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REVIEW PROTOCOL

# Molecular assessment of *Mycobacterium leprae* - infection and disease in contacts: a systematic review protocol

## Avaliação molecular do *Mycobacterium leprae* - infecção e adoecimento em contatos: um protocolo de revisão sistemática

Sarah Lamas Vidal[1]
**ORCID:** 0000-0002-9529-6830

Lavínia Cássia Ferreira Batista[2]
**ORCID:** 0000-0002-7469-9999

Daniele dos Santos Lages[1]
**ORCID:** 0000-0001-9375-2401

Bruna Eduarda Bortolomai[2]
**ORCID:** 0000-0002-8739-4713

Isabela de Caux Bueno[1]
**ORCID:** 0000-0003-4501-5989

Nathan Guilherme de Oliveira[2]
**ORCID:** 0000-0003-2407-5771

Francisco Carlos Félix Lana[1]
**ORCID:** 0000-0001-9043-3181

[1]Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

[2]Instituto Lauro de Souza Lima, Bauru, SP, Brasil

**Editors:**
Ana Carla Dantas Cavalcanti
**ORCID:** 0000-0003-3531-4694

Paula Vanessa Peclat Flores
**ORCID:** 0000-0002-9726-5229

Isabelle Campos de Azevedo
**ORCID:** 0000-0001-5322-7987

**Corresponding author:**
Sarah Lamas Vidal
**E-mail:** sarah_lamas@hotmail.com

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## ABSTRACT

**Objective:** To analyze the relationship between the presence and molecular viability of *Mycobacterium leprae* in the bodies of contacts of leprosy cases and the process of infection and disease in these contacts. **Method:** This systematic review protocol was developed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines and registered in PROSPERO under CRD42022381295. MEDLINE databases were searched via PubMed, Embase, Cochrane Library, LILACS via BVS, Scopus, and Web of Science. Search terms were selected from MeSH, DeCS, and Emtree thesauruses. The search strategy was designed to retrieve studies that included at least one of the following terms: "Leprosy," "Presence and Molecular Viability of *Mycobacterium leprae*," and "Contact Transmission." Two pairs of reviewers will select the study, with disagreements resolved by a third reviewer. Two independent reviewers will extract and enter data from the selected studies into a standardized table. A descriptive synthesis of the results will be performed narratively for each category formed. The methodological quality of the included studies will be assessed using the Newcastle-Ottawa scale.
**Descriptors:** Leprosy; Epidemiologic Monitoring; Molecular Epidemiology.

## RESUMO

**Objetivo:** Analisar a relação entre a presença e a viabilidade molecular do Mycobacterium leprae no organismo de contatos de casos de hanseníase e o processo de infecção e adoecimento desses contatos. **Método:** Este protocolo de revisão sistemática foi elaborado de acordo com as diretrizes *Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols* (PRISMA-P) e registrado no PROSPERO sob o número CRD42022381295. Serão realizadas buscas nas seguintes bases de dados: MEDLINE via PubMed, Embase, *Cochrane Library*, LILACS via BVS, Scopus e *Web of Science*. Os termos para elaboração da estratégia de busca foram selecionados no MeSH, DeCS e Emtree. A estratégia de busca foi planejada para recuperar estudos que abordem ao menos um termo de cada um dos seguintes conceitos: "Hanseníase", "Presença e Viabilidade Molecular do *Mycobacterium leprae*" e "Transmissão em Contatos". A seleção dos estudos será realizada por duas duplas de revisores, com as discordâncias sendo resolvidas por um terceiro revisor. Os dados dos estudos selecionados serão extraídos e registrados em uma tabela padronizada por dois revisores de forma independente. Será realizada uma síntese descritiva dos resultados, de maneira narrativa, a partir de cada categoria formada. A qualidade metodológica dos estudos incluídos será avaliada utilizando a ferramenta *Newcastle-Ottawa Scale*.
**Descritores:** Hanseníase; Monitoramento Epidemiológico; Epidemiologia Molecular.

## INTRODUCTION

Leprosy remains a public health problem in Brazil, among the three countries responsible for 78.1% of the world's new cases. The detection of new cases in children under 15 years of age and in cases with physical disabilities indicates the ongoing transmission chain and late diagnosis[1].

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Because *Mycobacterium leprae cannot be cultured* in vitro, there is still no widely available test to diagnose leprosy. However, in 1960, Shepard described a technique for enumerating *M. leprae* by direct microscopy in animal models[2]. Since then, significant investments have been made to overcome these limitations.

PCR and qPCR assays have been developed to amplify various targets, such as the RLEP sequence[3], sodA mRNA[4], and 16s rRNA[5]. In addition, techniques have been proposed to isolate the DNA and RNA of the bacillus simultaneously[6]. These techniques have been applied to various sample types, including intradermal scrapings[7], blood[8], skin biopsies[9], nerve biopsies[10], and nasal and oral swabs[11].

Close, prolonged contact with a person with a high bacillary load facilitates infection with *M. leprae*. Being in a household or social contact with an index case of leprosy carries a higher risk of developing the disease due to exposure to the bacillus[12]. In addition, a study of household contacts found a high prevalence of healthy individuals with bacilli present in their nasal mucosa, highlighting the role of asymptomatic carriers in the spread of *M. leprae* in the environment[13].

This protocol adheres to the basic principles of conducting a systematic review, such as relevance, transparency of conduct, and thorough and careful evaluation of published evidence, and produces a high-quality synthesis. This can assist researchers in making informed decisions regarding the maintenance of current molecular techniques or the need for new research development[14].

Because epidemiologic surveillance of household contacts is important for leprosy control, the systematic review aims to analyze the relationship between the presence and molecular viability of *M. leprae* in the bodies of contacts of leprosy cases and the course of infection and disease in these individuals.

## METHOD

This systematic review protocol was developed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines[15]. The protocol has been registered on the International Prospective Register of Systematic Reviews (PROSPERO) platform under CRD42022381295.

### Review question

To formulate the review question, the PECOT acronym was used:

- P (Population): Contacts of leprosy cases;
- E (Exposure): Mycobacterium leprae;
- C (Comparison): Not applicable;
- O (Outcome): Infection and/or illness due to leprosy;
- T (Type of study): Observational studies, both descriptive and analytical, including cross-sectional, case-control, and cohort studies.

The systematic review aims to answer the following question "What is the relationship between the presence and/or molecular viability of *M. leprae* and the course of infection and/or disease in contacts of leprosy cases?".

### Designing the search strategy

To develop the search strategy, the concepts "Leprosy," "Presence and Molecular Viability of Mycobacterium leprae," and "Transmission in Contacts" were selected. The strategy was designed to retrieve studies that address at least one term from each concept. Search terms were selected from MeSH, DeCS, and Emtree; significant free terms were included.

No restrictions were applied regarding language, date/period, or publication format. Figure 1 describes the search strategy developed for the MEDLINE database via PubMed. The other search strategies will be published concurrently with the systematic review.

**Figure 1 –** Search strategy on the MEDLINE database via PubMed. Belo Horizonte, MG, Brazil, 2023

| Database | Search strategy |
|---|---|
| MEDLINE via PubMed<br><br>Link to access:<br>https://pubmed.ncbi.nlm.nih.gov/ | ((((((“Leprosy”[Mesh]) OR (“Hansen’s Disease”[Title/Abstract] OR “Hansen Disease”[Title/Abstract] OR Leprosy*[Title/Abstract] OR Hansen*[Title/Abstract])) OR (“*Mycobacterium leprae*”[Mesh])) OR (“*Mycobacterium leprae*”[Title/Abstract]))) AND ((((((((((((Molecular Epidemiology[MeSH Terms]) OR (Molecular Epidemiology[Title/Abstract])) OR (Molecular Diagnostic Techniques[MeSH Terms])) OR (Molecular Diagnostic Techniques[Title/Abstract])) OR (Asymptomatic Infections[MeSH Terms])) OR (Asymptomatic Infections[Title/Abstract])) OR (Subclinical Infection[MeSH Terms])) OR (Subclinical Infection[Title/Abstract])) OR (Nasal Mucosa[MeSH Terms])) OR (Nasal Mucosa[Title/Abstract])) OR (Mouth Mucosa[MeSH Terms])) OR (Mouth Mucosa[Title/Abstract])) OR (“RNA, Ribosomal, 16S”[Title/Abstract] OR “Qpcr”[Title/Abstract] OR “RLEP”[Title/Abstract] OR “ARNr 16s”[Title/Abstract] OR “16s RNA”[Title/Abstract] OR “16Srna”[Title/Abstract] OR “PCR”[Title/Abstract] OR “Polymerase Chain Reaction”[Title/Abstract] OR “Rrna”[Title/Abstract] OR “DNA, bacterial”[Title/Abstract] OR “Real-Time Polymerase Chain Reaction”[Title/Abstract] OR “DNA determination”[Title/Abstract] OR “DNA extraction”[Title/Abstract] OR “DNA sequence”[Title/Abstract]))) AND (((((((“Disease Transmission, Infectious”[MeSH Terms]) OR (“Disease Transmission, Infectious”[Title/Abstract])) OR (“Contact Tracing”[MeSH Terms])) OR (“Contact Tracing”[Title/Abstract])) OR (Family Characteristics[MeSH Terms])) OR (Family Characteristics[Title/Abstract])) OR (“Infectious Disease Transmission”[Title/Abstract] OR “Household Contact”[Title/Abstract] OR “Household Contacts”[Title/Abstract] OR “Patient Contact”[Title/Abstract] OR “Patient Contacts”[Title/Abstract] OR “Peridomiciliary Contacts”[Title/Abstract])) |

**Conducting searches and selecting studies**

Searches will be conducted in the following databases: MEDLINE (via PubMed), Embase, Cochrane Library, LILACS (via BVS), Scopus, and Web of Science. Additionally, the reference lists of included studies will be checked. No search filters will be applied.

Immediately after the database searches are completed, the results are imported into Rayyan QCRI (Qatar Computing Research Institute) software, which manages the study selection process. Results from all databases are then combined, and duplicates are removed.

Study selection will be conducted independently by two pairs of review authors, with their decisions blinded to each other. A third review author will resolve discrepancies.

Initially, studies are selected based on title and abstract screening. After this stage, the selected texts are entered into the Mendeley software, which provides the reviewers with access to the full texts. This software will also manage the references to prepare the systematic review.

In the next step, the full texts of the articles will be read to confirm their eligibility. During this process, studies that were published in full, in Portuguese, English, or Spanish, or with translations into one of these languages, will be selected. Reasons for excluding each article will be recorded in a table containing the eligibility criteria.

Descriptive and analytical observational studies, including cross-sectional, case-control, and cohort studies, will be selected if they have infection and/or disease due to leprosy as an outcome, describe measures of incidence and/or effect and/or predictive measures in their results, and are published in full. Experimental studies, ecologically analytical observational studies, qualitative studies, reviews, and meta-analyses are excluded.

**Data extraction**

Studies selected for inclusion in the systematic review during the full-text reading stage will be re-read in their entirety, and relevant data will be extracted for the review. In addition, the included studies will undergo a methodological quality assessment using the Newcastle-Ottawa Scale (NOS).

Two independent review authors will enter the extracted data into a standardized table. After the tables are completed, they will be compared. In case of discrepancies in the extracted in-

formation, a third review author will decide which information will be included in the final table. The data extraction spreadsheet will include the following fields: authors; year of publication; journal; study design; study location (country); endemicity; study period; population (household or social contact); sample size (n); comparison group (if any); variables included in the study; biological material analyzed; collection site; marker used; analysis technique; effect measures assessed with confidence intervals and p-values (if available); statistical test; and outcome (n/%).

**Summary of results**

The primary outcomes to be studied are infection and disease due to leprosy, based on the presence of *M. leprae* and its viability in biological samples from contacts of leprosy cases.

The results will be synthesized descriptively. To achieve this, the extracted information will be aggregated and analyzed collectively.

Categories will be formed based on the relationships established between the presence and viability of *M. leprae* and the outcomes evaluated. The synthesized information will be described narratively based on each category formed.

## CONFLICT OF INTERESTS

The authors have declared that there is no conflict of interests.

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| **AUTHORSHIP CONTRIBUTIONS** |
|---|
| Project design: Vidal SL, Lana FCF |
| Data collection: Vidal SL, Batista LCF, Lana FCF |
| Data analysis and interpretation: Vidal SL, Batista LCF, Lana FCF |
| Writing and/or critical review of the intellectual content: Vidal SL, Batista LCF, Lages DS, Bortolomai BE, Bueno IC, Oliveira NG, Lana FCF |
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