# Metagenomics in the diagnosis of pneumonia: a systematic review protocol

Samuel Quarton, Alana Livesey, Charlotte Jeff, Christopher Hatton, Aaron Scott, Dhruv Parekh, David Thickett, Alan McNally, Elizabeth Sapey

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## *Abstract*

**Background:** Causative pathogens are currently only identified in a minority of cases of pneumonia, with implications for antimicrobial stewardship. Metagenomic next generation sequencing offers promise to improve this, as a sensitive and untargeted approach to identifying pathogens. However, while studies have shown improved sensitivity compared to conventional microbiological methods for pneumonia diagnosis, it remains unclear whether this can translate into clinical benefit. Much existing work has also been performed in ventilated patients, readily allowing for analysis of bronchoalveolar lavage fluid (BALF). The impact of sample type on the utility of metagenomic analysis remains poorly defined. Similarly, previous work has rarely distinguished between the type of pneumonia involved, whether community-acquired (CAP), hospital-acquired (HAP), or ventilator-associated (VAP), despite these having different clinical profiles.

**Objective:** .

**Methods:** We aim to review all studies (excluding case reports of series of less than 10 people) of adult patients with suspected or confirmed pneumonia that compare metagenomic analysis with traditional microbiology techniques, to include culture, antigen-based testing and PCR-based assays. Screening of titles and abstracts, and subsequent review of eligible full texts will be by two separate reviewers, with a third clinician providing adjudication in case of disagreement. Our focus is on the clinical utility of metagenomics for patients with CAP, HAP, and VAP, and data extracted will focus on clinically important outcomes - namely pathogen positivity rate, laboratory turnaround time, impact on clinical decision making, length of stay, and 30-day mortality. Sub-group analysis will be performed based on the type of pneumonia (CAP, HAP or VAP), and sample type used.

**Results:** Searches were completed on 13th January 2024, screening of returned papers ongoing.

**Conclusions:** Despite significant promise, it is unclear what the likely impact of metagenomic analysis will be on clinical pathways. Further, it is unclear whether the likely utility of this technique will alter depending on whether the pneumonia is a CAP, HAP or VAP or the sample type that is collected. This systematic review will assess the current evidence base to support benefit on clinical outcomes for metagenomic analysis depending on the setting of pneumonia diagnosis, or specimen type used. It will identify areas where further research is needed to advance this methodology into routine care. Clinical Trial: PROSPERO 2023 CRD42023488096 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023488096

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# Original Manuscript

# Metagenomics in the diagnosis of pneumonia: a systematic review protocol

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# Abstract

## Background

Causative pathogens are currently only identified in a minority of cases of pneumonia, with implications for antimicrobial stewardship. Metagenomic next generation sequencing offers promise to improve this, as a sensitive and untargeted approach to identifying pathogens. However, while studies have shown improved sensitivity compared to conventional microbiological methods for pneumonia diagnosis, it remains unclear whether this can translate into clinical benefit. Much existing work has also been performed in ventilated patients, readily allowing for analysis of bronchoalveolar lavage fluid (BALF). The impact of sample type on the utility of metagenomic analysis remains poorly defined. Similarly, previous work has rarely distinguished between the type of pneumonia involved, whether community-acquired (CAP), hospital-acquired (HAP), or ventilator-associated (VAP), despite these having different clinical profiles.

## Objective

To determine the clinical utility of metagenomic next-generation sequencing in community-acquired pneumonia, hospital-acquired pneumonia, and ventilator-associated pneumonia, compared to traditional microbiological methods.

## Methods

We aim to review all studies (excluding case reports of series of less than 10 people) of adult patients with suspected or confirmed pneumonia that compare metagenomic analysis with traditional microbiology techniques, to include culture, antigen-based testing and PCR-based assays. Relevant studies will be identified through systematic searches of the of Embase, MEDLINE, Scopus, and Cochrane CENTRAL databases. Screening of titles and abstracts, and subsequent review of eligible full texts will be by two separate reviewers, with a third clinician providing adjudication in case of disagreement. Our focus is on the clinical utility of metagenomics for patients with CAP, HAP, and VAP, and data extracted will focus on clinically important outcomes - namely pathogen positivity rate, laboratory turnaround time, impact on clinical decision making, length of stay, and 30-day mortality. Sub-group analysis will be performed based on the type of pneumonia (CAP, HAP or VAP), and sample type used. Risk of bias will be assessed using the QUADAS-2 tool for diagnostic accuracy studies. Outcome data will be combined in a random-effects meta-analysis, and where this is not possible a narrative synthesis will be undertaken.

## Results

Searches were completed with the assistance of a medical librarian on 13th January 2024, returning 5,750 records. Screening and data extraction are anticipated to be completed by September 2024.

## Conclusions

Despite significant promise, it is unclear what the likely impact of metagenomic analysis will be on clinical pathways. Further, it is unclear whether the likely utility of this technique will alter depending on whether the pneumonia is a CAP, HAP or VAP or the sample type that is collected. This systematic review will assess the current evidence base to support benefit on clinical outcomes for metagenomic analysis depending on the setting of pneumonia diagnosis, or specimen type used. It will identify areas where further research is needed to advance this methodology into routine care.

## Systematic Review Registration:

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## Keywords

Pneumonia, Metagenomics, CAP, HAP, VAP, Diagnosis.

# Introduction

## Rationale

Pneumonia is an acute infection of lung parenchyma [1], of key importance globally with high incidence and mortality [2]. Lower respiratory tract infection, encompassing pneumonia, is the leading infectious cause of death worldwide [3], although figures for incidence and mortality vary significantly with the population being studied. In adults, pneumonia is predominantly a disease of ageing, with incidence rising from 1.1-4.8/1,000 persons/year in all adults, to 6.7-42/1,000 persons/year in those over 65 years old [2]. At extreme age this increases further – a UK analysis found the rate of pneumonia episodes among 85-89 year olds was 7 times that among 65-69 year olds [4]. With populations ageing in many countries, there is therefore urgent need to improve outcomes for patients with pneumonia.

The diagnosis of pneumonia is not pathogen specific, encompassing a range of infections by varied bacterial, viral, and fungal causes [5]. Prognoses and optimal treatments will vary depending on the causative organism, and the likely pathogens change depending on the setting in which infection occurs, with pneumonia usually categorised into community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), or ventilator-associated pneumonia (VAP). Identifying the responsible pathogen carries importance in allowing targeted effective treatment, however with existing microbiological methods an organism is only identified in around half of cases of CAP [6], and between 4 and 27% of patients with HAP [7-9]. In addition, traditional culture-based techniques have a relatively slow turn-around time of 2-5 days from sample collection to antimicrobial sensitivity results [10]. As a result, treatment is largely empirical, based on common organisms and resistance patterns, with the risk of antibiotic prescriptions that are unnecessary, ineffective, or harmful [11]. Empiric treatment, especially with broad-spectrum antibiotics as recommended for HAP and VAP [12], also drives antimicrobial resistance which is a growing societal concern - an estimated 4.95 million deaths were associated with antibiotic resistance in 2019 [13]. With lower respiratory tract infections being the largest contributor towards this total [13], antibiotic resistance is both a consequence of, and cause of, inappropriate antibiotics in pneumonia. Timely identification of causative organisms and potential resistance patterns could lead to early rationalisation or escalation of antibiotics, and so improve outcomes and limit the impact on antimicrobial resistance.

Metagenomic next-generation sequencing is an emerging technology that is of considerable promise in pneumonia [14]. Current techniques to identify a causative pathogen in pneumonia rely largely on microscopy and culture. These traditional methods have significant limitations which contribute to the low rates of pathogen identification. Not all pathogens grow readily in standard culture media, and where organisms are identified results often take several days to be reported, leading to delays in optimising antimicrobials [10, 15]. Metagenomic sequencing offers a non-biased approach to pathogen identification by sequencing all nucleic acid present in a sample, and so in addition to identifying organisms, has the potential to provide data on likely resistance patterns or virulence. It also offers potential improvement in laboratory turn-around times – 24 hours has been achieved in clinical practice [16], with a 6 hour turnaround from sample to result in a research environment suggesting what may become possible with scale and optimisation [17].

Increasingly, studies are considering the role of metagenomics in pneumonia diagnosis [18-20]. This has led to systematic review of the sensitivity and specificity of metagenomics for pneumonia diagnosis [21]. However, work assessing the clinical utility of metagenomics is more limited. Lv et al. performed a recent systematic review assessing relative pathogen detection rates, together with some clinical outcomes, however this only considered severe pneumonia with the majority of included studies based exclusively within intensive care. Similarly, a recent pilot of metagenomics within a clinical service showed significant impact on antimicrobial prescribing, but was again based solely within intensive care [14]. Most patients with pneumonia are not treated within ICU, and so it is important to assess the potential benefit of metagenomic methods outside this setting. The Lv *et al.* review also made no distinction between CAP, HAP or VAP [22]. Given CAP, HAP and VAP result from a different range of pathogens, among differing cohorts of patients, it is possible that metagenomics may have clinical utility in one disease but be ineffective in another.

Studies have predominantly looked at the impact of metagenomic analysis of bronchoalveolar lavage (BAL) samples, and the relative sensitivity and specificity of other bodily fluids (such as expectorated sputum, pharyngeal swabs or peripheral blood sampling) remains uncertain for patients with pneumonia. Lower respiratory tract sampling may improve pathogen identification, but is impractical to obtain for many patients. Understanding the relative utility of less invasive sampling methods is therefore important.

While previous work has looked specifically at the sensitivity and specificity of metagenomics, the clinical relevance of this methodology remains unclear. To be routinely adopted, metagenomics will need to identify a causative pathogen from non-sterile samples, improve the timeliness of laboratory turnaround times, impact on clinical prescribing, and be associated with improvements in clinical outcomes. These may differ in CAP, HAP and VAP.

## Objectives

This systematic review aims to determine the clinical utility of metagenomic next-generation sequencing (mNGS) in community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP) among an adult population, compared to traditional microbiological methods. This will be through assessing the impact on frequency and speed of pathogen identification, as well as evidence of this translating to clinical outcomes such as change in antibiotic therapy. It will also include sub-group analysis on how the utility of mNGS is affected by sample-type used for metagenomic analysis.

# Methods

The review will be conducted and reported in keeping with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [23], and a completed PRISMA checklist will accompany any published results.

## Eligibility Criteria

Studies will be included comparing metagenomic analysis for identifying pathogens with traditional microbiological methods (to include culture, serum and urine antigen testing, and PCR based approaches). As clinical use of metagenomic analysis is an emerging field, case series and observational studies will be included as well as randomised controlled trials. No date or language restrictions will be applied.

Inclusion and exclusion criteria for studies are as below.

Inclusion criteria:

- Randomised-controlled trials, observational studies and case series of more than ten patients.
- Studies comparing metagenomic analysis with alternative methods of microbiological diagnosis.
- Studies reporting data for hospitalised patients with suspected pneumonia (community-acquired, hospital-acquired or ventilator associated).
- Studies that include adult patients only (aged eighteen years old and over).

Exclusion criteria

- Case series of less than 10 patients.
- Studies describing respiratory infection, but where the definition does not require any radiological changes in keeping with pneumonia.
- Animal or environmental studies

## Outcomes

The systematic review will include the following primary and secondary outcomes:

## Primary Outcome:

- Pathogen positivity rate of metagenomic analysis in confirmed CAP, HAP and VAP compared to standard methods (to include culture, antigen testing and PCR (polymerase chain reaction)).

## Secondary Outcomes:

- As for the primary outcome, analysed in sub-groups based on sample type (blood, sputum or bronchoalveolar lavage fluid (BALF).
- Laboratory turnaround time (defined as time from sample receipt to report of organisms identified (with antimicrobial sensitivities where available)).
- Impact on clinical decision making (to include proportion of patients in which antimicrobials were changed or rationalised based on metagenomic results).
- Length of stay (hospital admission)
- 30-day and 90-day mortality
- Sensitivity and specificity of metagenomic methods for diagnosing CAP, HAP, and VAP

## Search strategy:

A comprehensive search of Embase, MEDLINE (via the Pubmed interface), Scopus, and Cochrane CENTRAL databases will be performed, with support of a healthcare librarian, as well as grey literature. Reference lists will also be manually searched for appropriate studies. No date or language restrictions will be applied.

## Search Terms

Exact search strings will be optimised to the database being searched. The full search strategy used will be provided as supplementary material with the completed review.
The following provides the core terms around which searches will be based:
(pneumonia OR CAP OR HAP OR VAP OR “lung infection” OR “respiratory infection” OR “pulmonary infection”) AND (metagenomic* OR “next generation sequencing” OR “NGS” OR “mNGS”).

## Screening, data management and data extraction

Studies identified by searches will be stored and processed using the ‘Covidence’ software package (Covidence, Australia). Retrieved titles and abstracts will be independently assessed for eligibility by two reviewers, with a third independent clinician providing adjudication in cases of disagreement. The full text of eligible articles will be retrieved and data relevant to our primary and secondary outcomes extracted and recorded onto pre-designed forms. Where studies provide separate data for CAP, HAP, or VAP, or explicitly study only one of these, this will be recorded and combined outcomes reported for each pneumonia type. If the type of pneumonia is not clear, or the criteria used to define pneumonia does not allow separation into sub-types, data will still be collected to avoid the loss of meaningful information. These data will only be included in outcomes looking at the utility of metagenomics in pneumonia as a whole. Where reported, data relevant to diagnostic test accuracy (true positive, false positive, true negative and false negative) will be recorded, to enable pooled sensitivity and specificity calculations. Information will also be extracted regarding study design, including sample size and definitions of pneumonia used, methods used for randomisation where appropriate, and the criteria used for a reference standard of positive diagnosis, as well as declared funding sources.

For papers where the sample type used for analysis is not stated or only statistics of combined sample types are given, attempts will be made to contact the authors to retrieve this missing information. If this is not available, these data will not be used when analysing sub-groups by sample type, but still included for other outcomes. Other missing data will be recorded and reported on when discussing results.

As this is a systematic review of a diagnostic tool, the quality of included studies and risk of bias will be assessed using the QUADAS2 tool (University of Bristol, Bristol UK), as determined by two independent reviewers. Again, any disagreement during data extraction or in assessment of study quality will be discussed with a third reviewer and a consensus decision reached.

## Analysis of results

Statistical analyses will be conducted using R version 4.2 (The R Collaboration, Vienna, Austria). For outcomes where insufficient data is available to conduct meta-analysis, this will be highlighted, and data combined in a narrative synthesis.

## Reporting of outcome measures

Data from eligible studies will be pooled and, if possible, a random effects meta-analyses of outcome measures performed. For dichotomous outcomes we will calculate odds ratios together with 95% confidence intervals where possible. Laboratory turnaround times, as a continuous outcome, will be converted to standard units (hours) and mean difference calculated. Sub-group analysis will be performed to look at the type of sample used for metagenomic analysis, and analysis will also be performed separately for CAP, HAP and VAP.

## Missing data

Where standard deviations are not provided for continuous outcome data (laboratory turnaround time), study authors will be contacted up to three times in an attempt to obtain these. If this is not possible, attempts will be made to estimate standard deviations using recognised formulae from statistics provided (for example standard error or P values), or – if there is insufficient information to allow this, a standard deviation will be imputed based on that of other included studies. Where this is necessary, a sensitivity analysis of the results will be performed to identify if this has a meaningful impact.

## Assessing heterogeneity

A funnel plot will be performed to assess for publication bias, and statistical heterogeneity of studies calculated using $I^2$. Where substantial heterogeneity is found (>50%), data entry will be assessed for accuracy. Possible reasons for the observed heterogeneity will be investigated by identifying studies that are obvious outliers on visual inspection of the graphical data, and assessing for any methodological or population characteristics that account for the heterogeneity. These will be discussed alongside the results.

# Results

Searches were completed on 13th January 2024, returning 5,750 records. Screening of returned papers and data extraction are anticipated to be completed by September 2024.

# Discussion

Systematic review of the literature surrounding the use of metagenomics within the management of pneumonia should clarify what is already understood about a topic of importance in terms of clinical burden and current unmet need. Metagenomic sequencing has been shown previously to improve the rate of pathogen identification [21], however, how to interpret this in light of a non-sterile respiratory tract, and lack of gold-standard comparators is challenging. The clinical significance of this increased analytical sensitivity remains unclear. Assessing the impact on clinical outcomes will highlight the potential clinical relevance or otherwise of metagenomics in this context, and highlight where future studies may be needed to develop this further. Similarly it will clarify if mNGS may be of particular benefit in certain pneumonia sub-types, and by investigating the impact of different sample types it may identify the least invasive method to provide meaningful clinical data, given existing reviews have focused on the use of bronchoalveolar fluid [21]. If insufficient data currently exists to make clear conclusions, the review will highlight this, and so direct where future research is needed.

We feel these are questions of clinical importance, and this review will therefore offer a valuable contribution in shaping the use of an emerging technology in clinical practice. Results will be submitted for publication in peer-reviewed journals and presentation at local and international conferences. If, for any outcome, we are unable to identify sufficient studies to derive meaningful results, this will be reported, to help direct future avenues for research.

## Publication and dissemination

The review is registered prospectively on the PROSPERO database (https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=488096), and will be updated once complete. Results will be submitted for publication in peer-reviewed journals and presentation at local and international conferences. If, for any outcome, we are unable to identify sufficient studies to derive meaningful results, this will be reported, to help direct future avenues for research.

# List of abbreviations

BAL – Bronchoalveolar lavage
BALF – Bronchoalveolar lavage fluid
CAP – Community-acquired pneumonia
HAP – Hospital-acquired pneumonia
mNGS – Metagenomic next-generation sequencing
PCR – Polymerase chain reaction
VAP – Ventilator-associated pneumonia

# Declarations

## Acknowledgments

With thanks to the National Institute for Health and Care Research (NIHR) Birmingham Biomedical Research Centre.

## Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed. On completion of the study, datasets generated as part of the study will be included in any submissions for publication.

## Author contributions

SQ participated in planning the review and drafting the manuscript. ES participated in planning the review and providing critical appraisal of the manuscript. AL, CJ, CH, AS, DT, DP, and AM provided critical appraisal of the manuscript.

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## Competing interests

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