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Pulmonary microbiology and microbiota in adults with non-cystic fibrosis bronchiectasis: a systematic review and meta-analysis

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Abstract

Background Non-cystic fibrosis bronchiectasis is associated with frequent and diverse microbial infections, yet an overall understanding of microbial presence across different disease stages is lacking.

Methods A meta-analysis assessed lung microbes in adults with non-CF bronchiectasis, collecting data using both culture-based and sequencing approaches through three international databases and three Chinese databases. Subgroups were categorized by disease stage: the stable group (S), the exacerbation group (E), and unclassified data consolidated into the undetermined group (U). Culture data were analysed in random-effects meta-analyses while sequencing data were processed using QIIME 2.

Results A total of 98 studies were included with data from 54,384 participants worldwide. *Pseudomonas aeruginosa* was the most frequently isolated bacterium (S: 26[19–34]%, E: 23[20–25]%, U: 20[16–25]%), while not specified *Mycobacterium avium* complex exhibited the highest mycobacterial prevalence (S: 3[1–5]%, E: 4[2–5]%, U: 15[3–27]%). *Aspergillus* spp. (S: 15[−10–39]%, E: 2[1–3]%, U: 10[5–15]%) and *Candida* spp. (S: not applicable, E: 11[2–20]%, U: 10[−8–27]%) were predominant in fungi culture with variable distributions among groups. Rhinovirus was the most commonly detected virus with varying prevalence across airway sample types rather than disease stages (S-sputum: 18[−16–53]%, S-nasopharyngeal: 4[−1–9]%, E-sputum: 22[16–29]%, E-nasopharyngeal: 6[4–8]%). Sequencing results revealed notable antibiotic persistence of *Pseudomonas* in 16S, and significant domination of *Candida* in ITS.

Conclusion Our findings indicate consistent bacterial patterns throughout bronchiectasis stages in both culture and sequencing results. Viruses are extensively detected in stable patients but vary across different airway sample types. Lower bacterial diversity and higher fungal diversity may be associated with exacerbation risks.

Keywords Bronchiectasis, Exacerbations, Microbiology, Microbiota, Prevalence

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Background

Non-cystic fibrosis (non-CF) bronchiectasis is a progressive chronic respiratory condition characterized by bronchial dilation and impaired mucociliary clearance, resulting in intermittent pulmonary exacerbations [1–3]. Frequent exacerbations are closely associated with poor prognosis and increased mortality [4, 5] with microbial infections widely recognized as main causes requiring antimicrobial therapy [2, 6, 7]. Bacteria have traditionally been emphasized as the primary agents in exacerbations, while their patterns and spectra vary globally [8–10]. Moreover, emerging evidence highlights the substantial impact of viruses [11, 12], potentially contributing to the misuse or overuse of antibiotics. Additionally, microbial isolations are also observed during stable periods [10, 13], yet the prevalence of microbial colonization or chronic infections remain inadequately addressed.

Despite numerous studies exploring the prevalence of microbes in bronchiectasis, these investigations often focus solely on single types of microorganisms or primarily concentrate on a single disease stage. Some studies even yield conflicting results [14, 15]. Hence, we conducted a systematic review and meta-analysis assessing pulmonary microbes including bacteria, fungi, and viruses across all disease stages in patients with non-CF bronchiectasis. To gain a more comprehensive global perspective, we selected both international and Chinese databases. We focused on observational studies to minimize potential confounding effects, particularly those associated with antibiotics in interventional studies. This effort aims to enhance our understanding of complete lung microbiology and its variation across disease stages, providing a broader insight on the overall microbial contribution to exacerbations.

Methods

This meta-analysis was carried out in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis [16] (PRISMA) guidelines, and the protocol was registered in the NIHR PROSPERO database with ID number CRD42021269668. All data were from published studies; thus, no ethical approval was required.

Search strategy and inclusion criteria

A literature search was conducted in three English-language databases (PubMed, Embase, and the Cochrane Library) as well as three Chinese-language databases (Chinese Biomedical Literature Database, China National Knowledge Infrastructure, and Wanfang). To have a current and updated perspective, the search was restricted from January 1st, 2000 to August 1st, 2023. The studies were limited to publications in English or Chinese. The primary search terms included "bronchiectasis," "microbiology", "bacteria", "virus", "fungus", etc. The detailed search strategy is provided in e-Appendix 1.

Studies were included if they fulfilled the following: a. Participants:1) patients aged 18 years and above; 2) a clinical diagnosis of non-CF bronchiectasis confirmed by computer tomography; and 3) Patients without other pulmonary disease comorbidities, including asthma, chronic obstructive pulmonary disease (COPD), lung cancer, or other significant pulmonary conditions. Bronchiectasis cases not explicitly designated as cystic fibrosis were considered as non-cystic fibrosis bronchiectasis by default. b. Microbiological outcomes: 1) any microbiological isolation/detection, without restrictions on the methodology used; and 2) data or raw data available for analysis. c. Study design: original observational research including cross-sectional and cohort studies.

Data extraction and quality assessment

The search was conducted by an experienced epidemiologist, and included both electronic and manual components. Supplementary searches within article references ensured comprehensive coverage. Duplicate records were identified and removed using EndNote X8 (Clarivate Analytics, Philadelphia, PA, USA).

Two authors (Y. W. and J. X.) independently screened studies by titles and abstracts, and then through the full text against the predetermined criteria. Data were extracted including study identifiers, publication year, geographical location, study design, sample size, patient characteristics (age, sex, FEV1% predicted values), and microbiology information (sample types, number of samples, number of participants providing samples, sample collection period, sampling modality, microbial detection methods, microbial isolation rates, potential pathogen proportions). A few studies reported multiple isolations within single samples, for data consistency, these data were incorporated into the calculation of the prevalence for individual microorganisms. Authors of studies with incomplete or uncertain results were contacted for further information. Rare microorganisms with reported frequencies of less than 5% were excluded for the meta-analysis.

The data were categorized based on the disease stages: the stable (S) group or the exacerbation (E) group. If the stages were not defined in the original articles, the data were consolidated into the undetermined (U) group. Microbes were classified into bacteria, fungi, and viruses. Mycobacteria data was presented separately due to its clinical significance in bronchiectasis. Sequencing data was analysed independently to accommodate its different datatypes.

Discrepancies were resolved through discussion or, if necessary, with the involvement of a third-party adjudicator (L.W.). Two authors J. X., and X. Y., independently performed the quality assessment (risk of bias) of the eligible publications. The Newcastle–Ottawa scale [17] (NOS) was employed for cohort studies, and the Joanna Briggs Institute [18] (JBI) statement was utilized for cross-sectional studies (e-Appendix 2).

Statistical analysis

A series of meta-analysis of single proportions was conducted to assess the prevalence of microbes across all included studies, following the methodology outlined by Balduzzi et al [19]. The random-effects model [20] without transformation was employed. The same method was applied for the data of antimicrobial resistance. Subgroup analyses were performed, categorizing studies based on disease stages. All statistical computations were performed using R statistical software (version 4.3.1). The meta package was employed for meta-analyses, and the ggplot2 package utilized for visualizing the results.

Sequencing data acquisition and analysis

High throughput sequencing data, encompassing both the raw sequences and corresponding metadata, were either downloaded from the Sequence Read Archive (SRA) or obtained through the links provided in the publications.

Differing from the culture data, the sequencing data were classified into four groups as defined in the included studies: baseline (B), exacerbation (E), treatment (T), or recovery (R). The baseline group (B) were data collected from patients in stable periods. The exacerbation group (E) included data obtained during exacerbations—specifically prior to the initiation of antibiotic treatment. The treatment group (T) encompassed data when patients were under antibiotic therapy. The recovery group (R) comprised data from patients post-antibiotic therapy cessation within two weeks or 30 days [21, 22].

The 16S and ITS gene sequencing data were processed using Quantitative Insights into Microbial Ecology2 [23] (QIIME 2 (2021.4)). Briefly, the quality of the sequence reads was assessed with fastp [24] to remove low-quality regions. The remaining paired-end reads underwent clustering using the q2-dada2 denoising pipeline. After that, the 16S data were matched to the silva-13–8 -99 Operational Taxonomic Units (OTUs) reference (version 13.8), while the ITS data were assigned to sh_qiime_release_10.05.2021.tgz. It was accomplished using the q2-feature-classifier, classify-sklearn naïve Bayes taxonomy classifier, to generate an Amplicon Sequence Variant (ASV) table. The process was assisted by HMGA tools—a freely available online platform (http://www.gigaomics.com/).

Genus read counts were normalized into genus profiles by dividing them by the total read counts in one sample. The alpha diversity and community composition were assessed using permutational multivariate analysis of variance [25] (PERMANOVA) and was calculated using the vegan package [26]. Dirichlet multinomial mixtures [27] (DMM) were used to classify different bacteria community types. The read count at the genus-level were then combined, and the samples were clustered using the Dmn function in the Dirichlet Multinomial package [28]. Differential bacteria genus between groups was identified using linear discriminant analysis effect size [29] (LEfSe) with an LDA score > 2 and p values < 0.05, implemented on the Galaxy platform (http://huttenhower.sph.harvard.edu/galaxy). Visualization was done using the ggplot2 package in the R platform (v 4.2.1).

Results

The overall strategy of data search and selection is shown in Fig. 1; 98 studies were included with a total of 54,384 participants from 41 countries worldwide—predominantly in Asia and Europe (Fig. 2). The study characteristics and population demographics are listed in e-Table 1–3. The average age of participants was 61 ± 7 years with approximately 53% females. Lung function was reported in 26 studies, showing a mean FEV1 (% predicted) of 64.5 ± 11.

The quality assessment (e-Appendix 2) indicates that most included studies reported data completely with valid and reliable measurements. However, many of them did not specify confounding factors. No studies were excluded based on the quality assessment.

Microbiology patterns

Bacteria

The prevalence of bacteria (excluding mycobacterium) is shown in Fig. 3a. In total, 41,156 participants from 89 studies provided respiratory specimens for bacteria culture. Of these, 60 studies reported bacteria isolation from 11,015 participants during exacerbations, 14 studies identified bacteria from 11,372 stable patients, and 17 studies encompassing 28,053 patients did not specify disease stages, which were aggregated into the undetermined group. More than 35 bacteria were reported with some of them classified at the genus or family levels. To present the data more effectively, we conducted a meta-analysis on the top 10 most frequently reported bacteria at the species level, while all other bacteria were grouped under the "other bacteria" category.

Pseudomonas aeruginosa stood out as the most frequently isolated bacteria across all groups with a

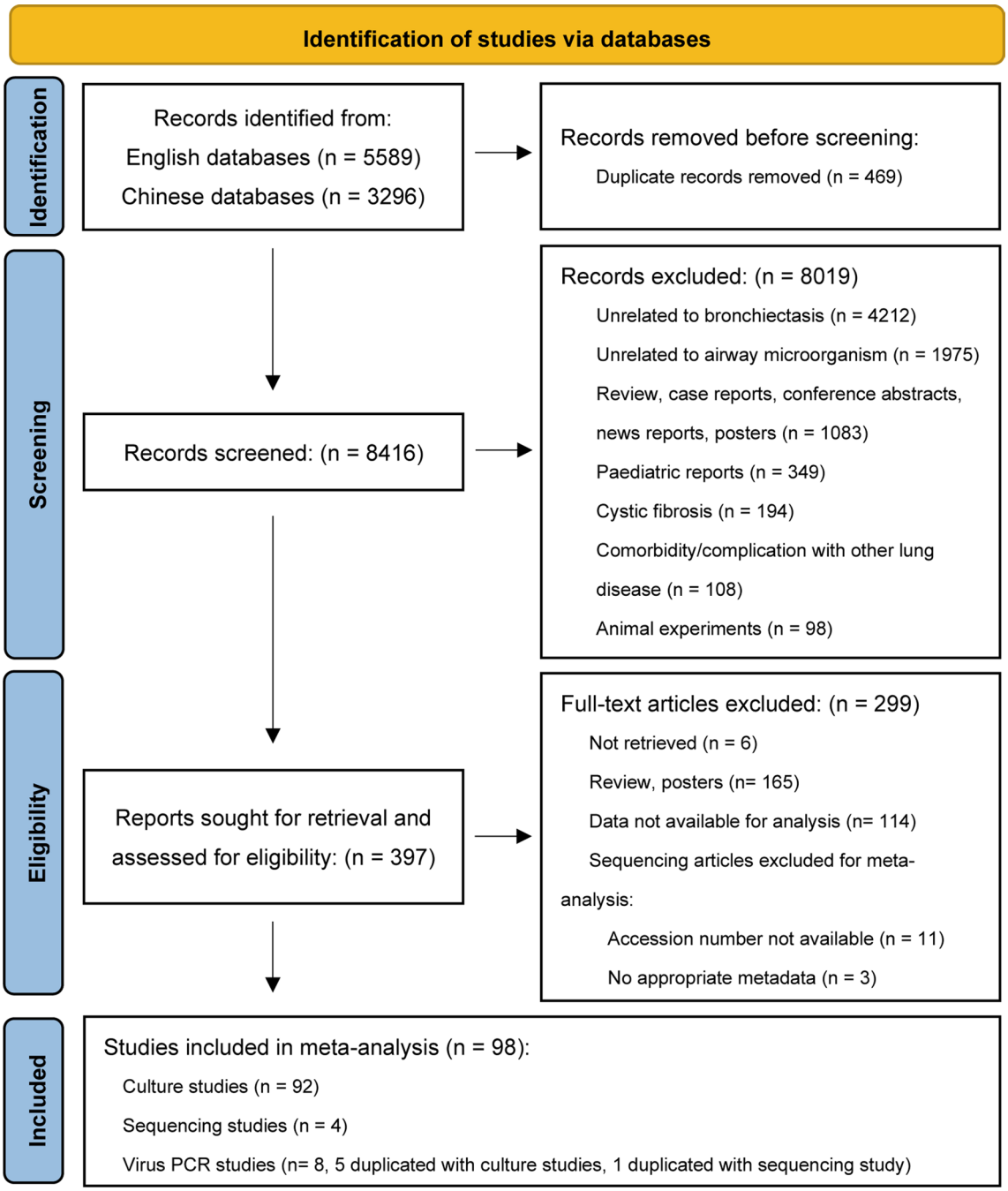

Fig. 1 Flowchart of study selection and inclusion

relatively consistent distribution regardless of the participants' stages (S: 26[19–34]%, E: 23[20–25]%, U: 20[16–25]%; $I^2 = 98\%$). The prevalence of Haemophilus influenzae (S: 21[10–31]%, E: 4[3–6]%, U: 15[9–21]%; $I^2 = 99\%$), and Streptococcus pneumoniae (S: 10[1–19]%, E: 3[2–5]%, U: 9[5–14]%; $I^2 = 98\%$) was higher in the stable group than the exacerbation group. In contrast, Klebsiella pneumoniae (S: 1[0–1] %, E: 7[5–8]%, U: 5[2–8]%; $I^2 = 87\%$) exhibited increasing rates in the exacerbation group. For Enterobacter cloacae (S: not applicable (N/A), E: 1[1, 2]%, U: 2[1–3]%; $I^2 = 33\%$), although the prevalence was low, no study reported its

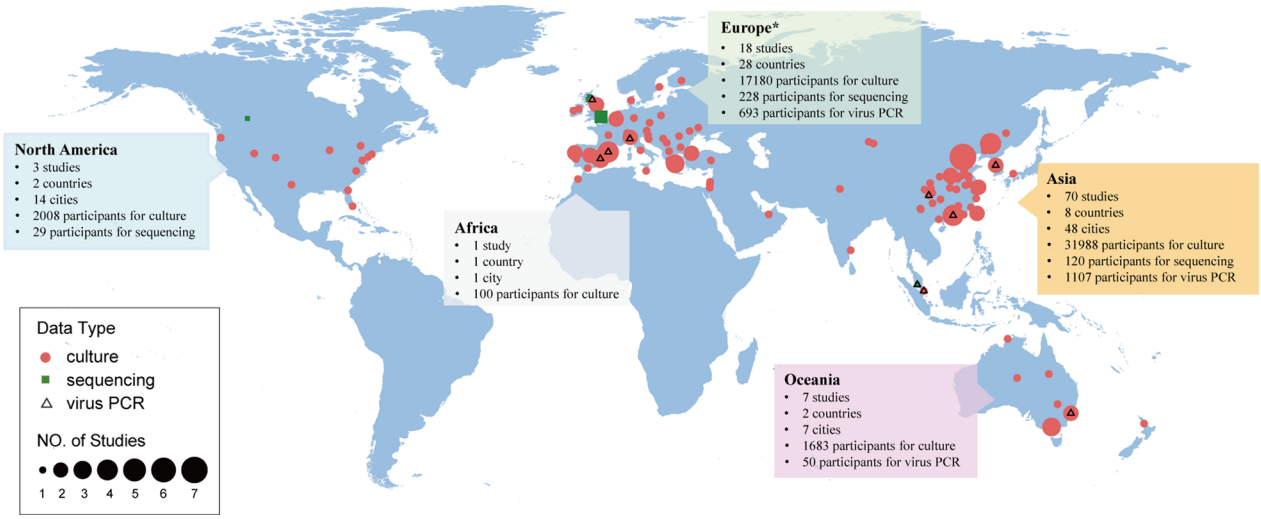

Fig. 2 Geographical distribution of study sites included in the meta-analysis. *Israel was counted as a European country because most included European multi-centre studies had Israel as a sub-centre

isolation in stable patients, while 23 reported it during the exacerbation stage.

Mycobacterium

Mycobacterium was less studied compared to other bacteria (Fig. 3b). A total of 23 studies involving 26,389 participants contributed samples for mycobacterium identification. Some studies reported Mycobacterium avium complex (MAC) and Nontuberculous mycobacterium (NTM) without distinguishing specific mycobacterium strains. The highest prevalence of mycobacterium species was not specified MAC (S: 3[1–5]%, E: 4[2–5]%, U: 15[3–27]%; I2 = 99%). This was followed by Mycobacterium intracellulare (S: 7[5–9]%, E: 6[1–12]%, U: 7[3–12]%; I2 = 0%) and Mycobacterium avium (S: 4[0–7]%, E: 6[1–12]%, U: 10[7–13]%; I^2 = 88%). Despite excluding tuberculosis-focused studies, there were still 6 studies identified tuberculosis in general bronchiectasis cases (S: N/A, E: 2[0–4]%, U: 1[1, 2]%; I^2 = 0%).

Fungus

Fungi were also investigated to a lesser extent (Fig. 3c) with a rough categorization. Thirty-five studies reported fungi isolation from 28,360 participants, with four involving stable participants (9791) and 20 conducted during exacerbations (3,887). *Aspergillus* spp. and *Candida* spp. were the two primarily identified species, although their distribution varied among groups. Candida spp. (not specified) was rarely reported in the S group, whereas its prevalence was 11[2–20]% in the E group and 10[-8–27]% in the U group (I2 = 96%). Candida albicans (S: N/A, E: 5[3–6]%, U: 7[2–12]%; I2 = 81%) was also frequently reported in patients experiencing exacerbations. Conversely, Aspergillus species (not specified) (S: 15[−10–39]%, E: 2[1–3]%, U: 10[5–15]%; I^2 = 95%) and Aspergillus fumigatus (S: 9[−3–21]%, E: 7[−1–15]%, U: 3[3, 4]%; I^2 = 91%) were mainly isolated from stable participants.

Virus

Viruses were mainly detected by polymerase chain reaction (PCR) techniques with specific virus panels (e-Table 2). Eight studies reported virus distribution in bronchiectasis, totalling 1850 participants (Fig. 3d). In the exacerbation group, 1169 participants provided nasopharyngeal samples, 99 provided sputum, and 58 provided both. For the stable group, 219 participants provided nasopharyngeal swabs, 217 provided sputum, and 146 provided both.

Rhinovirus was the most frequently detected virus with varying prevalence across airway sample types rather than disease stages (S-sputum: 18[−16–53]%, S-nasopharyngeal: 4[−1–9]%, E-sputum: 22[16–29]%, E-nasopharyngeal: 6[4–8]%; I^2 = 87%). Parainfluenza had the highest prevalence in sputum provided by stable patients but was rare in other groups (S-sputum: 31[−30–93]%, S-nasopharyngeal: 0[0–1]%, E-sputum: 3[0–6]%, E-nasopharyngeal: 2[1–3]%; I^2 = 98%). Influenza A was more commonly found in stable patients (S-sputum: 19[−15–52]%, S-nasopharyngeal: 10[−10–29]%, E-sputum: 8[2–14]%, E- nasopharyngeal: 6[1–11]%; I^2 = 89%), while the prevalence of Coronavirus was higher in the exacerbation

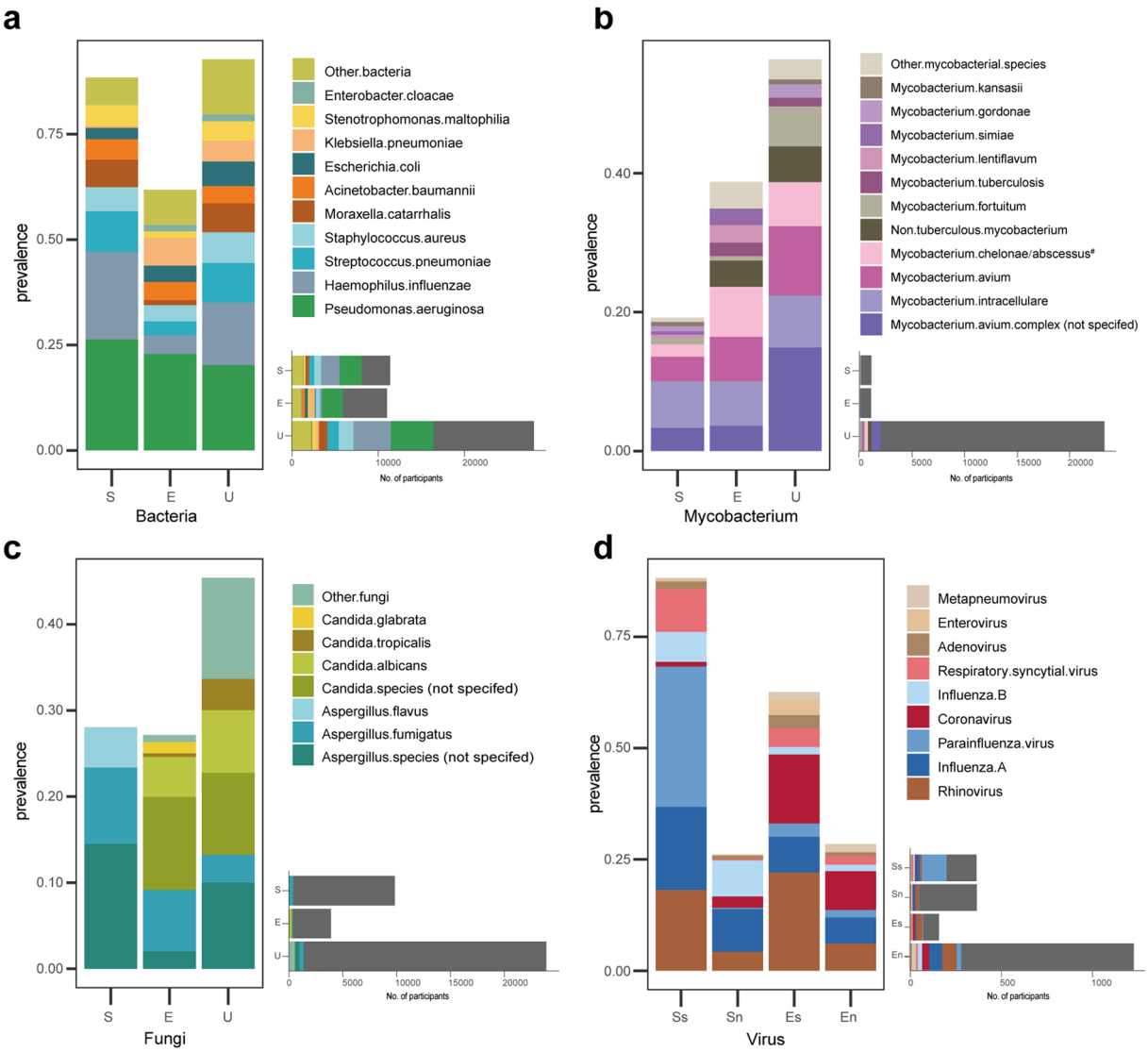

Fig. 3 The prevalence distribution of (**a**) bacteria, (**b**) mycobacterium, (**c**) fungal, and (**d**) virus following meta-analysis across the stable (S), exacerbation (**E**), and undetermined(U) groups. The prevalence data is presented in a stacked format for better comparison between the groups, each segment represents the prevalence of a specific microorganism independently. The accompanying horizontal bar charts display the total number of each microorganism isolations across all participants, the grey bars represent negative results. #Some studies counted *Mycobacterium chelonae* and *abscessus* together, to ensure comparability among groups, we merged the data and calculated them as a single category

group (S-sputum: 1[−1–3]%, S-nasopharyngeal: 3[0–5]%, E-sputum: 16[6–25]%, E-nasopharyngeal: 9[−6–24]%; $I^2 = 86\%$).

Microbiota profile

Sequencing techniques were underutilized in bronchiectasis microbial research. Among the included studies, we identified 4 16S rRNA gene datasets, 3 ITS gene datasets, and 1 metagenomics dataset. To maintain data consistency, we excluded the metagenomics dataset from our meta-analysis.

The 16S datasets included a total of 1,093 samples from 381 participants, but varied in sample origins, targeted hypervariable regions, and sequencing platforms (See e-Table 3 and **e**-Fig. 1). The Principal Coordinate Analysis (PCoA) on genus-level community revealed a distinct separation by study (PERMANOVA R [2] = 0.24, $p < 0.001$, e-Fig. 1d), indicating a study-related batch effect. A total of 331 bacterial genera were identified, with 43 genera having an average relative abundance over 0.001. The top 15 genera were illustrated in Fig. 4a, and others were grouped as "others". Among them,

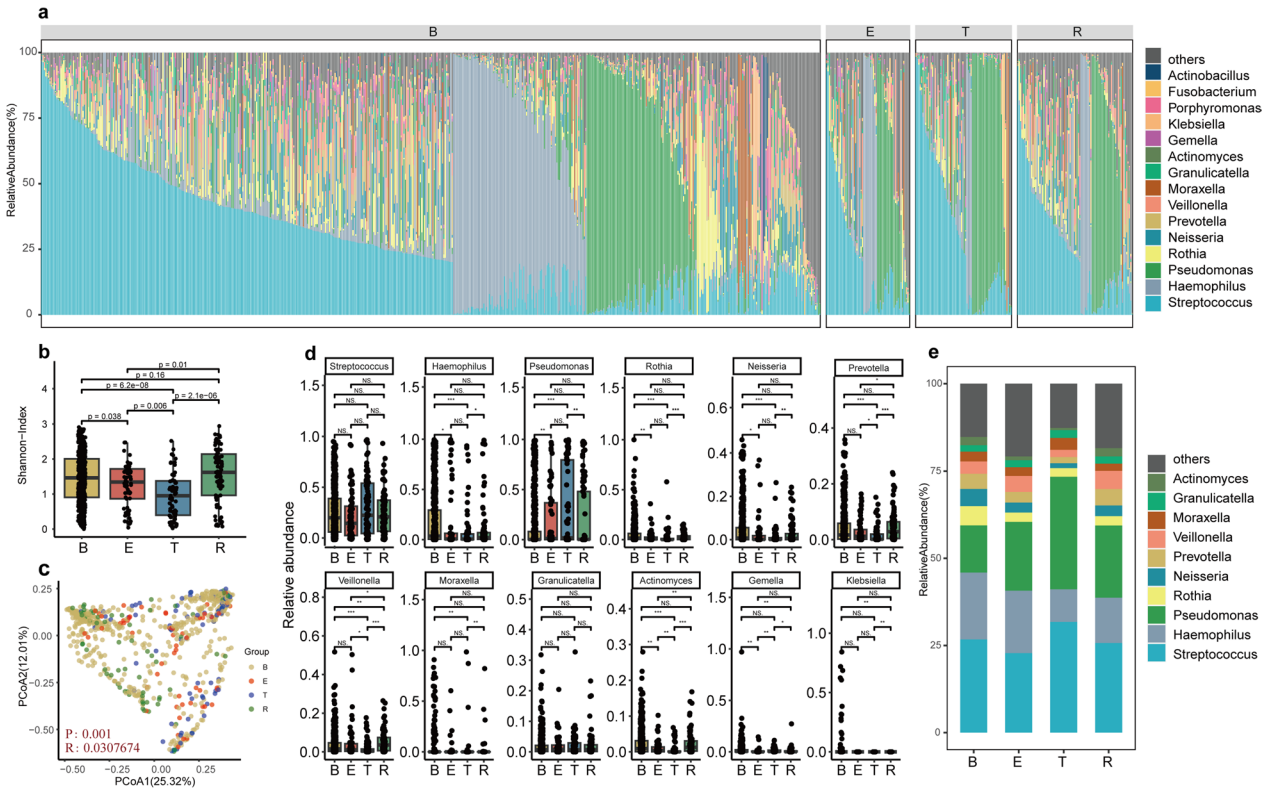

Fig. 4 **a.** Bacteriome at the genus level in bronchiectasis participants across different stages: baseline (B), exacerbation (E), treatment (T), and recovery (R) groups. **b.** Alpha diversity of bacterial microbiome represented using the Shannon diversity index. **c.** A PCoA based on Bray–Curtis dissimilarity illustrates a weak relationship between bacterial community variability and disease stages. The colour indicates groupings defined by disease stages. **d.** Comparison of the relative abundance of the top 12 bacteria among disease stages/groups. **e.** The distribution of the top 10 bacteria's relative abundance across the 4 groups. The remaining bacteria are collectively labelled as "others." Throughout, significance levels are denoted as follows: ns: non-significant; *p < 0.05; **p < 0.01; ***p < 0.001 (Wilcox test for multiple comparisons)

Streptococcus, Haemophilus, and *Pseudomonas* were the most dominant genera across the groups. The Shannon diversity index significantly decreased during exacerbations (E group), and further under antibiotic pressure (T group), compared to the B and R groups (Fig. 4b). Despite significant PERMANOVA results ($R^2 = 0.31$, $p < 0.001$, Fig. 4c), the PCoA figure did not show a clear clustering by disease stages, suggesting a weak relationship between bacterial community variability and disease stages. There was no significant difference in the relative abundance of *Streptococcus* across groups. However, *Pseudomonas* increased significantly in the E group, and even more pronounced in the T group. In contrast, many bacteria such as *Haemophilus*, *Rothia*, *Neisseria*, etc., were significantly more abundant in stable patients and less so in other stages, particularly in the T group (Fig. 4d, e).

For mycobiome, we included 311 available ITS reads from 276 participants across 3 datasets in two studies (Fig. 5). However, most reads originated from one study and primarily from stable patients, which may introduce bias into the analysis (**e**-Fig. 2a and b). The PCoA further confirmed a separation of samples based on datasets (PERMANOVA $R^2 = 0.16$, $p < 0.001$, Fig. 5c). In total, 52 mycobiome genera were identified, with 19 having an average relative abundance above 0.001. Notably, *Candida* was the overwhelmingly dominant species among all samples (Fig. 5a). It was followed by *Saccharomyces*, *Aspergillus*, *Clavispora*, and *Cryptococcus*. *Candida* and *Aspergillus* were significantly more abundant in stable patients, while *Cryptococcus* and *Curvularia* was higher in the E group (Fig. 5d, e). Additionally, in contrast to bacterial findings, fungal α diversity was significantly higher in the exacerbations and recovery groups compared to the stable group (Fig. 5b).

Discussion

To our knowledge, this is the first comprehensive, large-scale meta-analysis to assess the prevalence of bacteria, fungi, and viruses across different disease stages of non-CF bronchiectasis in global populations. While most studies continue to focus on patients during exacerbations, it is encouraging to see a growing recognition of

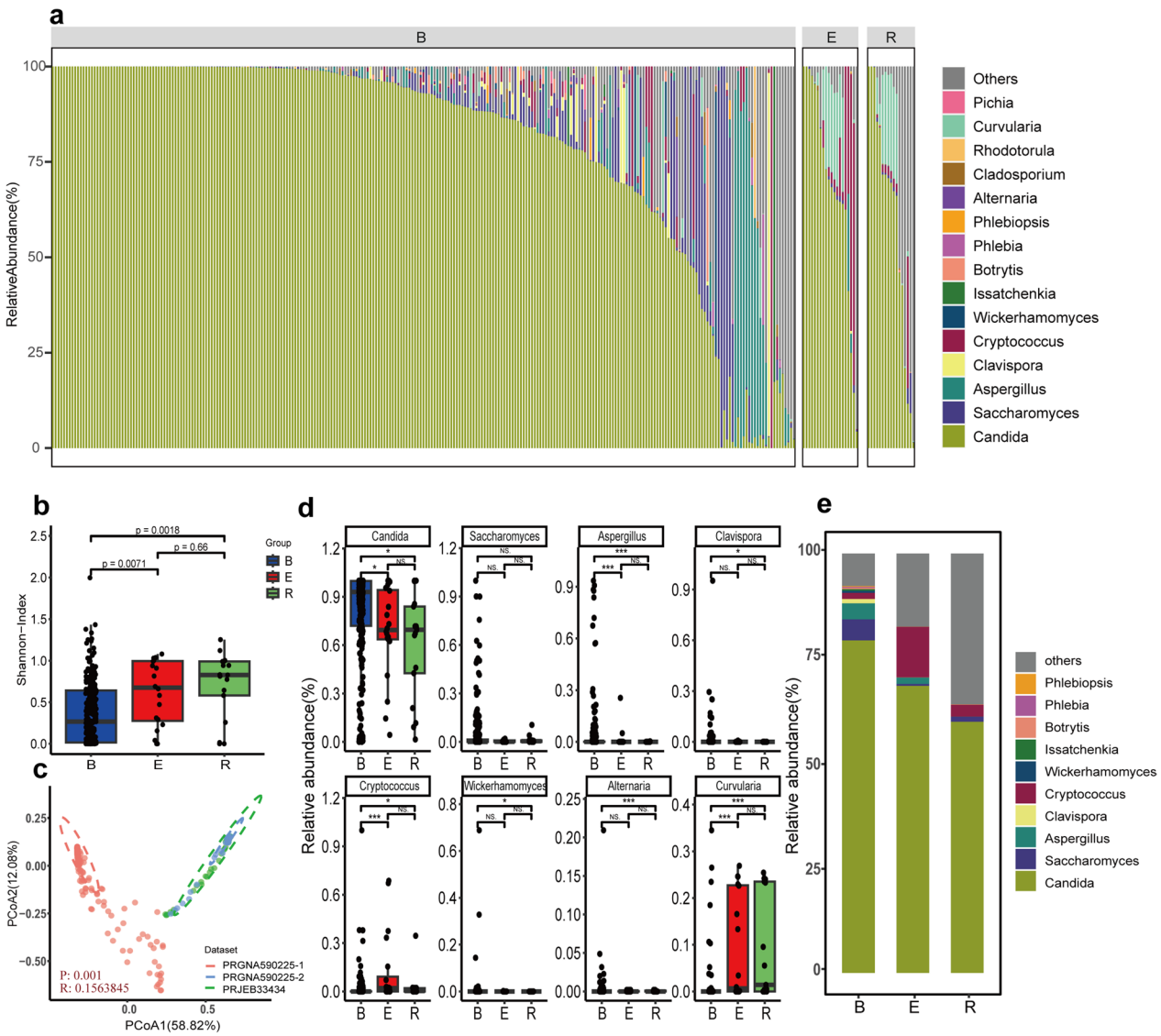

Fig. 5 **a.** Mycobiome profiles of bronchiectasis patients at the genus level across different stages: baseline (**B**), exacerbation (**E**), and recovery (**R**) groups. **b.** Alpha diversity of fungal microbiome represented by Shannon diversity index. **c.** A PCoA exhibits batch effects based on the individual datasets, two datasets from the same study shared the same accession number: PRGNA590225. **d.** Comparison of the relative abundance of the top 15 fungi between the B, E and R groups, only fungal genera with significant differences were shown. **e.** The distribution of the relative abundance of the top 10 fungi across the B, E, and R groups. The remaining fungi are collectively labelled as "others." Throughout, significance levels are denoted as follows: ns: non-significant; *p < 0.05; **p < 0.01; ***p < 0.001 (Wilcox test for multiple comparisons)

the importance of microbial colonization during the stable stage. Although the disease stage in the U group is unclear, it may better reflect the 'real' situation in clinical practice, as cultures are usually taken during symptom fluctuations, which can be challenging to classify as exacerbation or stable state. Our study encompasses various cohorts and employs diverse detection approaches.

While bacteria continue to play a significant role, an increasing variety of microorganisms have been identified as contributors to the progression of bronchiectasis. The most frequently isolated bacteria were *P. aeruginosa*, *H. influenzae*, and *S. pneumoniae* with prevalence metrics either higher or similar to the stable group versus the exacerbation group. *Aspergillus* spp. and *Candida* spp. were the predominant culture-identified fungi with varying distributions among subgroups. Rhinovirus, Influenza A, Parainfluenza, and Coronavirus were commonly reported viruses, but their prevalence exhibited greater

variability across airway sample types than between disease stages. Sequencing data yielded comparable results of culture but lower bacterial community diversity values were more associated with exacerbation risks.

The bacterial patterns in our study showed considerable similarity to other reported studies [8, 10]; however, the differences in bacterial prevalence across disease stages warrants further attention. The high colonization rates [30] account for the consistent presence of *P. aeruginosa* across different stages. Interestingly, *H. influenzae*, *S. pneumoniae*, and other bacteria such as *M. catarrhalis* and *S. maltophilia* were found to have a higher prevalence in the stable group. These organisms are all recognized as opportunistic pathogens [31–34]. For example, *H. influenzae* can persist within the host without causing invasive disease, becoming pathogenic only under specific conditions such as viral infections or immune suppression. Similarly, *S. pneumoniae* frequently colonizes the mucosal surfaces of the upper respiratory tract in healthy individuals but typically becomes invasive when it disseminates beyond this niche, causing diseases such as pneumonia. These findings underscore the need for further research to better understand the transition of these bacteria from a colonizing to a pathogenic state, as well as the role of inflammatory dysregulation and host–pathogen interactions in bronchiectasis exacerbations. Additionally, the significantly higher prevalence of *K. pneumoniae* and *E. cloacae* in the exacerbation group emphasizes the role of Enterobacteriaceae as pathogenic bacteria contributing to bronchiectasis exacerbations.

Sequencing data showed higher relative abundances of *Pseudomonas* genera during exacerbations and antibiotic treatment, likely due to its high resistance, allowing *Pseudomonas* to survive while other bacteria are eliminated. This bacterium is known to be difficult to eradicate. Clinical trials [35, 36] showed inconsistent results on the effectiveness of antibiotics against *P. aeruginosa*. Although *P. aeruginosa* was observed decrease in sputum during antibiotic treatment, it can rebound once treatment ceases [36]. The pathogenic role of *Pseudomonas* remains unclear. Aogáin [21] et al. presented a "*Pseudomonas*-interaction network model", which found that *Pseudomonas* in the high-frequency exacerbation cluster had more negative interactions with other microbes than those in the low-frequency cluster. Moreover, reduced bacterial richness were observed in the E and T groups. Considering the broader ecological context, there appears to be a competitive coexistence within the bacterial community. Disruptions in the coexistence balance may lead to exacerbations, and antibiotic strategies probably reshuffle the community to help restore balance.

Mycobacteria and fungi have been less studied than bacteria in bronchiectasis. It can be attributed to limitations in detection methods [37, 38] as well as their propensity for immunocompromised populations [39, 40], resulting in lower research visibility. In general, the meta-analysis revealed consistent mycobacteria patterns across disease stages. However, many organisms were isolated at a higher rate in the undetermined group. We reevaluated the original studies and found that data from this group often originated from registry-based research with large sample sizes. This may reduce random errors [41] and increase the likelihood of positive isolations, suggesting that mycobacterial distribution in bronchiectasis might be underestimated. Additionally, 16S sequencing showed limited sensitivity to mycobacteria, limiting its analytical utility for these organisms.

Fungal prevalence in bronchiectasis was generally low. The differing *Candida* profiles between culture isolation and sequencing underscore its role as an archetypal opportunistic pathogen [42]. While *Candida* spp. was relatively common cultured in the exacerbation group, they were rarely reported in stable patients. This discrepancy may be due to *Candida* often being regarded as an oral contaminant [43], its isolation in stable patients may not be considered clinically significant and therefore, not reported as a positive result. Although *Candida* spp. in the lower airways is generally interpreted with caution as a causative agent of lung infections [44], its higher prevalence in the exacerbation group may reflect its opportunistic pathogenic role during exacerbations. Meanwhile, its predominant relative abundance demonstrates its widespread presence and general harmlessness in patients' lungs [45]. The decreased *Candida*'s dominance in the exacerbation group may indicate an increased fungal competition during exacerbations. The *Aspergillus* abundance was consistent with the culture results, suggesting a weaker correlation with exacerbations. However, due to its association with hypersensitivity bronchopulmonary responses [46], its role requires further exploration beyond being a mere trigger for exacerbations.

The significance of viruses in bronchiectasis was often underestimated due to inadequate testing methods. Our meta-analysis provides a more comprehensive viral profile. We observed notable variability in virus prevalence depending on the type of specimen collected. While some viruses exhibited consistent detection patterns across different collection sites within the same disease state, others did not. Evidence remains inconclusive regarding whether viral prevalence is higher in nasal or sputum samples. Some studies [47, 48] have reported that sputum samples yield significantly higher detection rates compared to nasopharyngeal swabs when tested

simultaneously, a finding that is also reflected in our results. Given that bronchiectasis exacerbations are typically associated with lower respiratory tract infections, sputum samples are particularly relevant. Lower respiratory tract samples are more recommended as optimal samples for virus detection in severe lung infections, as viral replication in the lower respiratory tract often persists longer than in the upper respiratory tract and nasopharyngeal swabs may yield false negative results [49]. However, the notable higher prevalence of some viruses in stable patients challenges the assumption that viral levels increase during exacerbations [11, 50]. The pathogenic role of viruses may vary across different respiratory tracts, necessitating further research into their transmission and interactions with other microbes. Importantly, all studies included in our analysis were conducted before the Covid-19 pandemic, thus the isolation of coronaviruses does not reflect the impact of SARS-CoV-2.

Our study has several limitations. First, we integrated microbiology data from studies conducted over 20 years worldwide, with most data originating from Chinese and European populations. This introduces potential inter-study variability due to differences in detection techniques, geographic regions, and facility standards, which may limit global representativeness. Second, our quality assessment revealed that many studies did not report on the identification or control of potential confounding factors. Additionally, inconsistent definitions of exacerbations, seasonal variations, and differing antibiotic treatment statuses across studies could introduce bias. Many included studies focus on microbiology and lack antibiotic therapy details, limiting our analysis of specific treatments. Few studies reported disease severity metrics, such as lung function, limiting our ability to evaluate its impact on microbiology findings. Third, sequencing data were limited compared to culture-based data. Data heterogeneity, arising from variations in experimental procedures, sequencing platforms, and sample sizes, may have affected the robustness of our analyses. Lastly, viral prevalence in our results may be underestimated. Examination methods often targeted only specific, common viruses, which likely do not capture the full viral spectrum present in bronchiectasis.

Conclusion

The multi-microorganism meta-analysis highlights the ubiquitous presence of various microorganisms in stable bronchiectasis states, with similar patterns observed during exacerbations. Exacerbations were found to negatively correlate with bacteriome diversity but might positively correlate with mycobiome diversity. Different sample collection sites may result in varied virus patterns, but the underlying mechanisms remain unclear.

Abbreviations

ASV	Amplicon sequence variant
CF	Cystic fibrosis
DMM	Dirichlet multinomial mixtures
MAC	*Mycobacterium avium* Complex
NTM	Nontuberculous mycobacterium
N/A	Not applicable
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
QIIME 2	Quantitative Insights into Microbial Ecology2
SRA	Sequence read archive

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03140-w.

Additional file 1.

Additional file 2. e-Figure 1. Bacterial microbiome at the genus level for each dataset, presented by projecting accession numbers across different stages: a. PRJEB14304 b. PRJNA590225 (contain two datasets) c. PRJNA514329. d. A PCoA demonstrates batch effects among these datasets (PERMANOVA $R^2 = 0.24$, $P < 0.001$).

Additional file 3. e-Figure 2.Mycobiome datasets identified by project accession numbers, illustrated independently: a. PRJNA590225 (contain two datasets) b. PRJEB33434.

Author contributions

Study concept and design: Y. Wang, J. Xiao, Z. Zhang, L. Wu and C. Stålsby Lundborg; Literature searches, data extraction and quality assessment: J. Xiao, X. Yang, Y Wang, and X. Zhang. Sequencing data analysis: Y. Liu and J. Du. Drafting of the original manuscript: Y. Wang. Revising the manuscript and supervision: C. Stålsby Lundborg, Z. Zhang, G. Su, J. Du and A. Bossios. C. Stålsby Lundborg and Z. Zhang take responsibility for the content of the manuscript, including the data and analysis. All authors provided critical review of the manuscript and approved the final version.

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Availability of data and materials

All data used in this meta-analysis are sourced from previously published studies, appropriately cited in the reference list included with the supplementary materials. The datasets analysed during this study can be found in the publications cited, along with their supplementary materials. The accession numbers for sequencing datasets are provided in Supplementary Table 3. Further details about data sources or analysis methods are available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate
This meta-analysis utilized data from previously published studies; therefore, no ethical approval was required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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