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MAJOR ARTICLE

Metagenomic next-generation sequencing (mngs) of bronchoalveolar lavage fluid on antimicrobial stewardship in patients with lower respiratory tract infections (lrtis): a retrospective cohort study

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Objectives: The impact of metagenomic next-generation sequencing (mNGS) on antimicrobial stewardship in patients with lower respiratory tract infections (LRTIs) is still unknown.

Methods: In this retrospective cohort study, patients diagnosed with LRTIs and underwent bronchoalveolar lavage (BAL) were included between September 2019 to December 2020. Individuals with mNGS and conventional microbiologic tests were classified as mNGS group, while patients only with conventional tests were included as control group. A 1:1 propensity score

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match for baseline variables was conducted, after which changes in antimicrobial stewardship between the two groups were assessed.

Results: 681 patients with an initial diagnosis of LRTIs who underwent BAL were evaluated. A total of 306 patients were finally included, with 153 in each group. mNGS was associated with lower rates of antibiotic escalation (adjusted odds ratio [OR] 0.466 [95% CI 0.237-0.919], p=0.02) than control group, while no association with antibiotic de-escalation. Compared to the control group, more patients discontinued the use of antivirals in the mNGS group (17/153, 11.1% vs. 5/153, 3.3%, p=0.008).

Conclusions: The use of mNGS was associated with lower rates of antibiotic escalation and may facilitate the cessation of antivirals, but not contribute to antibiotic de-escalation in LRTIs patients.

Keywords: metagenomic next-generation sequencing, lower respiratory tract infection, antimicrobial stewardship, antibiotic de-escalation, antibiotic escalation

INTRODUCTION

Lower respiratory tract infections (LRTIs) affected 489 million people globally and were responsible for >2.49 million deaths worldwide [1]. Accurate etiological diagnosis of LRTIs is crucial for targeted antibiotic therapy, prevention of antimicrobial resistance, and reduced healthcare-associated costs [2-3]. However, microbiological diagnosis was only achieved in less than 50% of patients with community-acquired pneumonia (CAP) by using conventional clinical microbiologic tests, including culture, serologic testing, antigen testing, and polymerase chain reaction (PCR) testing in the U.S [4]. In China, only 30~60% of LRTIs patients have pathogens found by conventional methods [5-6].

Metagenomic Next-Generation Sequencing (mNGS) is a novel technology for high-throughput sequencing of the total nucleic acid of all microorganisms in a sample, which can theoretically detect all pathogens in a sample independent of a pre-set pathogen suspected [7-9]. Attributed to the advantages that mNGS test has a high true negative rate and is less susceptible to clinical judgment [10], it could be assumed that the application of mNGS would drive a shift from empirical therapy to targeted antimicrobial therapy, and hence improve the outcomes of patients with LRTIs. However, the high sensitivity of mNGS is accompanied by a high false-positive rate, which means that mNGS has difficulties in interpretation [11-12]. Currently, the clinical benefit of mNGS on LRTIs is still unknown. We here initiated a retrospective cohort study to evaluate whether adding mNGS tests to conventional microbiologic tests versus conventional microbiologic tests alone could change antimicrobial stewardship in LRTI patients.

METHODS

Study design and population

This was a retrospective cohort study which enrolled hospitalized patients with LRTIs in China-Japan Friendship Hospital (CJFH) between September 2019 to December 2020. Patients were included if they met the following criteria: (I) aged ≥18 years; (II) with an initial diagnosis of LRTIs; (III) underwent bronchoscopy and bronchoalveolar lavage (BAL) for microbiological diagnosis during the hospitalization. The exclusion criteria were as follows: (I) incomplete clinical data; (II) mNGS test was performed on specimens other than bronchoalveolar lavage fluid (BALF), such as sputum and nasopharyngeal swabs. An episode of LRTIs in this study was defined as: (I) new or progressive infiltration, consolidation, ground-glass opacity, or interstitial changes on chest radiograph; (II) new-onset cough with sputum production, or exacerbation of the existing respiratory symptoms, with or without phlegm, chest discomfort, dyspnea, or hemoptysis; (III) fever; (IV) signs of lung consolidation and/or auscultatory findings such as altered breath sounds and/or localized rales; (V) peripheral blood WBC > 10×10^9 /L or < 4×10^9 /L. If meet (I) and any of (II) ~ (IV), an initial diagnosis of LRTIs would be established [13-15]. The researcher would made an initial diagnosis of LRTIs based on the above criteria, whereafter the final diagnosis of LRTIs would be confirmed according to the discharge diagnosis provided by the clinicians. Eligible patients were divided into the mNGS group and control group according to whether mNGS was performed. To assure patients in the mNGS and control groups have similar baseline clinical features, a 1:1 propensity score (PS) based matched analysis was conducted in the two groups for the following variables: age, sex, admission of ICU or not at disease onset, comorbidity (with or without), immune status (compromised or competent), the final diagnosis of non-severe community-acquired pneumonia (non-severe CAP), severe community-acquired pneumonia (SCAP), hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP). The study was approved by the ethics committee of CJFH (2022-KY-052).

Microbiologic methods and data collection

For patients in the mNGS group, bronchoscopy and BAL were performed and the BALF was collected and transported to four in vitro diagnostics laboratories (BGI Genomics, Shenzhen, China; Genskey Laboratory, Beijing, China; VISION Medicals, Shenzhen, China; HUGO Biotech, Beijing, China) for mNGS tests, which reported bacteria, fungi, mycobacteria and viruses in the samples that detected. In both the mNGS and control group, conventional microbiologic tests of BALF were performed, including smear stain and culture of bacteria, fungal and mycobacteria, PCR assays, and antigen tests. Sputum samples could also be performed for the same conventional microbiologic testing as BALF. The culture results of lower respiratory tract specimens were determined according to the standard operating procedures (SOP) of CJFH (see supplementary data). Blood samples were available for culture and cryptococcus antigen tests, while streptococcus pneumoniae antigen tests could be performed on urine samples. The available conventional microbiologic tests were listed in table S1 (see Supplementary data). The attending

physicians determined which conventional microbiologic tests should be performed according to patient's clinical manifestation. During the study period, all the patients were required to have PCR assay of oropharyngeal/nasopharyngeal swabs for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) before admission in CJFH according to the epidemic prevention policy of China, only those with negative results could be hospitalized in our hospital.

Definition and outcomes

The clinical information was collected from the electronic medical record system of CJFH. In this study, patients were diagnosed with immunosuppression when one of the following conditions presented: congenital/genetic immunocompromise, active malignancy or malignancy within one year of LRTIs, malignancies receiving chemotherapy, HIV infection, solid organ transplantation, hematopoietic stem cell transplantation, chronic steroid use, immunosuppressive drugs use and biological drug use [16]. The diagnosis of SCAP were based on the guideline from Infectious Diseases Society of America/American Thoracic Society [17]. Patients with either one major criteria: septic shock with need for vasopressors, respiratory failure requiring mechanical ventilation, or three or more minor criteria: respiratory rate ≥30 breaths/min, PaO2/FIO2 ratio ≤250, multilobar infiltrates, confusion/disorientation, blood urea nitrogen level>20 mg/dl, white blood cell count <4,000 cells/μl, platelet count <100,000/μl, core temperature <36℃, Hypotension requiring aggressive fluid resuscitation, were diagnosed with SCAP. The results of mNGS were usually returned 2 days after the bronchoscopy (Figure S1), while the results of conventional microbiologic tests reported within a week after the bronchoscopy performed. Possible colonized microbes detected by mNGS test and conventional microbiologic tests were excluded in determining the microbiological diagnoses of LRTI patients in this study based on the reference index of established lower respiratory tract pathogens [18-22]. We assessed the proportion of patients with antibiotic de-escalation or escalation in the mNGS and control group. The definition of antibiotic de-escalation and escalation refers to a previous study [23], which assigned frequently-used antibiotics into four ranks based on their antibacterial spectrum: Narrow spectrum (rank 1), Broad spectrum (rank 2), Extended spectrum (rank 3) and Protected (rank 4). Antibiotic de-escalation was defined as the discontinuation of one or more kinds of antibiotics in therapy, and/or the degradation of antibiotic of rank (from a broad-spectrum to a narrower-spectrum antibiotic). Antibiotic escalation was defined as adding one or more antibiotics to the current therapy or upgradation of the rank of antimicrobial agents, while unchanged was defined as either no change or a change in the opposite direction in the number and rank of antibiotics. In addition, the proportion of patients adding other antimicrobial agents (including antiviral agents, antifungal agents, and antitubercular agents, all counted separately) and the percentage of patients reducing other antimicrobial agents were also compared between the two groups. All the antimicrobial outcomes were measured within a week after bronchoscopy and BAL in the two groups. The treatment of CAP in CJFH were usually based on guideline from Chinese Thoracic Society [24], in which the treatment course of non-severe CAP is 5-7 days. In patients with severe CAP or with extrapulmonary complications, the duration of antibiotic therapy may be longer. The antimicrobial

stewardship program (ASP) team of CJFH would provide guidance on antimicrobial treatment according to the needs of clinicians during the antimicrobial therapy. Weekly holds multi-disciplinary treatment (MDT) discussions by ASP were also available for clinicians to acquire advises on antimicrobial stewardship. Moreover, clinical outcomes including length of hospital stay, duration of ICU stay, ICU admission rates, in-hospital mortality, and duration from bronchoscopy to discharge were also compared between the two groups.

Statistical analysis

Continuous data were expressed as medians and interquartile ranges (IQRs) or mean ± standard deviation depending on their distribution, while categorical data as frequency distributions. Mann-Whitney U test, Student t test, or chi-square test was performed for difference analysis depending on the data type and distribution. Multivariable logistic regression analyses were performed for antibiotic de-escalation and escalation separately. We also conducted multivariable logistic regression analyses to determine the effect of mNGS on the administration of other antimicrobial agents (including antiviral, antifungal, and antitubercular agents), ICU admission rate and in-hospital mortality rate. Multiple linear regressions were performed to assess the association of mNGS with length of hospital stay, days from bronchoscopy to discharge and length of ICU stay. All the analyses were done using SPSS 26.0, and $p < 0.05$ were considered statistically significant.

RESULTS

A total of 681 patients who underwent bronchoscopy and BAL with a diagnosis of "pneumonia", "pulmonary infection", "chronic obstructive pulmonary disease (COPD)", "lung abscess", or "bronchiectasis" in CJFH were assessed; 202 and 219 patients were included in the mNGS and control group, respectively. After the PS matching, 153 patients in both mNGS and control groups were included in the final analysis (Figure 1).

The baseline characteristics were similar between the mNGS group and the control group, but some differences remained in several items (Table 1). More patients in the control group had respiratory diseases than the mNGS group (48.4%, 74/153 vs. 28.8%, 44/153, $p<0.001$). The reasons for immunocompromise status were also different between the two groups: more patients in the mNGS group had hematologic cancer and fewer with solid organ transplantations than the control group (6.5%, 10/153 vs. 1.3%, 2/153, p=0.039; 0.7%, 1/153 vs. 9.2%, 14/153, p=0.001, respectively). In the mNGS group, 79.7% of patients had pathogens detected by mNGS plus conventional methods, compared to 34.6% in the control group by conventional methods (see Supplementary Table S2). Moreover, patients in the mNGS group have longer duration of antibiotic exposure prior to sampling (3.0 [1.0-6.0] days vs. 2.0 [1.0-4.0] days, p=0.01) than the control group.

Comparison of antimicrobial stewardship changes between mngs and control group

No significant difference was achieved in the proportion of patients with antibiotic de-escalation or antibiotic escalation between mNGS and control group (32%, 49/153 vs. 26.8%, 41/153, p=0.316; 19%, 29/153 vs. 26.8%, 41/153, p=0.102, respectively. Table 2). Multivariable logistic regression analysis showed that mNGS test was independently associated with lower rates of antibiotic escalation (adjusted odds ratio [aOR] 0.466 [95% CI 0.237-0.919], p=0.027; Table 3), but was not associated with antibiotic de-escalation (aOR 1.319 [0.756-2.302], p=0.330; Table S3). Pathogens detected by mNGS and/or conventional methods in patients with antibiotic de-escalation and escalation are shown in Table S6 and Table S7 (see Supplementary data). Most of the patients who were de-escalated with antibiotics had no bacteria detected in both mNGS group and control group (55.1%, 27/49 vs. 65.9%, 27/41, p=0.3, respectively). What's more, the median duration of antibiotic therapy was 15.0 (9.5-23.5) days in the mNGS group, longer than the 12.0 (7.0-18.0) days in the control group (p=0.01).

The proportion of patients who were newly added antiviral agents in the mNGS group was less than that in control group (5.2%, 8/153 vs. 12.4%, 19/153, p=0.027; Table 2). In detail, ganciclovir was the only antiviral agent newly added to the mNGS and the control group (see Supplementary Table S4). Meanwhile, a higher proportion of patients in the mNGS group had their antiviral therapies ceased than that in the control group (11.1%, 17/153 vs. 3.3%, 5/153, p=0.008; Table 2); the most discontinued antiviral drug in both groups was also ganciclovir, followed by oseltamivir (see Supplementary Table S4). Multivariable logistic regression analyses showed that mNGS test had no significant impact on the addition of antivirals, antifungals, and antitubercular agents (aOR 0.845 [0.466-1.533], p=0.580; see Supplementary Table S5), but was independently associated with the cessation of antiviral agents and/or antifungal agents (aOR 2.523 [1.143-5.568], p=0.022; Table 4, Table S8 (see Supplementary data).

Comparison of clinical outcomes between mngs and control group

The median length of hospital stay was longer in the mNGS group than in the control group (18.0 [13.0-30.0]) days) vs.14.0 [9.5-21.0 days], p=0.01). Furthermore, the median duration from bronchoscopy to discharge was 14.0 (8.5-24.5) days in the mNGS group, longer than the 12.0 (6.0-19.0 days) days in the control group (p=0.007). Patients in the mNGS group also had a longer duration of ICU stay compared with the control group (14.0 days [10.0-28.0 days] vs. 11.9 days [6.0-20.8 days], $p<0.001$). Multiple linear regressions showed that mNGS were not associated with length of hospital stay, days from bronchoscopy to discharge, and length of ICU stay (B=11.246, p=0.120; B=8.288, p=0.248; and B=-0.848, p=0.875, respectively). The proportion of patients admitted to ICU during hospitalization was similar between the two groups (45.1%, 69/153 vs. 43.8%, 67/153, p=0.818). There were 16 (10.5%) patients in mNGS group and 24 (15.7%) in the control group died during the hospitalization, with no significant difference between the two groups (p=0.175). mNGS test was not associated with ICU admission rate and in-hospital

mortality rate according to multivariable logistic regression models (aOR 0.950 [0.272-1.280], p=0.893 for ICU admission rate; aOR 0.650 [0.285-1.480], p=0.305 for in-hospital mortality rate).

DISCUSSION

This study found that the combination of mNGS test and conventional microbiologic methods is associated with lower rate of antibiotic escalation and may contribute to the cessation of antiviral drugs in patients with LRTIs compared with conventional methods alone. However, the addition of mNGS to conventional microbiologic methods was not associated with the de-escalation rates of antibiotics, or associated with length of hospital stay, length from bronchoscopy to discharge, duration of ICU stay, ICU admission rate, or in-hospital mortality rate of LRTI patients.

The impact of mNGS on antibiotic stewardship is ambiguous based on previous studies. Zhou et al. found that the application of mNGS led to antibiotic de-escalation in 25.2% of 159 patients with pneumonia in a before-after study without a control group [25]. Meanwhile, Liang et al found that only five (3.6%) patients underwent antibiotic de-escalation by using mNGS test in 140 patients with suspected LRTIs [26]. However, these studies lacked a strictly matched control cohort and the definitions of antibiotic de-escalation and escalation were ambiguous. This study found that the use of mNGS was associated with decreased rates of antibiotic escalation. The pathogen detection rate of mNGS test was relatively high, not to mention its wide-field pathogen detection range and rapid turn-around time [25,27-30]. For common pathogenic bacteria infections, the decision not to escalate antibiotics might be reasonable when empirical antimicrobial therapy has covered the pathogens detected by mNGS tests. By contrast, this study observed no association of antibiotic de-escalation and mNGS test, probably due to the difficulty in interpreting mNGS results, as mNGS reported all the microorganism detected in the sample, including opportunistic pathogen and resident flora in the respiratory tract. Currently, there is no good approach to distinguish pathogens from colonized microbes and the organisms detected by mNGS may differ from those detected by conventional microbiologic tests. As a result, clinicians are likely to adopt relatively conservative antibiotic regimens relying on conventional methods result, rather than antibiotic de-escalation. To better utilize the advantages of mNGS, it is urgent to develop approaches that can distinguish causative pathogens from commensal microbes in the respiratory tract. Therefore, the application of mNGS should be limited to appropriate clinical scenarios and in accordance with indications, but not be used routinely [31].

There are few studies on the impact of mNGS on other antimicrobial agents other than antibiotics in LRTI patients. The hypothesis-free diagnostic properties of mNGS give it the advantage of identifying rare as well as unexpected pathogens, including viruses, fungi, and parasite [32-33]. Thus, we focused on the addition and reduction of antivirals, antifungal and antitubercular agents in this study. No virus detected by either mNGS or conventional test confirmed the absence of viral infection, which makes empirical antiviral usage ceased, consistent with our findings that the combination of mNGS and conventional tests may promote the discontinuation of antivirals.

Studies have shown that mNGS tests have low sensitivity in the diagnosis of fungal infection [30,34], which may explain why the combination of mNGS and conventional tests may not influence the use of antifungal agents.

This study was a retrospective study with biases in data collection and analysis. Though a PS matching was conducted to equilibrate potential factors influencing antimicrobial strategies between the mNGS and control groups, there were still differences remained in the proportion of patients with respiratory disease, causes of immunocompromised status, and the duration of antibiotic exposure prior to sampling in the two groups, which could affect the reliability of the study results. Hence, further prospective studies are needed to confirm the results.

In conclusion, adding mNGS tests to conventional microbiologic methods was associated with lower rates of antibiotic escalation and may facilitate the cessation of empirical antiviral therapies in LRTIs patients. However, the combination of mNGS tests and conventional methods may not change antibiotic de-escalation rate in patients with LRTIs. The clinical scenarios in which LRTI patients would benefit from mNGS need to be clarified by strictly designed clinical studies.

Conflict of Interest. All authors declare no competing interests in this paper.

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Author's contributions

Conceptualization: BC; Patient enrolment and data collection: MWY, XHZ, CHW, ZBL, XJC; Analysis: MWY, YeMW, LHS, YiMW; Writing: MWY, YeMW, XHZ.

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Table 1. Baseline characteristics of patients with lower respiratory tract infections

	mNGS (n=153)	Control (n=153)	p value
Male	102 (66.7%)	93 (60.8%)	0.285
Age (years)	64.0 (56.8-71.0)	63.0 (54.0-72.0)	0.371

Comorbidity	134 (87.6%)	132 (86.3%)	0.734
Cardiovascular disease	66 (43.1%)	62 (40.5%)	0.643
Respiratory disease*	44 (28.8%)	74 (48.4%)	<0.001
Diabetes	32 (20.9%)	33 (21.6%)	0.889
Rheumatic disease	19 (12.4%)	22 (14.4%)	0.615
Renal disease	12 (7.8%)	6 (3.9%)	0.145
Cancer	30 (19.6%)	21 (13.7%)	0.167
Liver disease	6 (3.9%)	3 (2.0%)	0.499
ICU at disease onset	52 (34.0%)	52 (34.0%)	> 0.99
Immunocompromised	48 (31.4%)	50 (32.7%)	0.806
Receiving corticosteroid therapy	25 (16.3%)	33 (21.6%)	0.243
Receiving immunosuppressive drugs	20 (13.1%)	33 (21.6%)	0.05
Hematologic cancer*	10 (6.5%)	2 (1.3%)	0.039
Solid organ transplantation*	1 (0.7%)	14 (9.2%)	0.001
HIV infection with CD4-lymphocyte count < 200 cells/μL or percentage < 14%	0 (0.0%)	2 (1.3%)	0.498
Laboratory tests			
White cell count (×10⁹ per L)	8.2 (5.6-11.5)	8.3 (5.6-11.9)	0.620
Neutrophils (×10⁹ per L)	6.7 (3.7-9.4)	6.6 (3.7-10.2)	0.535
lymphocyte count (×10⁹ per L)	1.0 (0.5-1.6)	1.1 (0.8-1.6)	0.270
hemoglobin (g/L)	115.0 (101.5-132.0)	117.0 (102.0-128.0)	0.585
platelet count (×10⁹ per L)	211.0 (151.0-279.5)	215.5 (160.3-284.0)	0.972
PCT (ng/ml)	0.1 (0.1-0.5)	0.1 (0.1-0.6)	0.596
CRP (mg/L)	55.0 (8.3-191.0)	52.5 (5.0-162.2)	0.926
Final diagnosis			
CAP	100 (65.4%)	96 (62.7%)	0.634
non-severe CAP	61 (39.9%)	63 (41.2%)	0.816
SCAP	39 (25.5%)	33 (21.6%)	0.419
HAP	13 (8.5%)	16 (10.5%)	0.558
AECOPD	5 (3.3%)	10 (6.5%)	0.186
Bronchiectasis	4 (2.6%)	8 (5.2%)	0.377

VAP	3 (2.0%)	4 (2.6%)	1.0
Lung abscess	1 (0.7%)	6 (3.9%)	0.126
Other[a*]	27 (17.6%)	12 (7.8%)	0.01
Other infectious diseases	8 (5.2%)	13 (8.5%)	0.258

Data are expressed as n (%) or median (IQR). mNGS=metagenomic next-generation sequencing. ICU=Intensive Care Unit. HIV=human immunodeficiency virus. PCT=procalcitonin. CRP=c-reaction protein. CAP=community-acquired pneumonia. SCAP=severe community-acquired pneumonia. HAP=hospital-acquired pneumonia. VAP=ventilator-associated pneumonia. AECOPD=acute exacerbation of chronic obstructive pulmonary disease.

* Represents a statistically significant difference with $p < 0.05$.

[a] Includes infective exacerbation of interstitial lung diseases, pulmonary mycobacteria infection, bronchitis, and infective exacerbation of bronchial asthma.

Table 2. Comparison of the use of antimicrobial agents

Variables	mNGS (n=153)	Control (n=153)	p value
Antibiotic therapy before bronchoscopy	130 (85.0%)	133 (86.9%)	0.622
Duration of antibiotic exposure prior to sampling*	3.0 (1.0-6.0)	2.0 (1.0-4.0)	0.01
Duration of antibiotic therapy*	15.0 (9.5-23.5)	12.0 (7.0-18.0)	0.01
Antimicrobial agents change	115 (75.2%)	110 (71.9%)	0.517
Antibiotic change			0.237
De-escalation	49/130 (32.0%)	41/133 (26.8%)	0.316
Escalation	29 (19.0%)	41 (26.8%)	0.102
Add other antimicrobial agents	39 (25.5%)	38 (24.8%)	0.895
Antiviral agents*	8 (5.2%)	19 (12.4%)	0.027
Antifungal agents	31 (20.3%)	24 (15.7%)	0.297
Antitubercular agents	2 (1.3%)	2 (1.3%)	>0.99
Reduce other antimicrobial agents*	28 (18.3%)	14 (9.2%)	0.02
Antiviral agents*	17 (11.1%)	5 (3.3%)	0.008
Antifungal agents	13 (8.5%)	11 (7.2%)	0.671

Data are expressed as n (%). mNGS=metagenomic next-generation sequencing.

* Represents a statistically significant difference with $p < 0.05$.

Table 3: Univariate and multivariate logistic regression analyses for antibiotic escalation of patients with LRTIs

Variable	Univariable[a]		Multivariable	
	OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age≥65y	1.337 (0.783-2.284)	0.287	1.489 (0.766-2.896)	0.241
Male sex	1.215 (0.691-2.136)	0.499	1.118 (0.570-2.196)	0.745
ICU at disease onset	1.404 (0.809-2.434)	0.228		
Respiratory disease	1.168 (0.678-2.012)	0.575	0.894 (0.455-1.755)	0.744
Diabetes	1.131 (0.596-2.145)	0.707		
Rheumatic disease	1.477 (0.710-3.074)	0.297		
Hematologic cancer	0.296 (0.038-2.337)	0.248	0.614 (0.073-5.171)	0.654
Solid organ transplantation	1.738 (0.574-5.267)	0.328	1.626 (0.450-5.876)	0.459
non-severe CAP	0.769 (0.442-1.337)	0.351		
SCAP	1.415 (0.774-2.587)	0.259		
HAP/VAP	0.959 (0.416-2.210)	0.921		
Duration of antibiotic exposure prior to sampling	1.010 (0.994-1.027)	0.221	1.009 (0.992-1.027)	0.294
mNGS test*	0.639 (0.372-1.096)	0.104	0.466 (0.237-0.919)	0.027

LRTIs=lower respiratory tract infections. mNGS=metagenomic next-generation sequencing. ICU=intensive care unit. non-severe CAP=non-severe community-acquired pneumonia. SCAP=severe community-acquired pneumonia. HAP=hospital-acquired pneumonia. VAP=ventilator-associated pneumonia.

* Represents a statistically significant difference with $p < 0.05$.

[a] Variables with a univariable $p < 0.05$ or with potential clinical relevance were included in the multivariable model.

Table 4: Factors associated with other antimicrobial agent[a] reduction in patients with lower respiratory tract infections

Variable	Univariable[b]		Multivariable	
	OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age≥65y	1.326 (0.689-2.549)	0.398	1.150 (0.554-2.387)	0.707
Male sex	1.326 (0.689-2.549)	0.398	1.315 (0.615-2.809)	0.480

ICU at disease onset	1.740 (0.899-3.366)	0.100		
Respiratory disease	0.596 (0.292-1.217)	0.155	0.462 (0.202-1.059)	0.068
Diabetes	0.854 (0.375-1.948)	0.708		
Rheumatic disease	0.856 (0.316-2.321)	0.760		
Hematologic cancer	2.179 (0.565-8.402)	0.258	1.530 (0.356-6.578)	0.568
Solid organ transplantation*	2.421 (0.734-7.991)	0.147	5.440 (1.344-22.023)	0.018
non-severe CAP	0.474 (0.229-0.984)	0.045	0.555 (0.251-1.225)	0.145
SCAP	1.360 (0.656-2.818)	0.408		
HAP/VAP	1.016 (0.371-2.778)	0.976		
Duration of antibiotic exposure prior to sampling	1.016 (0.989-1.044)	0.258	1.016 (0.994-1.038)	0.152
mNGS test*	2.224 (1.121-4.414)	0.022	2.523 (1.143-5.568)	0.022

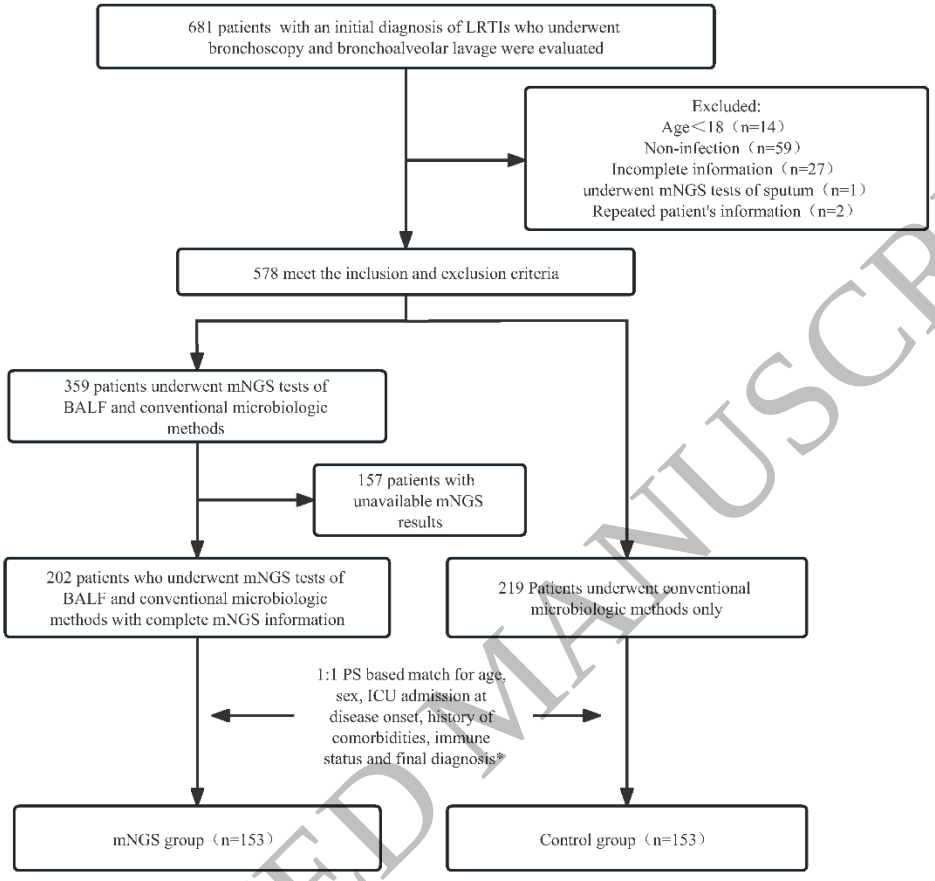
mNGS=metagenomic next-generation sequencing. ICU=intensive care unit. non-severe CAP=non-severe community-acquired pneumonia. SCAP=severe community-acquired pneumonia. HAP=hospital-acquired pneumonia. VAP=ventilator-associated pneumonia.

* Represents a statistically significant difference with $p < 0.05$.

[a] Other antimicrobial agent includes antivirals and antifungal agents.

[b] Variables with a univariable $p < 0.05$ or with potential clinical relevance were included in the multivariable model

Figure 1. Flowchart of the patients included in the study.



Note. mNGS=metagenomic next generation sequencing. LRTIs=lower respiratory tract infections. BALF= bronchoalveolar lavage fluid. PS=propensity score.

* Final diagnosis includes non-severe community-acquired pneumonia, severe community-acquired pneumonia, hospital-acquired pneumonia, and ventilator-associated pneumonia.