Association between host genetic polymorphisms and susceptibility to *Helicobacter pylori* infection: a systematic review protocol

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ABSTRACT. *Helicobacter pylori* is a gram-negative, microaerophilic bacterium and an etiological agent of gastroduodenal diseases, including gastritis, peptic ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. *H. pylori* affects approximately half of the global population. Most infected patients remain asymptomatic or exhibit non-severe gastric diseases. The clinical outcome of the infection is intricately associated with a delicate host-parasite relationship. Infected individuals may present a variety of immune and inflammatory responses influenced by genes that either attenuate or exacerbate the infection. Characterizing potential molecular biomarkers of the host could provide a significant contribution to precision medicine, assisting in the diagnosis, prognosis, and personalized therapeutic approaches for *H. pylori* infected patients. This systematic review protocol aims to provide a comprehensive and critical synthesis of the scientific evidence regarding genetic polymorphisms and their association with host

susceptibility to *H. pylori* infection. This protocol has been registered in the International Prospective Register of Systematic Reviews with number CRD42023409085. It was prepared in accordance with the guidelines of the Joanna Briggs Institute for systematic review protocols concerning etiology and risk. The literature searches will be conducted in electronic bibliographic databases. The selection process will be conducted in pairs, using a double-blind format, and any discrepancies will be resolved by a third reviewer. After selection, the relevant data will be extracted and recorded in a designated form. This is designed to find genetic polymorphisms associated with specific clinical outcomes, potentially helping provide valuable insights for precision medicine, allowing the development of personalized and effective therapeutic approaches in patient treatment.

Key words: Bacteria; Precision genetics; Precision medicine; Systematic review

INTRODUCTION

Helicobacter pylori, a prevalent gram-negative bacterium, is estimated to infect more than half of the global population. While most *H. pylori* carriers remain asymptomatic, the infection can lead to various gastrointestinal diseases, such as gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphomas (Papaefthymiou et al., 2019; Malfertheiner et al., 2023).

The variability in clinical outcomes arises from a complex interplay of environmental factors, the virulence of the infecting strain, host immune response, and genetic predisposition (Mohammadi et al., 2022; Malfertheiner et al., 2023). Genetic susceptibility is linked to host gene polymorphisms, particularly those involved in the immune response to *H. pylori.* These polymorphisms can trigger a cascade of cytokines that accelerate the inflammatory process and promote the development of malignant conditions (Chmiela et al., 2017).

Genetic variability, which comprises genetic polymorphisms, is responsible for generating variations in morphology and physiology between individuals belonging to the same species. These factors play an extremely important role in regulating disease severity, especially when they are associated with the host's genetic components (Kalsoom et al., 2020; He and Jiang, 2022). An interaction between two polymorphisms can lead to a synergistic effect, resulting in a phenotypic alteration that increases cytokine production at the cellular level. This phenomenon can trigger exacerbated immune responses and contribute to the severity of certain clinical conditions (Kalsoom et al., 2020).

The objective of this systematic review is to characterize potential host molecular biomarkers associated with susceptibility to *H. pylori* infection. We will identify genetic variability in genes of the immune system components related to increased susceptibility to infection and construct a panel of genetic variants that may contribute to precision medicine, aiding in the diagnosis, prognosis, and personalized therapy for patients.

Therefore, the guiding question for this study is: "Which host genetic polymorphisms are associated with a higher susceptibility to *H. pylori* infection?".

MATERIAL AND METHODS

This systematic review protocol was developed following the guidelines provided by the Joanna Briggs Institute (JBI) for the Etiology and Risk chapter, considering its focus on susceptibility to infection (Moola et al., 2020). We have registered our systematic review protocol in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42023409085. This registration is crucial for reducing duplication of efforts, ensuring transparency, facilitating updates and monitoring of the review, and preventing biases.

Eligibility criteria

The PICO (Population, Intervention, Comparison, and Outcome) format, traditionally used in studies evaluating therapeutic interventions, is not suitable for etiology and risk studies. For this type of research, the PEO (Population, Exposure, and Outcome) format is utilized, which is more appropriate for exploring causes and risk factors. Therefore, a systematic review assessing these factors should encompass these components (Moola et al., 2020).

Articles describing host gene polymorphisms associated with *H. pylori* infection will be included in the study, with no restrictions on time or language. Studies involving animals, duplicate articles, articles without abstracts, review articles, protocols, conference abstracts, and articles that do not address the review's guiding question will not be included. Inclusion details are provided in Table 1.

Table 1. Inclusion and exclusion criteria according to Population, Exposure and Outcome (PEO).

PEO Acronym	Description	Inclusion criteria	Exclusion criteria
P	Population	Population infected with *H. pylori*.	Studies that do not involve humans, such as *in vitro* investigations or research focused on animal experimentation.
E	Exposure	Presence of genetic polymorphisms susceptible to *H. pylori* infection.	Not applicable.
O	Outcome	Associated with *H. pylori* infection.	Studies with no or insufficient data on infection or polymorphism associated with protection or population improvement.

Search methods

Strategies for search will be created using specific subject heading indexes such as MESH terms, and the strategy will be adapted for each database. The search terms/keywords will be combined using Boolean operators 'AND' and 'OR'.

Search strategy

Searches will be conducted in the respective bibliographic databases Web of Science (Science and Social Science Citation Index), Scopus, National Center for Biotechnology Information (NCBI)/PubMed, Portal Regional da BVS, and EMBASE. These searches will be focused on key terms aligned with the previously conducted PEO. Additionally, the references of included studies will be assessed to ensure that no relevant article is omitted during the selection process. The search strategy, which combines MeSH terms and keywords used in the preliminary search in MEDLINE/PubMed, is detailed in Table 2.

Table 2. Search strategy in the NCBI/PubMed database.

DATABASE	SEARCH STRATEGY
NCBI PubMed	(("*Helicobacter pylori*") OR ("*H. pylori*") OR ("*Campylobacter pylori*") OR ("*Campylobacter pyloridis*")) AND (("Polymorphisms, Genetic") OR ("Genetic Polymorphism*") OR ("Genetic susceptibility")) AND ("Infection")

Study selection

Manual and reference searches will be conducted by HCOS, followed by independent verification by team members (CCPC and RSS). The selection process will be carried out by two reviewers (HCOS and DNM), independently, and will be divided into two phases (Phases I and II), where articles will be evaluated based on inclusion and exclusion criteria. The web application Rayyan® will be utilized during Phase I of selection, aiding researchers in extraction and enhancing evaluation, with a focus on titles and abstracts. Articles selected in Phase I will proceed to Phase II, involving a comprehensive reading of the entire article. Both phases will follow a double-blind approach, ensuring that the two independent reviewers are unaware of each other's evaluations. The same reviewers will independently assess the eligibility of each article. In cases of duplicate publications, the article with the most comprehensive data will be used. In the event of disagreements, consensus will be reached between the reviewers at any stage of data extraction or resolved by a third reviewer (AFPLR). If the abstract or full-text article cannot be located, the corresponding author will be contacted twice via email to request the file or relevant information. If no success is achieved in obtaining the required materials, the study will not be selected for data extraction. The study selection process will be presented in a flowchart (Figure 1) following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Shamseer et al., 2015; Page et al., 2021).
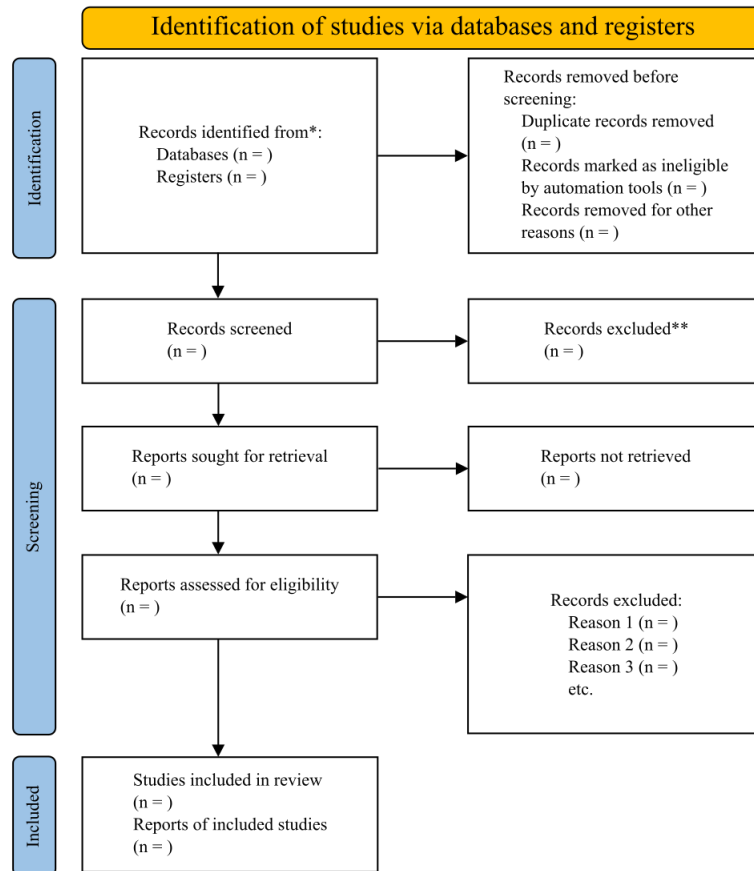
*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicate how many records were excluded by a hum an and how many were excluded by automation tools.

Figure 1. PRISMA flowchart, which documents the process of study selection accurately, clearly, and uniformly in a systematic review (Page et al., 2021)

Risk of methodological bias

The selected articles will be assessed for methodological quality using the JBI questionnaires. Each type of study has a questionnaire with questions that should be answered as "Yes", "No", "Unclear", or "Not applicable". Studies with more than 60% of "Yes" responses will be considered to have a low risk of bias. Two independent reviewers (HCOS and DNM) will assess the methodological bias independently. The third reviewer (AFPLR) will synthesize the results and resolve any discrepancies between the reviewers if there is no consensus. The funnel plot or Egger's test will be used to visualize and comprehensively capture the risk of methodological biases (Muka et al., 2020).

Data Extraction

Data extraction will be conducted by two reviewers (HCOS and DNM), independently, and any discrepancies will be resolved through consensus or by involving a third reviewer (AFPLR). The extracted and selected data will be organized and tabulated using Microsoft Excel®.

In case of any uncertainties regarding the data, the corresponding author will be contacted, and if no response is received after two attempts, the data will be excluded. The official names of genes and polymorphisms will be consulted on the NCBI database platform.

Data synthesis

The data will be presented according to the PEO format, characterizing the Population, Exposure, and Outcome under investigation. The information to be extracted from the articles includes study characteristics (publication year, study location, and design), genetic aspects (gene, genomic location, genotyping method, investigated polymorphism, type of genetic variation, sample size in case and control groups, genotypic or allelic comparisons, and their frequencies in the groups), genetic models, bacterial strain (possessing virulence factors), and statistics (p-value, calculation of odds ratio (OR) with 95% confidence interval (CI)).

If homogeneous studies are found, a meta-analysis of the polymorphisms will be conducted to assess the 95% odds ratios and confidence intervals, grouping them into genetic models. The results will be presented in a table, and forest plots will be used to demonstrate the findings of the meta-analysis (Muka et al., 2020). Furthermore, the potential presence of publication bias will be assessed through a funnel plot calculated using the Egger's test. All statistical analyses will be carried out using RStudio software (version 4.3.2).

Ethics

In studies where no consensus or informed consent is required, and data collection does not involve patients, submission to a research ethics committee is not necessary.

DISCUSSION

So far, this represents the first protocol of a systematic review focused exclusively on susceptibility to *H. pylori* infection, without any association with the resulting pathologies of the host-parasite interaction. The occurrence of this infection in the population reaches around 90% in developing countries, making the study highly relevant in these communities, and less than 40% in developed countries (González et al., 2014; Bassagh et al., 2019). Investigating susceptibility is important to understand this imbalance in the host-parasite relationship. Genetic variability will possibly elucidate the reasons why some hosts present severe diseases, others non-severe diseases, and others remain asymptomatic.

Through the systematic review, we aim to list the main host genetic polymorphisms in the worldwide population infected with *H. pylori* and detect possible polymorphisms or allelic combinations associated with the risk of infection by the microorganism, either individually or in combination.

This systematic review protocol of etiology and risk provides a detailed and comprehensive description of the procedures to be followed in the study, addressing aspects such as which guidelines to use, how to assemble the search strategy, statistical analysis, elements to be collected for the Microsoft Excel® spreadsheet, as well as detailed explanations of each stage of the process. Additionally, it offers recommendations for databases and web applications that will serve as auxiliary tools during the review process.

AUTHOR CONTRIBUTIONS

Conceptualization: MSB, RSS. Formal analysis: HCOS, DNM, AFPLR, CCPC, RSS, MSB. Methodology: HCOS, DNM, AFPLR, CCPC, SBS, RSS, MSB. Resources: HCOS. Software: HCOS. Supervision: MSB, RSS. Writing – original draft: HCOS, DNM, RSS, MSB.

FUNDING

DATA AVAILABILITY STATEMENT

There is no supplementary information accessible beyond the included file, as it serves as a protocol for conducting system reviews.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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