Research Square

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mNGS-Based Profiling Reveals Antiretroviral Therapy Selectively Modulates Pulmonary Pathogen Profiles in People Living with HIV

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Research Article

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Abstract

Background

Pulmonary complications are leading causes of poor prognosis in people living with HIV (PLWH). Though antiretroviral therapy (ART) recovers systemic immunity, its effects on lung pathogens remain unclear. This retrospective research explored how ART shapes lung pathogen profiles and loads in PLWH complicated with pulmonary infection.

Methods

This retrospective study enrolled 62 PLWH with suspected pneumonia. Patients were categorized into ART (n = 29) and ART-naïve (n = 33) groups. Metagenomic next-generation sequencing (mNGS) of bronchoalveolar lavage fluid was performed to profile bacterial, fungal, and viral communities. Microbial features, pathogen burden and clinical indicators were compared statistically.

Results

The ART group exhibited significantly higher $CD4^+$ T cell counts (median 131 vs. 44 cells/μL, P = 0.0049) and suppressed HIV viral load (median 185 vs. 259,000 copies/mL, P < 0.0001). mNGS revealed a distinct shift in co-infection patterns: ART recipients showed more bacterial-viral co-infections, while ART-naïve patients had more fungal-viral co-infections. ART significantly reduced the total pathogen burden (mean 3.7 ± 2.0 vs. 5.0 ± 2.6, P < 0.05), primarily through the suppression of fungal (P < 0.05) and viral loads (P < 0.05), with no significant effect on bacterial counts. Clinically, the ART group had significantly better outcomes (93.1% vs. 72.7% improvement, P = 0.036) and a trend toward shorter hospital stays.

Conclusion

ART remodels the pulmonary microbial community in PLWH and lowers total pathogen burden through targeted suppression of opportunistic fungi and viruses. These findings underscore that ART plays a vital role in managing pulmonary co-infections, with therapeutic benefits extending well beyond systemic HIV viral suppression alone.

Introduction

Human Immunodeficiency Virus (HIV) infection remains a global public health challenge. People living with HIV (PLWH) have a significantly increased susceptibility to pulmonary complications, which can seriously affect their morbidity and mortality[1]. The lung has historically been one of the organs most severely affected by HIV-induced immune deficiency. It is not only an important immune organ but also a

common target for inhaled pathogens such as bacteria, viruses and pneumocystis[2]. Due to continuous exposed to such microbes, the lungs rely on the coordinated function of innate and adaptive immune systems to maintain host-microbial homeostasis[3]. However, HIV infection can damage the pulmonary immune system at multiple key stages. The most significant mechanism is the exhaustion of $CD4^+$ T cells, which in turn disrupts the immune surveillance function. This immune regulatory disorder can lead to significant changes in the pulmonary microbiome, thereby increasing the risk of opportunistic infections and co-infections with mixed pathogens in PLWH[4].

Antiretroviral therapy (ART) has revolutionized the management of HIV infection by suppressing viral replication and restoring systemic immune function, particularly through the recovery of $CD4^+$ T cell counts[5, 6]. While ART's role in reducing HIV-related mortality and opportunistic infections is well established, its specific impact on the pulmonary microenvironment and microbial community structure remains incompletely elucidated. Previous studies have suggested that the severity of HIV-associated immune dysfunction correlates with alterations in lung microbiota[7], but inconsistencies persist regarding how ART modulates distinct pathogen classes (bacteria, fungi, viruses) within the pulmonary niche. Notably, PLWH with pulmonary infections often present with mixed-pathogen profiles, with fungi such as *Pneumocystis jirovecii* and *Talaromyces marneffei*, and viruses including *Human betaherpesvirus 5*, being among the most prevalent pathogens detected[8]. Understanding how ART reshapes these microbial communities is critical for optimizing clinical strategies for PLWH with pulmonary complications.

Traditional diagnostic methods for pulmonary infections in PLWH are often limited by low sensitivity, particularly for detecting mixed or opportunistic pathogens. Metagenomic next-generation sequencing (mNGS) has emerged as a powerful tool for comprehensive pathogen detection, capable of unbiased characterization of bacterial, fungal and viral communities in clinical samples such as bronchoalveolar lavage fluid (BALF)[9, 10]. Compared with conventional methods, mNGS has a significantly higher detection rate of pulmonary pathogens in PLWH[11, 12], making it an ideal approach to analyze the complex interactions between ART, immune reconstitution, and pulmonary microbiota.

To address the existing knowledge gap, this study aimed to systematically evaluate the impact of ART on the pulmonary microbiome composition, pathogen burden, and clinical outcomes in PLWH with pulmonary infections. Using mNGS, we characterized the bacterial, fungal, and viral profiles of PLWH who received antiretroviral therapy (ART) and those who did not. The differences between the two groups of people in terms of pathogen co-infection patterns, class-specific pathogen loads, and clinical outcomes were analyzed. We hypothesized that immune reconstitution induced by ART alters the structure of the pulmonary microbiota and reduces the total pathogen load, and has specific effects on bacterial, fungal and viral communities. The results of this study provide new insights into the ecological role of ART in shaping the pulmonary microenvironment and inform the development of targeted management strategies for PLWH with pulmonary infections.

Methods

Study subjects

In this retrospective study, we consecutively enrolled 62 PLWH with suspected lung infections at The Affiliated Changsha Hospital of Xiangya School of Medicine, Central South University (Changsha, China) from January 2020 to April 2024. The Affiliated Changsha Hospital of Xiangya School of Medicine is a Grade-A tertiary general hospital and also a national major infectious disease prevention and control base in China. Recruitment involved HIV-positive patients who were suspected of having pneumonia, based on new clinical symptoms and imaging findings. Subsequent inclusion required their consent for bronchoscopy, the acquisition of BALF samples suitable for mNGS analysis, and the availability of comprehensive medical records.

Data Collection

The following clinical and laboratory data were collected from all enrolled patients: age, sex, duration of antiretroviral therapy (ART), clinical manifestations, laboratory examination results (including complete blood count, neutrophil count, lymphocyte count, and $CD4^+$ T cell count), and patient outcomes.

BALF extraction and mNGS analysis

Samples were collected and processed according to established protocols. In brief, bronchoalveolar lavage fluid (BALF) was obtained via bronchoscopy following aerosol anesthesia with 2% lidocaine. Sterile physiological saline (pre-warmed to 37°C) was instilled and aspirated to recover the fluid, which was promptly transferred to a sterile, silicon-coated container. For nucleic acid extraction, BALF was centrifuged to collect a pellet. For nucleic acid extraction, BALF was centrifuged to collect a pellet. DNA extraction was performed using a dual lysis strategy combining enzymatic digestion and bead-beating to ensure thorough cell wall disruption, whereas RNA extraction included an initial RNA virus enrichment step prior to isolation. Sequencing libraries were prepared through fragmentation, end repair, adapter ligation, and PCR amplification, followed by quality control and sequencing on the BGISEQ-50/MGISEQ-2000 platform. After removing low-quality and human-derived reads, remaining sequences were aligned against a custom pathogen metagenomic database (PMDB) covering bacteria, fungi, viruses, and parasites.

Criteria for positive mNGS results.

Positive mNGS findings were defined based on established criteria: (i) documented pathogenicity; (ii) for bacteria (except mycobacteria), viruses, and parasites, a ≥ 10-fold higher coverage compared to conspecific microbes; (iii) for *Mycobacterium tuberculosis*, ≥ 1 read mapped to species/genus level, and for nontuberculous mycobacteria, reads ranking in the top 10 among bacteria; (iv) for fungi, a ≥ 5-fold coverage advantage. Common oropharyngeal commensals were excluded from positive calls regardless of read count. Final interpretation of infection versus colonization or contamination integrated clinical, radiographic, laboratory, and expert assessment.

Ethics approvals

This study conforms to the ethical norms of the 1975 Helsinki Declaration. Due to the retrospective nature of the study, written informed consent from the patients was not required. This study was approved by the Institutional Ethics Committee of the The Affiliated Changsha Hospital of Xiangya School of Medicine. The approval number is (2025) Lun KuaShen [Lin Wen] No. (15). All the data used for analysis were anonymized.

Statistical Analysis

All statistical analyses were performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA). The choice of statistical test was based on data properties. Continuous variables were first evaluated for normality and variance homogeneity. Comparisons between two groups were conducted using either the unpaired Student's t-test (for normally distributed data with equal variances) or the Mann-Whitney U test (for non-normally distributed or unequal-variance data). Categorical variables were analyzed with the chi-square test. Statistical significance was defined as a two-sided $P < 0.05$.

Results

1. Patient Characteristics

Among PLWH with pulmonary infection, this study compared the demographic and laboratory parameter characteristics between those receiving antiretroviral therapy (ART) and those not receiving ART (Table 1). The results showed that there were no statistically significant differences between the two groups in terms of age (median 50 years in the ART group vs. 47 years in the non-ART group, $P = 0.3136$) and gender composition (males accounted for 89.7% in the ART group and 75.8% in the non-ART group, $P > 0.9999$). However, the HIV viral load in the ART group was significantly suppressed (median 185 copies/mL vs. 259,000 copies/mL in the non-ART group, $P < 0.0001$), and the counts of white blood cells, neutrophils, lymphocytes, and $CD4^+$ T cells were all significantly higher in the ART group than in the non-ART group (for example, median $CD4^+$ T cell count was 131 cells/μL vs. 44 cells/μL, $P = 0.0049$).

Table 1
Contrast of characteristics of pulmonary infection patients among PLWH: those on antiretroviral therapy (ART) versus those without ART

Patient characteristicsa	On ART	NO ART	*P* value
	(*n* = 29)	(*n* = 33)	
Age (yrs, median [IQR])	50 (25 to 73)	47 (22 to 64)	0.3136
Male, n (%)	26 (89.7%)	25 (75.8%)	> 0.9999
HIV load (copies/mL, median [IQR])	185 (21 to 32800000)	259000 (29.7 to 23300000)	< 0.0001
WBC counts (per μL, median [IQR])	6380(3650 to11800)	4700(770 to 14240)	0.0022
Neutrophil counts (per μL, median [IQR])	4550(2010 to 10890)	2960(620 to 10980)	0.0162
Lymphocyte counts (per μL, median [IQR])	1090 (300 to 3310)	550 (60 to 2230)	0.0122
$CD4^+$ T cell count (per μL, median [IQR])	131(12 to 764)	44 (1 to 629)	0.0049

IQR, interquartile range.

2. Microbial Composition Profiles in PLWH With Pulmonary Infection

To elucidate the influence of ART on the pulmonary microbiota in PLWH with pulmonary infection, we employed mNGS to characterize the bacteria, fungi, and viral profiles in the lungs of infected patients receiving ART compared with ART-naïve individuals. The results showed that there were 31 positive and 2 negative microbial detections in the non-ART group, and 28 positive and 1 negative detections in the ART group (Fig. 1A), with similar positive detection rates between the two groups. The Venn diagram analysis revealed distinct patterns of mixed infections between the two groups. The ART group was predominantly characterized by bacterial-viral and bacterial-fungal-viral co-infections. In contrast, the non-ART group mainly presented with fungal-viral and bacterial-fungal-viral co-infections (Fig. 1B and 1C).

Specifically, for bacteria (Fig. 1D), *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* had equal counts in both groups. *Haemophilus influenzae* (3 in No ART group and 5 in On ART group) and *Staphylococcus aureus* (1 in No ART, 5 in On ART) were less frequent in the non-ART group. *Enterococcus faecium* (3 in No ART, 1 in On ART) was detected more frequently in the non-ART group. Lots of other bacteria had low numbers in both groups. In terms of fungal assemblages (Fig. 1E), *Pneumocystis jirovecii* and *Talaromyces marneffei* were found in higher amounts in the non-ART group, while the detected quantities of other fungi were relatively small. For viruses (Fig. 1F), Human beta-herpesvirus 5

(No ART: ~21, On ART: ~13) and Torque teno virus (No ART: ~15, On ART: ~4) were found more in the non-ART group. Human gamma-herpesvirus 4 had a higher number in the ART group (No ART: ~18, On ART: ~20). Other viruses had low numbers, and their detection patterns were different.

3. ART Reduces Overall Pathogen Burden Through Targeted Suppression of Fungal and Viral Loads

To further evaluate the impact of ART on the pulmonary microbiome in PLWH, we compared the pathogen loads between patients receiving ART and those not receiving ART. Patients on ART had a marked reduction in the total pulmonary pathogen burden, compared to those without treatment (mean ± SD: 3.7 ± 2.0 vs 5.0 ± 2.6, $P < 0.05$; Fig. 2A). This shows ART contributes to a decreased microbial load in the lung environment. ART worked really differently on different types of pathogens. Bacterial counts didn't show any significant difference between the two groups (ART group: 1.2 ± 1.0 vs non-ART group: 1.0 ± 1.2, $P > 0.05$; Fig. 2B). That means ART might not directly modulate bacterial colonization in the lungs here. But patients on ART had much lower fungal pathogen loads (0.8 ± 0.9) than those who didn't get treatment (1.5 ± 1.2; $P < 0.05$; Fig. 2C). This points to ART having a specific suppressive effect on fungal species. People on ART also had way lower viral pathogen loads (Fig. 2D). This shows ART works well to control virus replication or co-infections in the lungs.

4. Clinical Outcomes Differed Significantly Between ART and Non-ART Groups

The distribution of clinical outcomes differed significantly between the ART and non-ART groups (χ^2 test, $p = 0.036$) (Table 2). In the ART group (n = 29), 93.1% (27/29) of patients experienced clinical improvement, with no mortality (0.0%) and 6.9% (2/29) abandoning treatment. In contrast, the non-ART group (n = 33) showed a lower rate of improvement (72.7%, 24/33), with 3.0% (1/33) mortality and 24.3% (8/33) treatment abandonment. Regarding hospital stay, the ART group had a shorter mean duration (23 ± 13 days) than the non-ART group (30 ± 15 days). This difference was not statistically significant ($p = 0.069$).

Table 2
Comparison of clinical outcomes between patients on ART and no ART

Variables	**On ART (n = 29)**	**NO ART (n = 33)**	***P* value**
Hospital stay (Days, mean ± SD)	23 ± 13	30 ± 15	0.0687
Disease Outcome, n (%)			
1: Improved	27 (93.1)	24 (72.7)	0.036
2: Death	0 (0.0)	1 (3.0)	
3: Treatment Abandonment	2 (6.9)	8 (24.3)	

Discussion

Pulmonary complications remain a major determinant of outcomes in PLWH. Despite ART is established for systemic immune reconstitution, its specific impact on the pulmonary microenvironment has been incompletely understood [13, 14]. Using mNGS, this study comprehensively evaluated ART's impact on the pulmonary microbiome in PLWH with concurrent pulmonary infections, while systematically investigating its effects on clinical characteristics, pathogen loads, and clinical outcomes. Our core findings demonstrate that ART is closely associated with substantial alterations in the structure and taxonomic composition of the pulmonary microbial community and markedly reduces total pulmonary pathogen burden—an effect predominantly manifested by the effective suppression of fungal and viral loads. By integrating multi-dimensional immunological, microbiological, and clinical data, these results not only validate ART's established role in immune restoration but also delineate a novel "pathogen-class-specific regulatory pattern" of ART in shaping the pulmonary microenvironment, filling a critical gap in understanding how systemic immunotherapy remodels local organ microecology in immunocompromised populations and providing new insights for optimizing clinical management strategies for PLWH with pulmonary complications.

We first confirmed that ART significantly suppressed HIV viral load (median 185 vs. 259,000 copies/mL, $P < 0.0001$) and elevated peripheral immune cell counts, particularly $CD4^+$ T cells (median 131 vs. 44 cells/μL, $P = 0.0049$), without altering age or gender distribution. This finding confirms that ART enables effective systemic immune reconstitution in PLWH, aligning with its well-documented immunological benefits[15]. It further extends current knowledge by confirming that ART maintains immune-restorative effects even in the challenging context of secondary pulmonary infections[16, 17]. However, this rapid immune recovery is a double-edged sword. It can precipitate immune reconstitution inflammatory syndrome (IRIS), particularly in co-infected individuals such as tuberculosis, due to a brisk restoration of pathogen-specific responses[18, 19]. Despite this risk, the preponderance of evidence supports the early initiation of ART. The benefits—enhanced immune recovery, reduced HIV-related morbidity and mortality, and superior long-term outcomes—generally outweigh the manageable risks of IRIS[18, 20]. Consequently, clinical management must balance the imperative for early ART initiation with vigilant monitoring for IRIS.

Analysis using mNGS showed that ART changes the makeup of microbes in the lungs of PLWH in a pathogen-specific way. This change mainly happens because ART helps restore the immune system. A key thing we found is that common community-acquired pathogens—like *H. influenzae* and *S. aureus*—were found more often in the ART group. This shift signifies a transition toward an infection profile more characteristic of an immunocompetent host, where the partially restored immunity can suppress most opportunistic infections but remains susceptible to these common pathogens. In contrast, *S. pneumoniae* (which relies on biofilm formation)[21] and *M. tuberculosis* (which utilizes intracellular latency)[22] maintained similar detection frequencies between groups. The persistence of these pathogens highlights their capacity to evade even a partially recovered immune system, allowing for long-term colonization regardless of immune status. What's more, the total number of bacteria was about the same in both groups. This shows ART doesn't kill bacteria directly. It works by adjusting how

the immune system deals with them instead. The marked reduction in fungal pathogens following ART highlights their critical dependence on host immunosuppression.

Fungal germs dropped a lot after ART treatment. That tells us these fungi really depend on a weak immune system to grow. For example, *P. jirovecii* and *T. marneffei* are two big fungal threats for PLWH[8]. They were way more common in people who hadn't had ART. This makes sense because we already know ART helps the body make more $CD4^+$ T cells. Those cells boost the immune system's ability to fight fungi[23], and that stops these opportunistic fungi from multiplying.

When we analyzed the viral community, we found that ART treatment led to both expected and distinct changes in its composition. The overall number of viruses went down probably because ART directly stops HIV from copying itself. It might also have extra antiviral effects[24]. For example, CMV and TTV levels were much lower in ART group. However, EB virus maintained elevated levels despite therapy, underscoring the unique capacity of certain herpesviruses to sustain latency and reactivate, thereby evading even a partially reconstituted immune response[25]. Collectively, these findings illustrate that ART alleviates pulmonary dysbiosis. It cuts down on certain pathogens, shifts the community structure (β-diversity) to a better state and lowers overall fungal and viral loads. But it can't make the lung microbiome completely normal again.

Clinically, ART was associated with significantly improved outcomes. The significantly more favorable distribution of clinical outcomes in the ART group, characterized by superior clinical improvement, absent mortality, and reduced treatment abandonment, strongly affirms the critical role of antiretroviral therapy in enhancing clinical recovery and survival for hospitalized AIDS patients with pulmonary infections. This is consistent with the paradigm that early ART mitigates the severity of opportunistic infections through immune reconstitution[2]. Although the observed 7-day reduction in mean hospital stay for the ART group was not statistically significant ($p = 0.069$), it represents a clinically substantial trend. This lack of significance may be attributable to limited statistical power and the potential bias introduced by the high rate of early discharge due to treatment abandonment in the non-ART group, which could artifactually shorten their mean stay. Collectively, these findings underscore that inpatient ART initiation is a pivotal strategy to improve survival and clinical outcomes, while its full impact on healthcare utilization metrics like length of stay warrants further investigation in larger, prospective cohorts.

A key limitation of this study is its relatively small sample size, which may have limited the statistical power to detect subtle differences. Furthermore, as an inherent constraint of the methodology, mNGS cannot distinguish active microbial replication from non-viable nucleic acid residues[26]. Future studies could be strengthened by incorporating targeted NGS (tNGS) for its higher sensitivity in detecting specific pathogens of interest[11], or by integrating serological assays to validate active infection. Despite these limitations, the use of mNGS represents a significant methodological strength by providing an unbiased overview of the pulmonary microbiome. The internal consistency observed across HIV viral

load, immune cell counts, and fungal/viral load measurements reinforces the reliability of our core findings.

In conclusion, this study reframes ART as an ecological modulator of the pulmonary microenvironment, which establishes a new immunologic equilibrium by selectively suppressing opportunistic fungi and viruses while maintaining bacterial communities. This pathogen-class-specific restructuring, driven by immune reconstitution, revises our understanding of the host-microbe dynamic in HIV and supports the development of immunologically informed, pathogen-directed management strategies for pulmonary co-infections. Future studies are needed to validate the causality and mechanisms of these observations.

Abbreviations

ART
Antiretroviral therapy
BALF
Bronchoalveolar lavage fluid
HIV
Human Immunodeficiency Virus
mNGS
Metagenomic next-generation sequencing
PLWH
People living with HIV.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Hospital of Changsha. The study was conducted in accordance with the ethical standards of the 1975 Helsinki Declaration and its later amendments. Due to the retrospective nature of the study, written informed consent was waived by the Ethics Committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare no competing interests.

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Authors' contributions

YZ conceived and designed the study. LL, JW, and JL performed the experiments and collected the data. YW and YZ analyzed and interpreted the data. LL and YZ drafted the manuscript. All authors read and approved the final manuscript.

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Figures

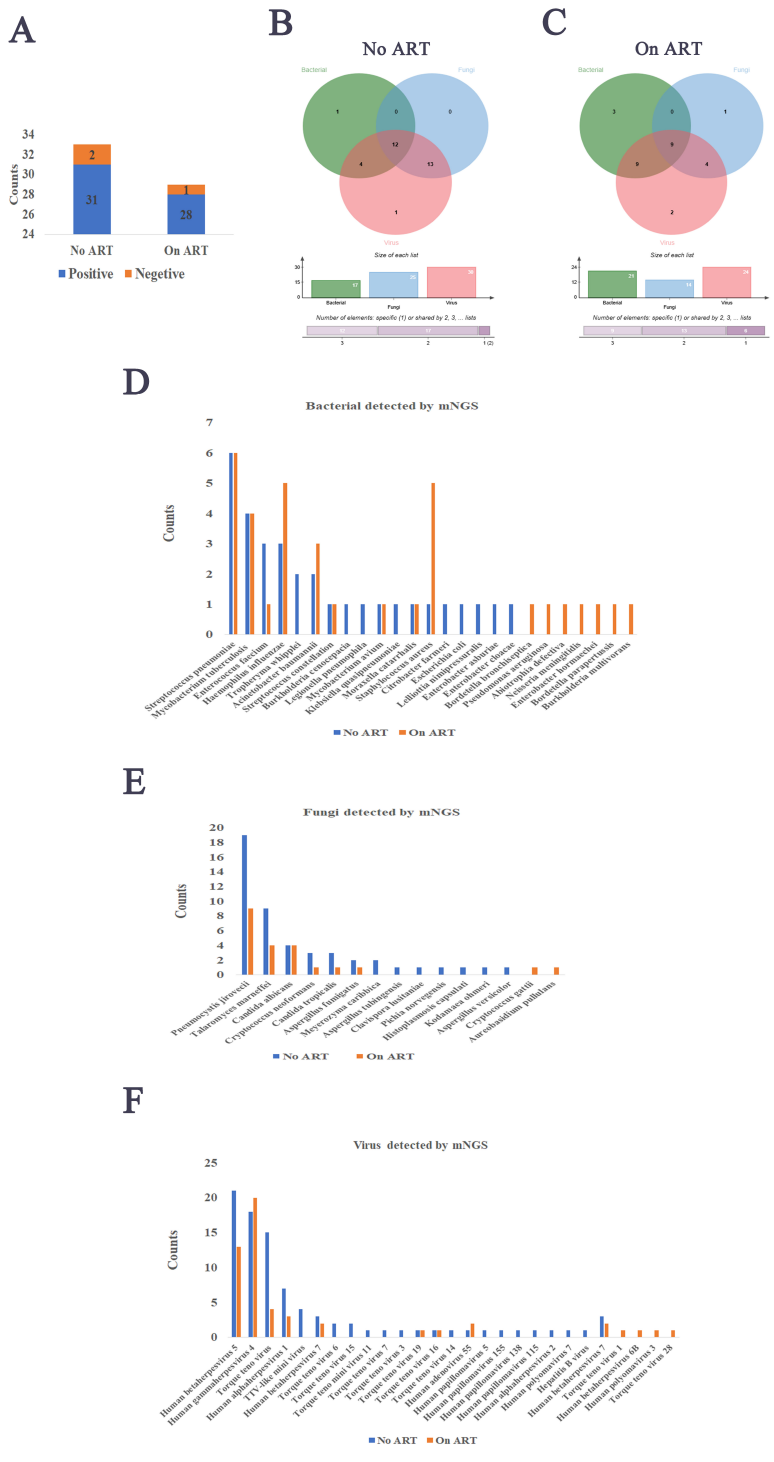


Figure 1

Microbial profiles detected by mNGS in PLWH with pulmonary infection, stratified by ART status. (A) Microbial detection results (positive or negative) in PLWH who didn't receive ART (No ART) and those who did (On ART). It gives a general look at the overall microbial detection rate for each group. **(B)** Venn diagram for No ART group. It shows how bacterial, fungal and viral taxa are distributed, plus how they appear together in the No ART group. **(C)** Venn diagram for On ART group. It shows the distribution and

co-occurrence of bacterial, fungal, and viral taxa in the On ART group, highlighting shared and unique taxa among different microbial categories. **(D-F)** Microbial community composition assessed by mNGS. They show the relative abundances of different **(D)**bacterial, **(E)** fungal, and **(F)** viral species in the lungs of PLWH from the No ART and On ART groups.

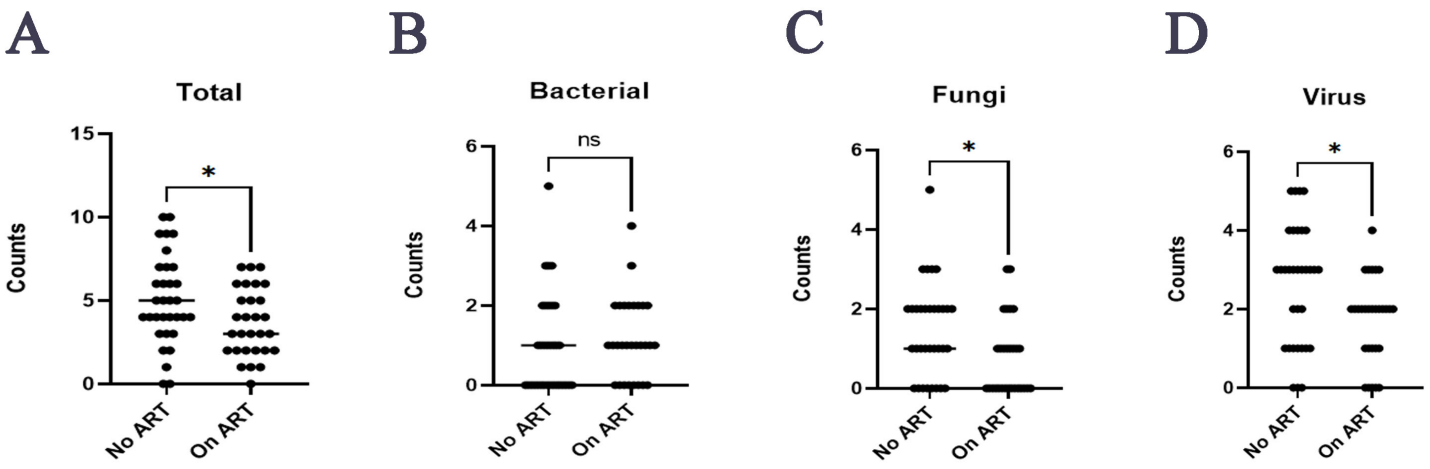


Figure 2

Assessment of pulmonary pathogen loads by ART status in PLWH. (A) Distribution of total pulmonary pathogen counts in people living with HIV (PLWH) with or without ART. (**B**) Analysis of bacterial pathogen counts in PLWH stratified by ART status. (**C**) Assessment of fungal pathogen counts in PLWH with or without ART intervention. (**D**) Evaluation of viral pathogen counts in PLWH according to ART receipt. Data are presented as mean ± standard deviation. *, $P < 0.05$; ns, no significant difference.