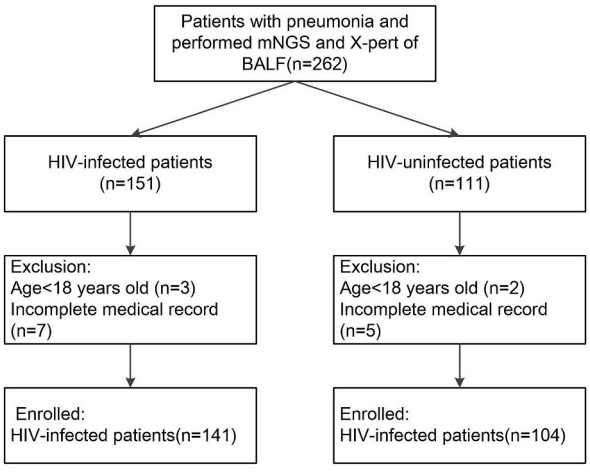

Fig. 1. Flowchart of study participants enrolled in the study.

Table 1
Clinical characteristics data summary.

Characteristics	HIV+(n = 141)	HIV-(n = 104)		*P*
Age, (years), mean ± SD	43.4 ± 13.9	56.1 ± 17.4	t = 3.925	<0.001[c]
Gender male (female)	136 (5)	70 (34)	$\chi^2 = 37.683$	<0.001[c]
Onset of symptoms/signs				
Fever, n (%)	83 (58.87)	52 (50.00)	$\chi^2 = 1.901$	0.168
Cough, n (%)	87 (61.70)	44 (42.30)	$\chi^2 = 9.049$	0.003[b]
Expectoration, n (%)	65 (46.10)	34 (32.69)	$\chi^2 = 4.468$	0.035[a]
Hypoxemia, n (%)	57 (40.43)	22 (21.15)	$\chi^2 = 10.175$	0.001[b]
Chest pain, n (%)	2 (1.42)	0 (0.00)	–	0.509
Hemoptysis, n (%)	2 (1.42)	7 (6.73)	–	0.039[a]
Duration of symptoms, (median, IQR)	21.00 (8.00, 60.00)	14.00 (4.00, 30.00)	U = 6135	0.035[a]
<2 week, n (%)	42 (29.79)	45 (43.27)	$\chi^2 = 4.751$	0.029[a]
2 weeks–2months, n (%)	70 (49.65)	39 (37.50)	$\chi^2 = 3.575$	0.059
>2 months, n (%)	29 (20.57)	20 (19.23)	$\chi^2 = 0.067$	0.796
Duration of ART				
ART naive, n (%)	86 (60.99)	NA	–	–
ART<6 months, n (%)	39 (27.66)	NA	–	–
ART>6 months, n (%)	16 (11.35)	NA	–	–
Laboratory test				
CD4 T-cell count (cells/mm3) (median, IQR)	31 (8117)	412 (250,560)	U = 1063	<0.001[c]
CD8 T-cell count (cells/mm3) (median, IQR)	446 (252, 696)	486 (154, 548)	U = 6542	0.150
CD4/CD8 ratio (median, IQR)	0.056 (0.026, 0.166)	0.900 (0.704, 1.441)	U = 984	<0.001[c]
White blood cells ($\times 10^9$/L), mean ± SD	5.423 ± 2.506	6.537 ± 3.161	t = 1.852	0.067
Neutrophils (%)	68.85 ± 15.69	65.27 ± 16.11	t = 1.017	0.312
Hemoglobin (g/L), mean ± SD	117.5 ± 23.09	116.6 ± 25.38	t = 0.1783	0.859
Platelet ($\times 10^9$/l), mean ± SD	215.8 ± 105.0	209.5 ± 81.02	t = 0.2877	0.774
Comorbidities present				
Chronic lung disease (%)	18 (12.77)	16 (15.38)	$\chi^2 = 3.343$	0.558
Heart disease (%)	2 (1.42)	16 (15.38)	–	<0.001[c]
Hepatopathy (%)	0 (0.00)	4 (3.85)	–	0.031[a]
Gastroenteritis or ulcer (%)	15 (10.64)	12 (11.54)	$\chi^2 = 0.049$	0.824
Renal transplant status (%)	0 (0.00)	1 (0.96)	–	0.424
Chronic kidney disease (%)	5 (4.26)	23 (23.08)	$\chi^2 = 20.389$	<0.001[c]
Thyroid disease (%)	0 (0.00)	4 (3.85)	–	0.031[a]
Old cerebral infarction (%)	0 (0.00)	8 (7.69)	–	0.001[b]
Diabetes (%)	7 (4.96)	19 (18.27)	$\chi^2 = 11.169$	<0.001[c]
Hypertension (%)	15 (10.64)	19 (18.27)	$\chi^2 = 2.916$	0.088
Hormone application (%)	0 (0.00)	8 (7.69)	–	0.001[b]
SLE (%)	0 (0.00)	4 (3.85)	–	0.031[a]
Malignant tumors (%)	4 (2.84)	7 (6.73)	$\chi^2 = 0.111$	0.212

note: SD: Standard Deviation; IQR: interquartile range; ART: antiretroviral therapy; SLE: systemic lupus erythematosus.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

cells/μL and >200 cells/μL groups ($P < 0.001$). Although the proportion of single infections did not significantly differ among CD4 T-cell count groups, a notably higher proportion of single infections (33.33 %) was observed in the >200 cells/μL group ($P = 0.321$). Moreover, the rate of negative results (defined as inability to detect pathogens by mNGS and X-pert) was higher in patients with CD4 T-cells >200 cells/μL compared to those with CD4 T-cells in the 1–100 cells/μL and 101–200 cells/μL ranges ($P < 0.001$). Notably, TB infections were detected by both mNGS and X-pert in the group with CD4 T-cell counts below 100 cells/μL, whereas no detections were observed in the other two groups (Table 4).

3.4. Distribution of single and mixed pathogenic microorganisms in ART-naïve or ART-treated HIV-infected patients with pneumonia

Untreated HIV-infected patients exhibited significantly lower CD4 T-cell counts, with a median of 16 cells/μL. As the duration of antiretroviral therapy (ART) increased, CD4 T-cell counts significantly rose, while CD8 T-cell counts remained relatively stable. The CD4/CD8 ratio also increased significantly with longer ART duration, indicating improved immune function in these patients. Mixed infections of bacteria, fungi, and viruses were most common in ART-naïve patients. With prolonged ART treatment, the proportion of mixed infections decreased, and the incidence of single-pathogen detections tended to increase, suggesting potential beneficial effects of ART in reducing pathogen diversity, although these differences were not statistically significant ($P = 0.197$ and 0.485, respectively). TB detection rates through mNGS or X-pert did not significantly differ with ART treatment duration. Both mNGS and X-pert techniques showed advantages and limitations in TB detection, with combined use significantly enhancing accuracy (Table 5).

Table 2
Distribution of single and mixed pathogenic microorganisms in HIV infected and uninfected patients with pneumonia.

	HIV+ (n = 141)	HIV- (n = 104)	*P*
NTM + virus, n (%)	3 (2.13)	0 (0.00)	0.264
TB, n (%)	0 (0.00)	7 (6.73)	0.002[b]
TB + NTM, n (%)	0 (0.00)	2 (1.92)	0.179
TB + virus, n (%)	3 (2.13)	0 (0.00)	0.264
Virus, n (%)	5 (3.55)	4 (3.85)	1.000
Bacterium, n (%)	6 (4.26)	25 (24.04)	<0.001[c]
Bacteria + NTM, n (%)	4 (2.84)	7 (6.73)	0.212
Bacteria + NTM + Virus, n (%)	1 (0.71)	0 (0.00)	1.000
Bacteria + TB, n (%)	0 (0.00)	4 (3.85)	0.031[a]
Bacteria + TB + NTM, n (%)	1 (0.71)	0 (0.00)	1.000
Bacteria + viruses, n (%)	11 (7.80)	0 (0.00)	0.003[b]
Bacteria + fungi, n (%)	12 (8.51)	23 (22.12)	0.003[b]
Bacteria + fungi + NTM, n (%)	3 (2.13)	4 (3.85)	0.462
Bacteria + fungi + NTM + viruses, n (%)	3 (2.13)	0 (0.00)	0.264
Bacteria + fungi + TB, n (%)	2 (1.42)	1 (0.96)	1.000
Bacteria + fungi + TB + NTM + virus, n (%)	2 (1.42)	0 (0.00)	0.509
Bacteria + fungi + TB + viruses, n (%)	3 (2.13)	0 (0.00)	0.264
Bacteria + fungi + TB + Rickettsia, n (%)	1 (0.71)	0 (0.00)	1.000
Bacteria + fungi + viruses, n (%)	16 (11.35)	0 (0.00)	<0.001[c]
Bacteria + fungi + viruses + mycoplasma, n (%)	1 (0.71)	0 (0.00)	1.000
Bacteria + fungi + mycoplasma, n (%)	1 (0.71)	0 (0.00)	1.000
Bacteria + mycoplasma, n (%)	2 (1.42)	0 (0.00)	0.509
Fungi, n (%)	17 (12.06)	9 (8.65)	0.393
Fungi + NTM, n (%)	9 (6.38)	0 (0.00)	0.011[a]
Fungi + NTM + Virus, n (%)	2 (1.42)	0 (0.00)	0.509
Fungi + TB + NTM + Virus + Mycoplasma, n (%)	1 (0.71)	0 (0.00)	1.000
Fungi + viruses, n (%)	11 (7.80)	6 (5.77)	0.536
Fungi + Mycoplasma, n (%)	0 (0.00)	3 (2.88)	0.075
NTM, n (%)	0 (0.00)	3 (2.88)	0.075
Mixed infection, n (%)	92 (65.25)	50 (48.08)	0.007[ba]
Single infection, n (%)	28 (19.86)	48 (46.16)	<0.001[c]
Negative, n (%)	21 (14.89)	6 (5.77)	0.024[a]
TB by mNGS only, n (%)	3 (2.13)	2 (1.92)	1.000
TB by X-pert only, n (%)	6 (4.26)	7 (6.73)	0.393
TB by mNGS and X-pert, n (%)	4 (2.84)	5 (4.81)	0.501

note: NTM: nontuberculous mycobacteria; TB: tuberculosis.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

4. Discussion

Our study revealed significant differences in age and comorbidities between HIV-infected and HIV-uninfected patients presenting with unexplained pneumonia. HIV-uninfected patients tended to be older and had a higher prevalence of comorbidities such as heart disease, hepatopathy, chronic kidney disease, thyroid disease, old cerebral infarction, diabetes, hormone use, and SLE. This aligns with previous research indicating that older age and underlying health conditions contribute to increased susceptibility to pneumonia and higher mortality rates among elderly populations [14–17]. Although HIV-uninfected patients in our study had higher CD4 T-cell counts and CD4/CD8 ratios compared to HIV-infected individuals. However, the older individuals, who experienced immune decline associated with aging, were more susceptible to pathogen infections [18–20]. Our research findings also indicate that the HIV-uninfected but older patients' lungs were particularly vulnerable to infections, characterized by a complex and varied pathogen composition.

Moreover, our findings highlight the significant burden of TB among HIV-uninfected patients with pneumonia. Despite X-pert's specificity for TB detection due to its ability to disrupt TB's thick cell wall effectively [21–23], our study found a lower detection rate for TB using both mNGS and X-pert compared to previous reports [6]. Specifically, among 104 HIV-uninfected pneumonia patients, 14 were TB-positive, with X-pert detecting 7 cases (6.73 %), mNGS identifying 2 cases (1.92 %), and both methods detecting 5 cases (4.81 %). Similarly, among 141 HIV-infected pneumonia patients, 13 were TB-positive, with X-pert detecting 3 cases (2.13 %) and mNGS identifying 6 cases (4.26 %), while both methods detected 4 cases (2.84 %). These detection rates were lower than the reported sensitivity of X-pert (100 %) in BALF for HIV-infected pneumonia patients [24]. Challenges in mNGS, such as difficulties in DNA extraction from TB due to its unique cell wall composition, may explain the suboptimal TB detection rates observed [25,26]. Thus, the combined use of X-pert and mNGS in BALF remains essential for timely and accurate TB detection, particularly given TB's status as a common opportunistic infection in HIV-positive individuals [27–30].

Previous studies have consistently reported high rates of mixed-pathogen infections among HIV-infected patients with community-acquired pneumonia (CAP), especially in those with low CD4 T-cell counts [10,31–34]. Our study confirmed these findings, with a

Table 3
Distribution of single and mixed pathogenic microorganisms in HIV infected and uninfected male patients across age groups.

Categoryies	18–64 years (Non old age)			≥65 years (Old age)		
	HIV+	HIV-	*P*	HIV+	HIV-	*P*
	(n = 128)	(n = 43)		(n = 8)	(n = 27)	
Age, (years), mean ± SD	41.9 ± 11.3	50.7 ± 10.8	<0.001[c]	70.3 ± 6.1	75.0 ± 3.9	0.086
TB, n (%)	0 (0.00)	7 (16.28)	0.001[c]	0 (0.00)	0 (0.00)	–
TB + NTM, n (%)	0 (0.00)	0 (0.00)	–	0 (0.00)	0 (0.00)	–
TB + virus, n (%)	3 (2.34)	0 (0.00)	0.573	0 (0.00)	0 (0.00)	–
Virus, n (%)	5 (3.91)	2 (4.65)	1.000	0 (0.00)	0 (0.00)	–
Bacterium, n (%)	6 (4.69)	5 (11.63)	0.109	0 (0.00)	20 (74.07)	<0.001[c]
Bacteria + NTM, n (%)	2 (1.56)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + NTM + Virus, n (%)	1 (0.78)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + TB, n (%)	0 (0.00)	4 (9.30)	0.004[b]	0 (0.00)	0 (0.00)	–
Bacteria + TB + NTM, n (%)	1 (0.78)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + viruses, n (%)	11 (8.59)	0 (0.00)	0.067	0 (0.00)	0 (0.00)	–
Bacteria + fungi, n (%)	11 (8.59)	7 (16.28)	0.155	1 (12.50)	4 (14.81)	1.000
Bacteria + fungi + NTM, n (%)	3 (2.34)	0 (0.00)	0.573	0 (0.00)	0 (0.00)	–
Bacteria + fungi + NTM + viruses, n (%)	3 (2.34)	0 (0.00)	0.573	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB, n (%)	2 (1.56)	1 (2.33)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB + NTM + virus, n (%)	2 (1.56)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB + viruses, n (%)	3 (2.34)	0 (0.00)	0.573	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB + Rickettsia, n (%)	1 (0.78)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + fungi + viruses, n (%)	14 (10.94)	0 (0.00)	0.022[a]	2 (25.00)	0 (0.00)	0.047[a]
Bacteria + fungi + viruses + mycoplasma, n (%)	1 (0.78)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + fungi + mycoplasma, n (%)	1 (0.78)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Bacteria + mycoplasma, n (%)	2 (1.56)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Fungi, n (%)	15 (11.72)	8 (18.60)	0.252	2 (25.00)	0 (0.00)	0.047[a]
Fungi + NTM, n (%)	9 (7.03)	0 (0.00)	0.114	0 (0.00)	0 (0.00)	–
Fungi + NTM + Virus, n (%)	2 (1.56)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Fungi + TB + NTM + Virus + Mycoplasma, n (%)	1 (0.78)	0 (0.00)	1.000	0 (0.00)	0 (0.00)	–
Fungi + viruses, n (%)	11 (8.59)	6 (13.95)	0.310	1 (12.50)	0 (0.00)	0.229
Fungi + Mycoplasma, n (%)	0 (0.00)	0 (0.00)	–	0 (0.00)	0 (0.00)	–
NTM, n (%)	0 (0.00)	0 (0.00)	–	0 (0.00)	0 (0.00)	–
Mixed infection, n (%)	84 (65.63)	18 (41.86)	0.006[b]	4 (50.00)	4 (14.81)	0.060
Single infection, n (%)	26 (20.31)	22 (51.16)	<0.001[c]	2 (25.00)	20 (74.07)	0.032[a]
Negative, n (%)	18 (14.06)	3 (6.98)	0.289	2 (25.00)	3 (11.11)	0.568
TB by mNGS only, n (%)	3 (2.34)	0 (0.00)	–	0 (0.00)	0 (0.00)	–
TB by X-pert only, n (%)	6 (4.69)	7 (16.28)	0.013[a]	0 (0.00)	0 (0.00)	–
TB by mNGS and X-pert, n (%)	4 (3.13)	5 (11.63)	0.045[a]	0 (0.00)	0 (0.00)	–

note: NTM: nontuberculous mycobacteria; TB: tuberculosis.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

mixed-pathogen infection rate of 65.25 % among HIV-infected pneumonia patients, higher than the 50.96 % observed in HIV-uninfected pneumonia patients. Although statistical significance was not reached, single-pathogen infections were significantly less frequent in HIV-infected pneumonia patients compared to their HIV-uninfected counterparts, particularly bacterial infections. Previous research has also noted the presence of dual-pathogen (17.0 %) and triple-pathogen (0.6 %) infections exclusively among HIV-infected pneumonia patients, underscoring the impact of severe immunosuppression and longer disease duration on pathogen complexity [35]. The challenges posed by distinguishing between pathogenic infection and colonization using mNGS further complicate the interpretation of results [31], with our study indicating that 14.89 % of HIV-infected and 5.77 % of HIV-uninfected pneumonia patients had negative BALF cultures.

Our investigation examined pathogen prevalence among HIV-infected pneumonia patients stratified by CD4 T-cell counts and antiretroviral therapy (ART) status, revealing significant insights. We observed a higher incidence of mixed infections as CD4 T-cell counts declined, consistent with existing literature associating severe immunosuppression and prolonged pre-hospitalization symptom duration with heightened vulnerability to mixed infections [31]. Conversely, higher CD4 T-cell counts correlated with fewer overall cases, resulting in non-significant differences in pathogen types among groups with higher CD4 T-cell counts. Among ART-treated patients, those receiving therapy for over 6 months exhibited fewer cases, although ongoing immunodeficiency persisted despite treatment, with no significant variations in pathogen infection rates across different ART durations. Previous studies have highlighted elevated proportions of Pneumocystis jirovecii and Talaromyces marneffei in fungal infections among HIV-infected individuals compared to non-infected counterparts, alongside reduced incidences of Candida, Aspergillus, Tropheryma whipplei, TB, Pneumocystis jirovecii, Talaromyces marneffei, and cytomegalovirus in ART-treated HIV patients [36].

However, our study's limited sample size restricted detailed analysis of pathogen types based on CD4 T-cell counts and ART durations, underscoring the need for larger-scale investigations to ensure robust statistical power and definitive conclusions in future

Table 4
Distribution of pathogenic microorganisms with different CD4 T-cell count in HIV-infected patients with pneumonia.

CD4 T-cell count (cells/μL)	1-100 (n = 98)	101-200 (n = 28)	>200 (n = 15)	*P*
NTM + virus, n (%)	3 (3.06)	0 (0.00)	0 (0.00)	–
TB + virus, n (%)	2 (2.04)	1 (3.57)	0 (0.00)	0.667
Virus, n (%)	3 (3.06)	1 (3.57)	1 (6.67)	0.609
Bacterium, n (%)	4 (4.08)	1 (3.57)	1 (6.67)	0.806
Bacteria + NTM, n (%)	1 (1.02)	3 (10.71)	0 (0.00)	0.049*
Bacteria + NTM + Virus, n (%)	0 (0.00)	1 (3.57)	0 (0.00)	–
Bacteria + TB + NTM, n (%)	1 (1.02)	0 (0.00)	0 (0.00)	–
Bacteria + viruses, n (%)	7 (7.14)	4 (14.29)	0 (0.00)	0.261
Bacteria + fungi, n (%)	9 (9.18)	3 (10.71)	0 (0.00)	0.572
Bacteria + fungi + NTM, n (%)	3 (3.06)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + NTM + viruses, n (%)	3 (3.06)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB, n (%)	2 (2.04)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB + NTM + virus, n (%)	2 (2.04)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB + viruses, n (%)	3 (3.06)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + TB + Rickettsia, n (%)	1 (1.02)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + viruses, n (%)	16 (16.33)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + viruses + mycoplasma, n (%)	1 (1.02)	0 (0.00)	0 (0.00)	–
Bacteria + fungi + mycoplasma, n (%)	1 (1.02)	0 (0.00)	0 (0.00)	–
Bacteria + mycoplasma, n (%)	0 (0.00)	2 (7.14)	0 (0.00)	–
Fungi, n (%)	12 (12.24)	2 (7.14)	3 (20.00)	0.461
Fungi + NTM, n (%)	6 (6.12)	2 (7.14)	1 (6.67)	1.000
Fungi + NTM + Virus, n (%)	2 (2.04)	0 (0.00)	0 (0.00)	–
Fungi + TB + NTM + Virus + Mycoplasma, n (%)	0 (0.00)	0 (0.00)	1 (6.67)	–
Fungi + viruses, n (%)	10 (10.20)	0 (0.00)	1 (6.67)	0.223
Containing NTM (%)	21 (21.43)	6 (21.43)	2 (13.33)	0.764
Containing TB (%)	11 (11.22)	1 (3.57)	1 (6.67)	0.437
Mixed infection, n (%)	73 (74.49)	16 (57.14)	3 (20.00)	<0.001[a]
Single infection, n (%)	19 (19.39)	4 (14.29)	5 (33.33)	0.321
Negative, n (%)	6 (6.12)	8 (28.57)	7 (46.67)	<0.001[a]
TB by mNGS only, n (%)	3 (3.06)	0 (0.00)	0 (0.00)	–
TB by X-pert only, n (%)	4 (4.08)	1 (3.57)	1 (6.67)	0.806
TB by mNGS and X-pert, n (%)	4 (4.08)	0 (0.00)	0 (0.00)	–

note: NTM: nontuberculous mycobacteria; TB: tuberculosis.
[a] $P < 0.001$.

research.

In conclusion, our study underscores the complexity of pneumonia pathogens in both HIV-infected and HIV-uninfected patients, emphasizing the efficacy of mNGS and X-pert in BALF for accurate pathogen identification. Timely application of these diagnostic tools facilitates precise treatment planning, particularly crucial for HIV-infected patients susceptible to multi-pathogen infections. While challenges persist in distinguishing pathogenic infections from colonization using mNGS, experienced clinical judgment remains invaluable in clinical decision-making. Future studies should address limitations such as small sample sizes, especially in the HIV-uninfected group, to mitigate intergroup biases and enhance the generalizability of findings.

5. Conclusions

In patients with pneumonia, whether HIV-infected or HIV-uninfected, pathogens often exhibit complexity, underscoring the critical role of timely mNGS and X-pert analysis of BALF for early pathogen detection.

Ethics statement

This study adhered to the ethical principles outlined in the 1975 Helsinki Declaration. The research protocols were approved by the Research Ethics Committee of Nanjing Second Hospital (Approval number: 2023-LS-ky047). All patients had previously provided broad consent upon admission. The study was retrospective, anonymized, and utilized only existing data.

Submission statement

All authors declare that this manuscript was submitted solely to this journal, and the work described therein is original research that has not been published previously, and is not under consideration for publication elsewhere in whole or in part.

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Table 5
Distribution of single and mixed pathogenic microorganisms in ART naïve or ART HIV infected patients with pneumonia.

Characteristics	ART naïve (n = 86)	ART<6 months (n = 39)	ART>6 months (n = 16)		*P*
Age, (years), mean ± SD	41.4 ± 11.6	44.7 ± 13.6	45.4 ± 12.1	F = 0.454	0.636
Gender male (female)	84 (2)	36 (3)	16 (0)	–	0.226
CD4 T-cell count (cells//μL) (median, IQR)	16 (5,36)	84 (35,141)	195 (56,273)	–	<0.001[b]
CD8 T-cell count (cells/μL) (median, IQR)	382 (229,667)	509 (318,853)	499 (382,618)	–	0.052
CD4/CD8 ratio (median, IQR)	0.034 (0.020,0.076)	0.125 (0.099,0.315)	0.405 (0.089,0.681)	–	<0.001[b]
TB + virus, n (%)	2 (2.33)	1 (2.56)	0 (0.00)	–	1.000
Virus, n (%)	1 (1.16)	4 (10.26)	0 (0.00)	–	0.034[a]
Bacterium, n (%)	5 (5.81)	1 (2.56)	0 (0.00)	–	0.487
Bacteria + NTM, n (%)	4 (4.65)	0 (0.00)	0 (0.00)	–	–
Bacteria + NTM + Virus, n (%)	0 (0.00)	1 (2.56)	0 (0.00)	–	–
Bacteria + TB + NTM, n (%)	0 (0.00)	1 (2.56)	0 (0.00)	–	–
Bacteria + viruses, n (%)	8 (9.30)	1 (2.56)	2 (12.50)	–	0.250
Bacteria + fungi, n (%)	6 (6.98)	5 (12.82)	1 (6.25)	–	0.499
Bacteria + fungi + NTM, n (%)	3 (3.49)	0 (0.00)	0 (0.00)	–	–
Bacteria + fungi + NTM + viruses, n (%)	1 (1.16)	1 (2.56)	1 (6.25)	–	0.197
Bacteria + fungi + TB, n (%)	2 (2.33)	0 (0.00)	0 (0.00)	–	–
Bacteria + fungi + TB + NTM + virus, n (%)	2 (2.33)	0 (0.00)	0 (0.00)	–	–
Bacteria + fungi + TB + viruses, n (%)	2 (2.33)	1 (2.56)	0 (0.00)	–	1.000
Bacteria + fungi + TB + Rickettsia, n (%)	1 (1.16)	0 (0.00)	0 (0.00)	–	–
Bacteria + fungi + viruses, n (%)	9 (10.47)	5 (12.82)	2 (12.50)	–	0.793
Bacteria + fungi + viruses + mycoplasma, n (%)	0 (0.00)	1 (2.56)	0 (0.00)	–	–
Bacteria + fungi + mycoplasma, n (%)	1 (1.16)	0 (0.00)	0 (0.00)	–	–
Bacteria + mycoplasma, n (%)	1 (1.16)	0 (0.00)	1 (6.25)	–	0.290
Fungi, n (%)	10 (11.63)	5 (12.82)	2 (12.50)	–	1.000
Fungi + NTM, n (%)	5 (5.81)	2 (5.13)	2 (12.50)	–	0.523
Fungi + NTM + Virus, n (%)	1 (1.16)	1 (2.56)	0 (0.00)	–	0.630
Fungi + TB + NTM + Virus + Mycoplasma, n (%)	1 (1.16)	0 (0.00 %	0 (0.00)	–	–
Fungi + viruses, n (%)	6 (6.98)	3 (7.69)	2 (12.50)	–	0.723
Mixed infection, n (%)	58 (67.44)	23 (58.97)	11 (68.75)	–	0.623
Single infection, n (%)	16 (18.60)	10 (25.64)	2 (12.50)	–	0.485
Negative, n (%)	12 (13.95)	6 (15.38)	3 (18.75)	–	0.880
TB by mNGS only, n (%)	2 (2.33)	1 (2.56)	0 (0.00)	–	1.000
TB by X-pert only, n (%)	5 (5.81)	1 (2.56)	0 (0.00)	–	0.839
TB by mNGS and X-pert, n (%)	3 (3.49)	1 (2.56)	0 (0.00)	–	1.000

note: SD: Standard Deviation; IQR: interquartile range; ART: antiretroviral therapy; NTM: nontuberculous mycobacteria; TB: tuberculosis.
[a] $P < 0.05$.
[b] $P < 0.001$.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Rentian Cai: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Fengxue Yu:** Writing – original draft, Conceptualization. **Jian Cheng:** Writing – review & editing, Investigation, Data curation. **Chen Chen:** Writing – review & editing, Investigation, Data curation. **Yuan Liu:** Writing – review & editing, Investigation, Data curation. **Ru Lv:** Writing – review & editing, Investigation, Data curation. **Zi Ye:** Writing – review & editing, Investigation, Data curation. **Yin Yuan:** Writing – review & editing, Investigation, Data curation. **Zhengjie Li:** Writing – review & editing, Investigation, Data curation. **Cong Cheng:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Hongxia Wei:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors 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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