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# Comparison of Microbiota in the Upper Versus Lower Respiratory Tract in Children During Health and Respiratory Disease: a Protocol for a Systematic Review

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**Protocol**

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# Abstract

**Background:** Several species of organisms reside in the upper airways of healthy infants and children. In most cases they do no harm to the host despite being potentially pathogenic. In contrast, the lung in healthy people was considered to be a sterile environment, but sophisticated techniques have recently demonstrated colonization by a complex population of microorganisms in healthy adults. It is unclear if a similar situation happens in healthy children. In children with respiratory disease, several micro-organisms can be recovered from the upper as well as lower respiratory tracts. However, the correlation between organisms recovered from the two sites is unclear. This systematic review is designed to explore the microbial composition of the respiratory system in apparently healthy asymptomatic children, comparing the organisms identified in the upper airways versus the lungs. We also intend to compare the site-specific prevalence and pattern of organisms in healthy children versus those with various respiratory diseases. We will also compare the organism identified in the upper airway versus the lungs in children with respiratory disease.

**Methods:** We will search the following electronic databases: Medline, Embase and the Cochrane Library. Reference list of relevant studies will be examined for links to potential related articles. Two reviewers will independently determine study eligibility. The methodological quality of the observational included studies will be scored using the Newcastle Ottawa Scale tool for assessing risk of bias. We will extract data from included studies and perform meta-analysis where feasible.

**Results:** The search strategy will be refined and the literature search will take place independently by two authors. Final stage will include analyses and writing.

**Discussion:** Through the publication of this protocol, readers will be able to assess the research question and methods presented in this protocol. Upon publication of the review, readers will be able to assess whether the review was conducted according to pre-defined plan. Researchers will be aware that the review is underway, thereby avoid duplication, and be able to use it as a basis for planning similar reviews.

**PROSPERO registration number:** CRD42020202115


# Background

The “microbiota” consists of different species of microorganisms that live in a defined environment. In our body, microbiota are present in organs that are in contact with the outside environment, mainly the gut (1). Microbiota differs among various individuals and also varies according to pathological events or the person’s health state, and it can modulate immune responses. The term “microbiome” is considered to include the complete set of microorganisms (bacteria, viruses, and fungi) with their genomes (2). There are numerous mutually beneficial interactions between the human body and microbiota with metabolic reactions, which are important for our health and can contribute to the pathogenesis of some diseases (3). Until recently, the microbial structure of a human body remained poorly understood. However, large-

scale research conducted within the framework of the “Human Microbiome Project” (HMP 2007) provides us with knowledge on the diversity of human microflora (4).

Previously, the lower respiratory tract was assumed to be sterile, except during infection. This concept existed due to limited experimental access to the respiratory tract of healthy individuals, and limitations of classical methods of culturing organisms (5). Therefore, the study of lungs was initially not included in the original Human Microbiome Project. Limited preliminary data showed differences in the composition of respiratory tract microbiome in patients suffering from various respiratory diseases and relatively healthy volunteers (6).

The upper respiratory tract is colonized by a variety of different microbial species right after birth. It has been shown that the initial colonization depends on delivery mode (vaginal delivery or caesarean section) (7). More dramatic changes occur during the first year of life, probably driven by the maturation of the immune system (8), dietary practices etc. Later on, this first microbial community transforms into the adult upper respiratory tract microbiome, becoming less dense and more diverse (9). Recent studies, using culture-independent techniques, demonstrated the presence of *Actinobacteria, Proteobacteria, Bacteroidetes*, and *Firmicutes* ribosomal DNA in the lungs of healthy (10). Bacteria have been identified using sensitive identification techniques, including the 16S rRNA gene, which is specific for bacterial cells (11). Next generation sequencing (NGS) of 16S rRNA has allowed accurate identification of bacterial genetic material in the lung of healthy children (12–13). Sampling of the upper airways at any age using nasal swabs or nasopharyngeal aspirates or orophrayngeal swabs is fairly straight forward (14). The inaccessibility of the lungs poses significant challenges for researchers. Sampling from the lungs and distal airways in adults has involved using sputum or endoscopic Broncho alveolar lavage (15). Currently, there is no comprehensive data comparing the micro-organisms present in the upper respiratory tract of heathy children versus those in their lungs. Similarly, there is paucity of data on site specific comparison of organisms in healthy children versus those with respiratory diseases. This systematic review is being conducted to address these gaps in literature.

# Study Aim And Objectives

This systematic review aims to compare the microbial flora of upper respiratory tract versus lower respiratory tract of healthy infants and children versus those with respiratory diseases.

The specific review questions addressed are:

1. What are the microbial organisms present in the upper airway respiratory specimens of healthy children?
2. What are the microbial organisms present in the lungs of healthy children?
3. In healthy children, how does the microbial flora present in the upper airway respiratory specimens compare with the microbial flora present in the lung?

4. How does the upper airway microbial flora in heathy children compare with the upper airway microbial flora in children with various respiratory diseases?
5. How does the lung microbial flora in heathy children compare with the lung microbial flora in children with various respiratory diseases?
6. In children with various respiratory diseases, how does the microbial flora present in the upper airway respiratory specimens compare with the microbial flora present in the lung?

# Methods/design

### Types of studies

We will include all study designs that have the potential to address one or more of the review questions highlighted above. These include observational studies notably prospective cohort studies, retrospective cohort studies, case-control studies and case series. Data may be available from one or other arm of randomised and non-randomised clinical trials, hence these will be included as well. We will exclude narrative reviews that do not provide objective data, and also case series with less than 5 participants.

### Types of participants

The review will include studies conducted in children (age range birth to 12 years) who are normal or healthy or asymptomatic (as defined by authors of individual publications) as well as those with acute or chronic respiratory disease (as defined by the authors of publications).

### Inclusion criteria

Broadly, we expect to include the following types of studies viz.

- Those which describe micro-organisms in the upper airway respiratory tract and/or lungs of healthy children.
- Those which report micro-organisms in the upper airway respiratory tract and simultaneously the lungs of children with respiratory diseases.
- Those which compare micro-organisms in the upper airway respiratory tract of healthy children versus those with respiratory diseases.
- Those which compare micro-organisms in the lungs of healthy children versus those with respiratory diseases.

### Exclusion criteria

We will also exclude studies that report findings in participants older than 12 years of age, or where data of children and adults are presented together, without possibility of extracting data from children. We will also exclude post-mortem studies.  If potentially eligible studies report data from specimens such as

tracheal aspirate, tracheostomy tube secretions, endotracheal tube aspirates, these will be excluded (as it is difficult to classify them as clearly upper or lower respiratory specimens.

### Types of outcome measures

- Organisms (bacteria, viruses, fungi) identified in upper respiratory tract specimens of healthy children.
- Organisms (bacteria, viruses, fungi) identified in the lungs of healthy children.
- Comparison of organisms (bacteria, viruses, fungi) identified in upper respiratory tract specimens of healthy children versus organisms identified in their lungs.
- Comparison of organisms (bacteria, viruses, fungi) identified in upper respiratory tract specimens of children with respiratory diseases versus organisms identified in their lungs.
- Comparison of organisms identified in upper respiratory tract specimens of healthy children versus those with respiratory diseases.
- Comparison of organisms identified in lungs of healthy children versus those with respiratory diseases.

### Search Methods

Two authors will independently search the electronic database to identify relevant studies, the following electronic databases will be searched: MEDLINE, EMBASE and The Cochrane Library using search MeSH terms for the below mentioned keywords.

**Table 1.** The search strategies

| Search Strategy | | |
|---|---|---|
| Sr. No. | Database | Search Strategy |
| 1. | Medline | (((((((("organ"[All Fields] OR "organ s"[All Fields]) OR "organism"[All Fields]) OR "organism s"[All Fields]) OR "organisms"[All Fields]) OR "organs"[All Fields]) OR "micro-organism"[All Fields]) AND ((((((("child"[MeSH Terms] OR "child"[All Fields]) OR "children"[All Fields]) OR "child s"[All Fields]) OR "children s"[All Fields]) OR "childrens"[All Fields]) OR "childs"[All Fields])) AND "respiratory"[All Fields] |
| 2. | Cochrane Library | (organism OR micro-organism) AND child AND respiratory in: Title, Abstract, Keywords |
| 3. | Embase | (organism OR micro-organism) AND child AND respiratory |

### Data collection and analysis

Two review authors will independently assess the titles and abstracts of the identified records to evaluate eligibility. We will retrieve the full text of all the papers identified as potentially relevant by one or both review authors. Two review authors will independently assess these papers for inclusion. In case of disagreements/ discrepancies, the senior author will do arbitration.

### Data extraction and management

Data from included studies will be extracted independently by two review authors. Extracted data will include information on study identification data, date of publication, country, context/setting, study design or conceptual framework, participant information, bacteriological outcomes, specimen collection methods etc. Information will be cross-checked by the authors to ensure that relevant information will not be missed, and that the authors agree on the findings. A special Data Extraction form will be used to record the extracted data (Table 2).

**Table 2:** Screening of potentially eligible studies

| Field | Response | Additional Comments |
|---|---|---|
| **Citation** | Include details such as journal, title, author, volume, page numbers etc. | |
| **Objective** | Describe the study objective as stated by the authors | |
| **Population** | Demographic detail of the participants in the study | |
| **Intervention/ Exposure** | Describe the intervention or treatment | |
| **Comparison** | Describe the control group or comparison intervention (if any) | |
| **Outcome** | Record the results of the intervention and how measured | |
| **Type** | Study Type / Design | |
| **Comments** | Notes in regard to the study quality for grading | |
| **Is this study eligible for inclusion in the Review?** | | |
| **Which review question(s) does it address?** | Organisms in upper airway of healthy children. | |
| | Organisms in lungs of healthy children. | |
| | In healthy children, upper airway organisms vs lung. | |
| | Organisms in upper airway of healthy children vs those with respiratory diseases. | |
| | Organisms in lung in heathy children vs with various respiratory diseases. | |
| | In children with respiratory diseases, organisms in upper airway vs lung. | |
| **Reason(s) for exclusion** | | |

**Table 3:** Data Extraction Form

**General Information**

| |
|---|
| Name/ ID of person extracting data |
| Author and year of study |
| Publication type |
| Period of study |
| Country |

## Study characteristics

| Study characteristics | Eligibility criteria (Insert inclusion criteria for each characteristic defined in the protocol) | Eligibility criteria met (Yes, No or unclear) |
|---|---|---|
| Type of Study | Cohort Study | |
| | Case control | |
| | Case series | |
| | Narrative Study | |
| | Others | |
| Participants | Age | |
| | Normal/Healthy/Asymptomatic | |
| | Acute or Chronic respiratory diseases | |
| | Severe community acquired pneumonia | |
| | Cystic fibrosis with acute pulmonary exacerbation | |
| | Suspected airway malformation/anomaly requiring bronchoscopy | |
| | Chronic or recurrent or persistent respiratory disease requiring bronchoscopic evaluation | |
| | Other | |
| Healthy children | Age range, mean, median | |
| | Recruited from where? | |
| | Why samples taken? | |
| | What samples taken? | |
| Children with respiratory diseases | Suspected or confirmed hospital acquired infections | |
| | Previously hospitalized within 14 days of current presentation | |
| Population description (from which study participants are drawn) | | |
| Setting (including location and social context) | | |
| Inclusion criteria | | |
| Exclusion criteria | | |

| Method of recruitment of participants (e.g. phone, mail or clinic patient) | | |
|---|---|---|
| Informed consent obtained | ☐ Yes ☐ No ☐ Unclear | |
| Age | | |
| Sex | | |
| Race/Ethinicity | | |
| Healthy or asymptomatic children | | |
| Children with acute or chronic respiratory infections | | |

## Outcome measures:

## Comparison of upper and lower airway microbiota in healthy children and children with respiratory diseases

| | Healthy children (No.) | Children with respiratory disease (No.) |
|---|---|---|
| **Number of specimens** | | |
| **Site** | | |
| **Processing method** | | |

| |
|---|
| *Moraxella* |
| *Staphylococcus* |
| *Corynebacterium* |
| *Streptococcus* |
| *Dolosigranulum* |
| *Streptococcus* |
| *Prevotella* |
| *Neisseria* |
| *Veillonella* |

| |
|---|
| *Rothia* |
| *Leptotrichia* |
| *Haemophilus* |
| *Burkholderia cepacia* |
| *Klebsiella pneumoniae* |
| *Mycoplasma pneumoniae* |
| *Chlamydophila psittaci* |
| *Legionella pneumophilia* |
| *Prevotella spp.* |
| *Tropheryma whipplei* |
| **Viruses** |
| *Rhinovirus (HRV)* |
| *Parainfluenza virus* |
| *Human Meta Pneumovirus* |
| *Human bocavirus* |
| *Polyomavirus* |
| *Human adenovirus* |
| *Human coronavirus* |
| *Respiratory syncytial virus(RSV)* |

| |
|---|
| *Influenza virus* |
| *Enterovirus* |
| *Parechovirus* |
| *Cytomegalovirus* |
| *Influenza virus* |
| *Enterovirus* |
| *Parechovirus* |
| *Cytomegalovirus* |
| **Fungi** |
| *Aspergillus spp.* |
| *Penicillium spp.* |
| *Candida albicans* |
| *Alternaria spp.* |
| *Cladosporium* |
| *Ulocladium* |
| *Wallemia* |
| *Yeasts* |
| *Zygomycetes* |
| *Coelomyces* |
| *Aureobasidium* |
| *Trichoderma* |

## Assessment of risk of bias

The authors will independently assess methodological limitations for each study using the Newcastle Ottawa Scale (NOS) to appraise the quality of the included studies (16). NOS is used to assess the quality of non-randomised studies including case-control and cohort studies to be used in a systematic review. The NOS contains eight items, categorized into three broad perspectives: the selection of the study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies, respectively. We will rate the quality of the studies (good, fair and poor) by awarding stars in each domain following the guidelines of the Newcastle–Ottawa Scale. A

“good” quality score will require 3 or 4 stars in selection, 1 or 2 stars in comparability, and 2 or 3 stars in outcomes. A “fair” quality score will require 2 stars in selection, 1 or 2 stars in comparability, and 2 or 3 stars in outcomes. A “poor” quality score will reflect 0 or 1 star(s) in selection, or 0 stars in comparability, or 0 or 1 star(s) in outcomes (16).

### Data Synthesis

This will include synthesis of study characteristics and, potentially, statistical synthesis of study findings. Review authors will perform meta-analysis where data is amenable to pooling.

### Dealing with missing data

If there is insufficient data, we will try to contact the corresponding author or the first author to obtain the missing data.

### Sensitivity analysis

A sensitivity analysis will be done by excluding the studies rated as having poor methodological quality.

## Discussion

To our knowledge there is no systematic review in which comparison of micro-organisms present in the upper respiratory tract versus lungs of healthy children and children with respiratory diseases have done. This protocol provides a clear and structured procedure for maximizing the extraction of relevant information and provide the summarised information regarding the microbiome present in the upper and lower respiratory system of children. Through the publication of this protocol readers will be able to assess whether the review was conducted according to a pre-defined plan. Researchers will be aware that the review is underway, thereby avoid duplication. Researchers will also be able to use it as a basis for planning similar review. The findings of this systematic review could be of interest for practitioners.

## Abbreviations

PRISMA-P: Preferred Reporting Items for Systematic Review and Meta-Analysis, MeSH: Medical Subject Headings, NOS: Newcastle Ottawa Scale

## Declarations

### Acknowledgement

Not applicable.

### Funding

There was no funding.

### Availability of data and materials

The dataset used or analysed during the current study are available from the corresponding authors on reasonable request.

### Author's contribution

Both the authors provided the input into design and draft of the final protocol.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interest

There is no competing interests.

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18. Additional file 1 (PRISMA-P checklist)

## Supplementary Files

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- PRISMAPchecklist.docx