

Review

Not peer-reviewed version

Metagenomic Next-Generation Sequencing to Characterise the Respiratory Microbiome in Paediatric Patients with Cystic Fibrosis: A Scoping Review Protocol

[Holly Drummond](#)^{*}, Clare Mills, Emilie Clifton, [Thomas Waterfield](#), [Helen Groves](#)

Posted Date: 5 February 2026

doi: 10.20944/preprints202602.0459.v1

Keywords: cystic fibrosis; paediatric; metagenomic sequencing; microbiome; review



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a [Creative Commons CC BY 4.0 license](#), which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Review

Metagenomic Next-Generation Sequencing to Characterise the Respiratory Microbiome in Paediatric Patients with Cystic Fibrosis: A Scoping Review Protocol

Holly Drummond ^{1,*}, Clare Mills ¹, Emilie Clifton ^{1,2}, Thomas Waterfield ^{1,2} and Helen Groves ^{1,2}

¹ Wellcome-Wolfson Institute of Experimental Medicine, Queen's University Belfast, Belfast, BT9 6BL United Kingdom

² Royal Belfast Hospital for Sick Children, Belfast, BT12 6BA, United Kingdom

* Correspondence: hdrummond01@qub.ac.uk

Abstract

Objective: To summarise the evidence on the use of metagenomic next-generation sequencing to characterise the respiratory microbiome in paediatric patients with cystic fibrosis (CF), with a focus on early-life microbial evolution, development of the airway resistome and the clinical utility of sequencing-based approaches in practice. **Introduction:** Early-life development of the respiratory microbiome plays a significant role in shaping long-term pulmonary outcomes in children with CF. Conventional culture-based diagnostics lack the sensitivity required to capture the complexity of airway microbial communities. Metagenomic sequencing enables culture-independent identification of pathogens and antimicrobial resistance genes, resulting in an expansion of literature utilising sequencing-based approaches to characterise the CF respiratory microbiome. The clinical significance of early-life microbiome variation and the translational value of these data remain poorly understood. **Eligibility Criteria:** Inclusion- studies that utilise metagenomic sequencing, including targeted 16S rRNA amplicon sequencing and untargeted shotgun sequencing, to characterise the bacterial respiratory microbiome of children and young people up to their 18th birthday with CF. Exclusion- animal models; in vitro, tissue-based or in silico studies; case-studies <10 participants, editorials, conference abstracts and review studies. **Methods:** The review will be conducted in accordance with Joanna Briggs Institute and PRISMA-Scoping Review (ScR) methodology. The databases to be searched will include Medline, and Web of Science with an additional for grey literature search via Google and Google Scholar, with no time or language restrictions. Two independent reviewers will screen by title and abstract, then by full-text review. The results of the search, study selection process and reasons for exclusion at full-text review will be reported in the PRISMA-ScR flow diagram. Data will be extracted into a chart. Key study characteristics and findings will be presented and discussed descriptively, supported by tabular summaries; highlighting how the available evidence relates to the review question.

Keywords: cystic fibrosis; paediatric; metagenomic sequencing; microbiome; review

Introduction

Cystic fibrosis (CF) is a progressive genetic disorder caused by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene, resulting in impaired mucociliary clearance and accumulation of viscous airway secretions [1]. These altered airway characteristics promote chronic colonisation by opportunistic pathogens and alter interspecies microbial interactions. Consequently, recent studies have demonstrated that many airway microbes adapt to the unique

niche of the CF respiratory tract, resulting in a respiratory microbiome differing from that of otherwise healthy individuals [2–4].

Traditionally, culture-based methods have been the gold standard for pathogen detection in microbiome studies and clinical settings. In caring for individuals with CF, regular culture-based surveillance is considered essential, supporting the guidance of clinical management and enabling the identification of both new infections and colonisation [5]. However, culture-based methods lack the sensitivity required to detect all clinically relevant bacteria, as many airway community members are slow-growing, fastidious or unculturable under standard laboratory conditions. This is particularly significant in paediatric populations, where limited sample volumes are frequently obtained. As a result, culture cannot reliably exclude bacterial infection in clinical settings and there has been an underestimation of microbial diversity in previous microbiome studies [6,7]. Advances in sequencing technologies have improved our understanding of the complex CF respiratory microbiome, extending beyond the limitations of culture-based approaches. Metagenomic sequencing techniques (mNGS) enable the identification of microbes without a priori knowledge and permits the detection of previously unculturable taxa.

Consequently, there has been a rapid expansion of literature utilising mNGS to characterise the CF respiratory microbiome. These studies are highly heterogeneous with respect to methodologies utilised and outcomes investigated, making it difficult to draw conclusions from the existing evidence base. This variability in study designs, with the breadth of the research questions investigated, highlights the need and suitability of a structured scoping review to map how sequencing-based approaches have been used to characterise the paediatric CF respiratory microbiome. Importantly, this review will focus specifically on paediatric patients living with CF. Early-life microbial colonisation is increasingly recognised as a key determinant of long-term respiratory outcomes and understanding the development and dynamics of the paediatric CF respiratory microbiome may highlight a critical window for early therapeutic intervention before significant lung function decline.

The overall aim of this scoping review is to describe how sequencing-based approaches have been used to characterise the paediatric CF respiratory microbiome, summarising specimen types, sequencing methodologies and reported outcomes. This review will systematically map the available evidence and identify knowledge gaps in the existing evidence base.

A preliminary search of Medline identified no existing systematic reviews or scoping reviews addressing the research question of this scoping review. A search on PROSPERO to identify ongoing systematic reviews similarly found no registered reviews on the use of respiratory mNGS in paediatric patients with CF. The most recent relevant systematic review focused on the CF population, evaluated blood biomarkers of CF pulmonary exacerbations and was published in 2013 [8]. This extended period and absence of paediatric-focused synthesis further highlights the need for a scoping review focused on this vulnerable population.

Review Question

How have mNGS-based approaches been used to characterise the paediatric CF respiratory microbiome, and what do they reveal about early-life microbial evolution, airway resistome development, and the clinical utility of mNGS in routine practice?

Methods

The proposed scoping review will be conducted in accordance with the Joanna Briggs Institute (JBI) methodology for scoping reviews [9] and adhere to the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) [10]. The PRISMA-ScR checklist to be completed in full in the final scoping review can be found in Supplementary Materials S1.

Eligibility Criteria

Participants

The review will include participants up to their 18th birthday living with CF. Studies including exclusively adult populations (aged >18 years) will be excluded. Studies on mixed age populations will be included on the presumption that paediatric data can be extracted. Studies utilising animal models, in vitro, tissue samples or in silico analysis will be excluded. Studies that investigate sequencing techniques used solely to assist or enhance culture-based methods will be excluded, as these approaches do not reduce time to diagnosis and remain dependent on organism growth.

Concept

The review will include studies that characterise the bacterial respiratory microbiome of paediatric patients with CF using mNGS, either through targeted 16S rRNA amplicon sequencing and/or untargeted shotgun sequencing.

Context

Any healthcare setting, such as hospitals or CF clinics, globally where paediatric patients living with CF are recruited. No geographic limitations will be applied.

Types of Sources

This scoping review will consider primary research of any study design. Grey literature will also be considered against eligibility criteria. Case studies of <10 participants, editorials, conference abstracts or review articles will be excluded.

Search Strategy

An example search strategy is available in Supplementary Materials S2. The search strategy will be adapted for each included database and/or information source. The reference list of all included sources of evidence will be screened for additional studies. Eligible studies published in any language will be included and there will be no time restrictions applied. The databases to be searched include MEDLINE (PubMed) and Web of Science alongside searching for grey literature including Google and Google Scholar.

Source of Evidence Selection

Following the search, all identified citations will be collated and uploaded into Rayyan and duplicates removed [11]. Titles and abstracts will then be screened by two independent reviewers for assessment against the inclusion criteria for the review. The full text of selected citations will be assessed in detail against the inclusion criteria by two independent reviewers. Reasons for exclusion of sources of evidence at full text that do not meet the inclusion criteria will be recorded and reported in the scoping review. Any disagreements that arise between the reviewers at each stage of the selection process will be resolved through discussion, or with a third reviewer. The results of the search and the study inclusion process will be reported in full in the final scoping review and presented in a PRISMA flow diagram [10].

Data Extraction

Data will be extracted from papers included in the scoping review by the lead author using a data extraction tool developed by the reviewers. The data extracted will include title, year of publication, country of origin, study design, study setting, study population, sample size, sample type, study aim and outcomes, study methodology, and key study results relevant to the review question.

A draft extraction form is provided in Supplementary Materials S3. The draft data extraction tool will be modified iteratively during the process of extracting data from each included evidence source. Modifications will be detailed in the scoping review. Any disagreements that arise between the reviewers will be resolved through discussion, or with an additional reviewer. If appropriate, authors of papers will be contacted to request missing or additional data, where required.

Data Analysis and Presentation

The intention of the scoping review is to map and summarise available evidence, rather than to synthesise novel quantitative results. Key characteristics of the included studies and findings will be presented and discussed descriptively, supported by tabular summaries; highlighting how the available evidence relates to the review question.

Individual studies will not undergo formal critical appraisal, as a scoping review is providing an overview of the existing research rather than an assessment of quality. Instead, evidence will be classified descriptively according to study design using a predefined hierarchy (randomised controlled trials, non-randomised interventional studies, observational cohort and case-control studies, case series, and laboratory or translational studies), as well as by outcomes investigated, including the number of studies addressing each outcome domain.

Discussion

The present scoping review will conduct a structured search across several databases to provide a comprehensive overview of how mNGS has been utilised to characterise the respiratory microbiome of paediatric patients living with CF. In comparison with conventional microbiological methods such as culture, mNGS provides an unbiased means of characterising the CF respiratory microbiome, enabling the profiling of microbial community composition, diversity and changing dynamics directly from patient samples. These advantages are particularly relevant for children with CF, where chronic colonisation with bacterial pathogens and frequent antibiotic exposure can significantly impact the development and dynamics of the early-life respiratory microbiome [12]. It is expected that the main limitation of the planned review will be the substantial heterogeneity across study design, methodological approaches and reported outcomes, reflecting the rapid expansion of respiratory microbiome studies utilising mNGS. This scoping review aims to describe how sequencing-based approaches have been used to characterise the paediatric CF respiratory microbiome, summarising sequencing methodologies and reported outcomes, while identifying knowledge gaps in the existing evidence base. The findings of the review can be used to inform the design of future research and support the translation of mNGS-based microbiome profiling into clinically relevant investigations.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: HD conceptualised the research; HD and CM will act as reviewers; HD writing—original draft preparation; HD, CM, EC, TW and HG writing—review and editing; TW and HG supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This study has been supported by the Department for the Economy (Northern Ireland). As scoping reviews are not eligible for registration on PROSPERO, the protocol will be made publicly available as a pre-print prior to beginning the review.

Conflicts of interest: There are no conflicts of interest to declare.

References

1. Gray RD, Downey D, Taggart CC. Biomarkers to monitor exacerbations in cystic fibrosis. *Expert Rev Respir Med.* 2017;11(4):255–7.

2. Silo-Suh L, Suh SJ, Phibbs PV, Ohman DE. Adaptations of *Pseudomonas aeruginosa* to the cystic fibrosis lung environment can include deregulation of *zwf*, encoding glucose-6-phosphate dehydrogenase. *J Bacteriol.* 2005;187(22):7561–8.
3. Goerke C, Wolz C. Adaptation of *Staphylococcus aureus* to the cystic fibrosis lung. *Int J Med Microbiol.* 2010;300(8):520–5.
4. Camus L, Briaud P, Vandenesch F, Moreau K. How Bacterial Adaptation to Cystic Fibrosis Environment Shapes Interactions Between *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Front Microbiol.* 2021;12:617784.
5. Smyth AR, Bell SC, Bojcin S, Bryon M, Duff A, Flume P, et al. European Cystic Fibrosis Society Standards of Care: Best Practice guidelines. *J Cyst Fibros.* 2014;13 Suppl 1:S23–42.
6. Carroll KC. Laboratory diagnosis of lower respiratory tract infections: controversy and conundrums. *J Clin Microbiol.* 2002;40(9):3115–20.
7. Cookson W, Cox MJ, Moffatt MF. New opportunities for managing acute and chronic lung infections. *Nat Rev Microbiol.* 2018;16(2):111–20.
8. Shoki AH, Mayer-Hamblett N, Wilcox PG, Sin DD, Quon BS. Systematic review of blood biomarkers in cystic fibrosis pulmonary exacerbations. *Chest.* 2013;144(5):1659–70.
9. Peters MDJ, Marnie C, Tricco AC, Pollock D, Munn Z, Alexander L, et al. Updated methodological guidance for the conduct of scoping reviews. *JBIM Evid Synth.* 2020;18(10):2119–26.
10. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann Intern Med.* 2018;169(7):467–73.
11. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev.* 2016;5(1):210.
12. Muhlebach MS, Zorn BT, Esther CR, Hatch JE, Murray CP, Turkovic L, et al. Initial acquisition and succession of the cystic fibrosis lung microbiome is associated with disease progression in infants and preschool children. *PLoS Pathog.* 2018;14(1):e1006798.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.