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Protocol for a randomised 'screen-and-treat' *Helicobacter pylori* eradication trial in 14–18-years-old adolescents residing in three regions of Chile: effectiveness and microbiological host implications

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ABSTRACT

Introduction Gastric cancer is a major global health concern, being the final stage of a long-term process, primarily associated with *Helicobacter pylori* (*H. pylori*) infection. Early childhood acquisition of *H. pylori* with low spontaneous eradication rates underscores the need for preventive measures. Our previous pilot treatment study revealed high eradication rates, favourable tolerance profile and a decline in serum biomarkers indicative of gastric damage in asymptomatic school-aged children. The purpose of this study is to determine the potential benefit of a 'screen-and-treat' strategy targeting persistently infected, asymptomatic adolescents. Specific aims are to assess eradication efficacy, its clinical and molecular outcomes and potential clinical and microbiological side effects.

Methods and analysis The screening phase will involve testing 500–1000 asymptomatic adolescents aged 14–18 from three cities in Chile using the urea breath test (UBT) to identify 210 participants with persistent infection. They will proceed to a randomised, non-blinded, controlled trial, receiving either a sequential eradication scheme for *H. pylori* or no treatment. Follow-up will span up to 24 months post-treatment, involving UBT, gastroenterological assessments and blood and stool sample collections. Concurrently, a subset of 60 uninfected adolescents will undergo matched follow-up. Enzyme-linked immunosorbent assay (ELISA) commercial kits will evaluate gastric damage biomarkers in serum (pepsinogen I and II, gastrin-17, VCAM-1, CXCL13). Stool samples will be employed for *Escherichia coli* and *Enterococcus* spp—culture, assessing AMR via the disk diffusion method. *H. pylori* clarithromycin resistance will be determined by molecular method from stool samples. The gut microbiome will be characterised by amplifying and sequencing the 16S rRNA gene from stool samples, followed by bioinformatics analysis.

Ethics and dissemination Approved by the Human Research Ethics Committee at the Faculty of Medicine, University of Chile (073–2022). Findings will be disseminated in peer-reviewed journals and scientific meetings to guide future practices.

Trial registration number NCT05926804.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is a prospective and randomised clinical trial, with a design that will evaluate the effectiveness of eradication in a 'screen-and-treat' strategy in asymptomatic adolescents with persistent *H. pylori* infection.
⇒ While participants will not be blinded to their group assignments for practical reasons, the post-treatment gastroenterological evaluations will be conducted by blinded physicians. This approach aims to mitigate bias in the assessment of clinical outcomes.
⇒ A limitation of the study is that 25%–50% of subjects are expected to not provide one or more blood and/or stool samples, and 10% are expected to dropout. These potential failures have been considered in the sample size calculation.

INTRODUCTION

Despite a decreasing incidence in recent years, gastric cancer (GC) persists as a significant global health challenge.[1] In Chile, GC had the highest mortality rate among all cancers during the period 2002–2021,[2] positioning the country among the highest in GC mortality across Latin America.[3] *Helicobacter pylori* (*H. pylori*) infection, ubiquitous among Chilean adults,[4] is estimated to account for 89% of all adult GC cases, establishing it as the primary cause.[3] The progression from chronic gastritis to premalignant lesions (mucous atrophy, intestinal metaplasia and

dysplasia) and, ultimately, carcinoma is a protracted process.[5]

Eradication of *H. pylori* during the early stages of disease in young adults significantly influences outcomes, aiding in the healing of duodenal ulcers, preventing duodenal and gastric ulcer recurrence[6] and reducing GC incidence.[7] Notably, optimal benefits are observed when eradication occurs before the formation of precancerous lesions, suggesting a critical 'point of no return' in the carcinogenic process for certain patients.[8] In Asian regions with intermediate or high GC prevalence, adopting a 'screen-and-treat' approach targeting *H. pylori* infection in asymptomatic young adults has shown potential in reducing the risk of developing GC.[9–12]

However, *H. pylori* eradication has broader effects that require consideration. Immediate variations in the diversity and composition of the gut microbiome posteradication therapy are observed, with most changes returning to baseline levels within 8 weeks posteradication.[13–16] Additionally, antimicrobial use in *H. pylori* eradication therapies among adults has been linked to short- and long-term resistance in gut bacteria, persisting for up to 8 weeks in enteric Gram-negative bacteria and 3 years in faecal enterococcus.[15 17 18]

Our prior cohort studies consistently revealed that, in Colina, a metropolitan periurban area in Chile, primary acquisition of *H. pylori* infection occurs within the first 5 years of life, reaching an infection rate of approximately 25%. Furthermore, most infected children maintain persistent infection up to 12 years of age, displaying notably low rates of spontaneous clearance.[19 20] Similar trends of early childhood acquisition have been observed in other regions, such as Bangladesh and Ireland.[21 22]

Despite this, abdominal symptoms alone do not reliably predict *H. pylori* infection.[23–25] However, symptomatic children presenting with endoscopic gastroduodenal lesions are more likely to test positive for the infection.[26–28] An extensive review indicates significant underexpression, overexpression or hypermethylation of genes and proteins associated with GC in *H. pylori*-infected children.[29] This suggests that chronic infection during childhood, even in asymptomatic cases, might trigger the development of a progressive, potentially malignant condition later in life.

The 2017 and 2023 clinical guidelines from the European and North American Societies for Paediatric Gastroenterology, Hepatology and Nutrition recommend eradication treatment for children with gastric or duodenal peptic ulcer disease but do not advocate for a 'screen-and-treat' strategy due to unclear clinical benefits.[30 31] However, successful experiences in Japan implementing a two-step screening strategy among high-school students (initial non-invasive testing followed by confirmation via upper endoscopy) have led to estimates that a nationwide 'screen-and-treat' programme for Japanese adolescents could prevent the development of GC in approximately 6000 individuals over their lifetime.[32 33]

Our prior investigations into the dynamics of *H. pylori* infection during early childhood have also revealed that persistently infected children experience heightened abdominal complaints and elevated serum levels of pepsinogen (PG) II, indicating gastric damage.[34] In a pilot eradication trial involving approximately 60 school-aged healthy children with persistent *H. pylori* infection, a 2-week sequential triple therapy achieved eradication in 96.8% of cases. Moreover, this eradication correlated with reductions in PG I and II levels.[35] Notably, no significant decrease in symptoms was observed, likely due to the low frequency and limited sample size.

These findings have led to our proposal for a 'screen-and-treat' strategy, targeting individuals transitioning from childhood to adulthood in three Chilean regions, each exhibiting intermediate prevalence of *H. pylori* infection and intermediate-to-high GC prevalence. The primary objective is to assess eradication therapy's efficacy, clinical and molecular benefits and potential microbial effects.

The specific aims of the trial are as follows.

1. Determine the effectiveness of *H. pylori* eradication treatment in infected 14–18-year-old adolescents from three Chilean cities over 2 years of follow-up.
2. Determine the effect of successful eradication on clinical findings indicative of gastric disease and biomarkers indicative of 'gastric damage' (PG I, PG II, gastrin-17 and other potential biomarkers).
3. Determine the effect of eradication therapy on antimicrobial resistance (AMR) rates in selected gastrointestinal bacteria and gut microbiota composition over time.

The exploratory aims of the trial are as follows.

1. Determine the prevalence of *H. pylori* infection in three groups of 14–18-year-old adolescents from three Chilean cities.
2. Determine the presence of clarithromycin resistance genes in *H. pylori* by stool analysis of children not achieving eradication.
3. Determine the reinfection rates in adolescents with successful eradication, over 2 years of follow-up.
4. Determine the effects of reinfection on clinical findings indicative of gastric disease, biomarkers indicative of 'gastric damage', gut microbiota composition and AMR rates in selected gut bacteria.

MATERIALS AND METHODS

Study design and setting

This is a prospective, multicentre study conducted in Colina, Temuco and Coyhaique cities, from Chile. The primary purpose is treatment, employing a randomised, parallel assignment interventional model with single masking. The protocol is prepared in accordance with Standard Protocol Items: Recommendations for Interventional Trials guidelines (online supplemental table 1). The WHO Trial Registration Dataset items can be found in online supplemental file 1.

Recruitment and screening process

Invitations are being extended to 14–18-year-old students through collaboration with health and educational

authorities in the three cities until a cohort of 500–1000 recruited adolescents is achieved, to achieve a target of 210 *H. pylori* persistently infected individuals.

Educational institutions in each of the three cities in Chile were invited to participate by first contacting the relevant authorities and providing the necessary information. Once their authorisation was obtained, the invitation to students and their parents began and is ongoing. Students are being invited to participate through several strategies: in-person or online meetings with parents, on-site visits to schools to invite students to participate and the distribution of printed brochures containing relevant study information.

A questionnaire will be administered during the screening phase to determine eligibility criteria. On obtaining parental informed consent and adolescent assent (for those under 18) or consent (for those who are 18 years old), participants will undergo *H. pylori* screening using the urea breath test (UBT). Adolescents with negative results in the screening test will be discharged from the study, except for 60 subjects retained as a non-infected control group. Those with a positive test will undergo two additional tests, spaced 30 days apart, to confirm persistent infection. Persistently infected adolescents will be identified by at least two positive tests out of three (first and second, or first and third test). It is expected, based on our pilot study, that 20%–25% of screened adolescents will be positive for *H. pylori*, of which over 90% will be persistently infected.

Eligibility criteria

Inclusion criteria

1. Healthy adolescents 14–18 years of age from Colina, Temuco or Coyhaique.
2. At least one responsible adult family member accessible for phone contact.
3. Persistent *H. pylori* infection determined by at least two positive UBT tests in a 3-month period (except for non-infected controls).

Exclusion criteria

1. Adolescents not consenting to treatment will be invited to continue as non-treated controls.
2. Known allergy to any of the antimicrobials used in the trial protocol (except for non-infected controls).
3. Signs/symptoms compatible with organic abdominal pain according to Rome IV criteria: persistent right upper or right lower quadrant pain, dysphagia, odynophagia, persistent vomiting, gastrointestinal blood loss, involuntary weight loss, deceleration of linear growth and delayed puberty.
4. Prior *H. pylori* eradication therapy.
5. Antimicrobial course received during the previous month (at least 3 days of treatment at appropriate dosing, children meeting this criteria can be included at a later stage).
6. Pregnancy.
7. Use of immunosuppressive or biological drugs.
8. Children deemed 'not healthy' after review of the questionnaire by study physician.

Interventions

The 210 subjects with persistent *H. pylori* infection will be randomised in a 2:1 ratio for either antimicrobial eradication therapy (cases, n=140) or no treatment (controls, n=70). Additionally, a subset of non-infected subjects will be invited to continue in the study (non-infected controls, n=60).

Intervention for cases: 14 days of Lansoprazole (30 mg two times per day) (days 1–14), 7 days of Amoxicillin (1000 mg two times per day) (days 1–7), 7 days of Clarithromycin (500 mg two times per day) (days 8–14) and 7 days of Metronidazole (500 mg two times per day) (days 8–14).

Controls will not receive any intervention initially, but once they complete the initial 6-month follow-up (with their blood and stool samples taken), they will be offered the same treatment as cases, allowing adolescents and their parents to decide freely and informedly whether they wish to receive it or not.

Non-infected controls will not receive any intervention.

Early discontinuation of treatment (before completing 14 days) in subjects allocated to the intervention arm can occur in two scenarios: due to personal decision (eg, mild abdominal complaints) or at the discretion of the medical team (eg, severe intolerance or an allergic reaction), and considered as non-complete therapy subgroup for further analysis.

Allocation of intervention: randomisation sequence generation

We are applying block randomisation stratified by gender and city to assign each participant to one of the two trial arms. Blocked randomisation sequences using block sizes of 3 and 6 were generated for each stratum, with a 2:1 allocation ratio using R and assignment of trial arm was performed using randomisation tools of the REDCap software.[36 37]

Allocation of intervention: concealment mechanism and implementation

After the screening phase, a blind physician conducts a gastroenterological assessment during a pretreatment visit to confirm eligibility criteria (clinical evaluation 1). Following this, randomisation and treatment allocation are carried out by unblinded staff using the REDCap software with predefined parameters to ensure unbiased allocation. The participant is immediately informed of their group allocation by the unblinded physician. Subsequently, medications are delivered directly to participants in the intervention group by the study nurse during an additional scheduled appointment, which also includes education on drug administration and questionnaire completion.

Allocation of intervention: blinding/masking

Blinding of participants is not feasible, as the control arm patients will not receive any medication or placebo. As

Table 1 Protocol key concepts and definitions

Concept	Definition
Persistent *H. pylori* infection	At least two positive UBT tests in a 3-month period during the screening phase.
Non-infected adolescents	Those that have a maximum of one UBT sample positive for *H. pylori* during screening period.
Successful eradication	Negative UBT sample 30 days after treatment.
Reinfection	Adolescents in whom *H. pylori* is successfully eradicated post-treatment, but who then become persistently positive during the remainder of the follow-up period.

H. pylori, *Helicobacter pylori*; UBT, urea breath test.

described in the Surveillance Plan and Data Collection section, subsequent clinical evaluations (clinical evaluations 2–4) are performed by a blinded physician who has no access to information related to group allocation. Additionally, participants are instructed not to inform the blinded physician about their group allocation.

Outcomes measures

Key concepts and definitions for outcomes measures are described in table 1. The study endpoints are depicted in tables 2 and 3.

Surveillance plan and data collection

Study visits

One breath sample will be collected 1 month after the last antimicrobial dose and then every 6 months for five samples per subject to determine sustained eradication or reinfections (figure 1 and online supplemental table 2).

Samples will be collected at an equivalent timepoint for matched controls.

Subjects will undergo gastroenterological evaluation a month before treatment (clinical evaluation 1). Persistently infected adolescents will be randomly assigned in a 2:1 ratio (cases and controls, respectively), paired by gender and city of origin, via the REDCap online survey.[36 37] Randomisation, after the pretreatment gastroenterological evaluation, will be performed by a non-blind physician.

A daily online questionnaire will assess treatment-associated side effects and therapy adherence for 3 weeks (during the 2-week treatment and for days 1–7 after the final treatment dose), with indication to contact the study team in case of need. Solicited symptoms in the

Table 2 Primary and secondary outcomes measures description

Outcome measure	Measure description
Primary outcomes	
Efficacy endpoint: Percentage of persistently infected adolescents, who change UBT status from positive to negative 1-month post-treatment, as compared with non-treated subjects.	UBT samples will be obtained pretreatment and 1-month post-treatment.
Clinical endpoint: Change in the percentage of persistently infected adolescents, who have 'gastric disease' according to gastroenterologist examination from baseline (pretreatment) to 2–4 months postsuccessful eradication therapy, as compared with non-treated subjects.	Clinical evaluation by gastroenterologist or trained physician, blind to the treatment arm of the subject, for specific gastrointestinal (GI) signs/symptoms, will be performed at baseline (during the month prior to treatment) and post-treatment (2–4 months post-treatment).
Biomarkers endpoint: Change in blood levels of biomarkers indicative of gastric damage in adolescents with successful eradication after treatment as compared with non-treated subjects after 6-month follow-up.	Blood samples for PG I, PG II, gastrin and other potential biomarkers of gastric damage. Samples will be collected at baseline, 1 month and 6 months post-treatment.
Secondary outcomes	
Change in faecal *Escherichia coli* and *Enterococcus* AMR rates in treated subjects from baseline to 1 month and 6–12 months post-treatment, as compared with non-treated subjects.	Phenotypic antimicrobial susceptibility analysis of *Escherichia coli* and *Enterococcus* will be performed from stool samples. Samples will be collected at baseline, 1 month and 6–12 months post-treatment.
Change in gut microbiome alpha-diversity index in treated subjects from baseline to 1 month and 6–12 months post-treatment, as compared with non-treated subjects.	30 stool samples from each group (cases and controls) will be obtained at baseline, 1 month and 6–12 months post-treatment. Changes in gut microbiota composition in stool samples will be analysed by sequencing of the 16S rRNA V3–V4 hypervariable region using Illumina.

AMR, antimicrobial resistance; PG, pepsinogen; UBT, urea breath test.

Table 3 Other outcomes measures description

Outcome measure	Measure description
Prevalence of *H. pylori* persistent infection in adolescents in Colina, Temuco and Aysén.	Two initial UBT tests will be performed separated by 1 month; a third UBT test will be obtained if there is a discordance in results of the first two tests.
Effect of treatment on frequency of clarithromycin resistance comparing those subjects who would not eradicate with non-treated individuals.	Stool evaluation for presence of Clarithromycin resistance genes will be performed in stool samples obtained before eradication (30 in each group), and within 6 months after the positive UBT sample indicating non-eradication in conjunction with testing of a similar number of non-treated UBT positive subjects (20 in each group, including 10 treated and non-eradicated subjects).
Overall reinfection rates in adolescents with successful eradication after treatment.	UBT test performed 1 month after the last day of treatment to confirm eradication, and then 6 months after treatment and, subsequently, every 6 months to evaluate reinfection up to 24 months post-treatment.
Change in the percentage of adolescents, which have 'gastric disease' according to gastroenterologist examination from posteradication to postreinfection.	Clinical evaluations by a gastroenterologist or trained physician, blinded to the subject's treatment arm, will be conducted after successful eradication (2–4 months post-treatment), and subsequently, at 9–12 months and 18–24 months post-treatment to assess specific GI signs and symptoms and their changes in relation to reinfection during this follow-up period.
Change in blood levels of biomarkers indicative of gastric damage postreinfection as compared with postsuccessful eradication.	A blood sample will be collected within 6 months after the positive UBT sample indicating reinfection. Biomarkers will be analysed as described in primary endpoints.
Change in gut microbiome alpha-diversity index postreinfection as compared with postsuccessful eradication.	A stool sample will be collected within 6 months after the positive UBT sample indicating reinfection. Gut microbiome analysis will be performed according to what is described in secondary endpoints.
Change in faecal *Escherichia coli* and *Enterococcus* AMR rates postreinfection as compared with postsuccessful eradication.	A stool sample will be collected within 6 months after the positive UBT sample indicating reinfection. AMR will be analysed as described in secondary endpoints.

AMR, antimicrobial resistance; *H. pylori*, *Helicobacter pylori*; UBT, urea breath test.

questionnaire include upper abdominal pain, nocturnal abdominal pain, vomits, nausea, regurgitation, early satiety, bad taste in the mouth and diarrhoea. This questionnaire includes a three-point Likert scale to estimate severity of each symptom from no reaction (zero point) to severe (three points). Though safety is not included in this trial's outcomes, medical monitoring of adverse events will be performed, with the possibility to indicate early discontinuation of treatment due to severe intolerance according to medical criteria.

Failure to complete the survey or record therapy adherence for two consecutive days will prompt contact via phone.

Study subjects will be followed every 6 months by a study nurse, who will record relevant variables in REDCap (other antimicrobial use, referred adverse effects), conduct a UBT test and request a stool sample.

All subjects will be assessed by a gastroenterologist or trained paediatrician, blinded to the subject's treatment arm, at 1 month (if persistent treatment side effects are detected), 2–4 months (clinical evaluation 2), 9–12 months (clinical evaluation 3) and 18–24 months (clinical evaluation 4) for specific GI signs/symptoms. Equivalent assessments will be done for matched controls.

Blood samples will be obtained from infected children within 2 weeks before eradication treatment initiation and at similar time frames in non-treated age-matched controls (presample), 1-month post-treatment termination (postacute) and at 6 months (postdelayed). Approximately, 150 pre- and postacute samples (75% acceptance) and 100 postdelayed samples (50%) are expected.

Stool samples for microbiome and AMR studies will be obtained from consenting adolescents. Based on the prior studies, 50% adherence with one or more stool samples is anticipated. Samples will be collected from both treated and non-treated groups within 1-month pretreatment (presample), 1-month post-treatment termination (postacute) and at 6–12 months (postdelayed).

In cases of reinfection after successful eradication, a blood sample and stool sample will be collected within 6 months after the positive UBT sample indicating reinfection.

Sample collection

Breath samples will be collected using the Heliforce kit, which includes breath collection bags and 50 mg orange-flavoured granulated ^{13}C-urea. Once administered orally, granules will dissolve in 80–100 mL of fresh water. Each

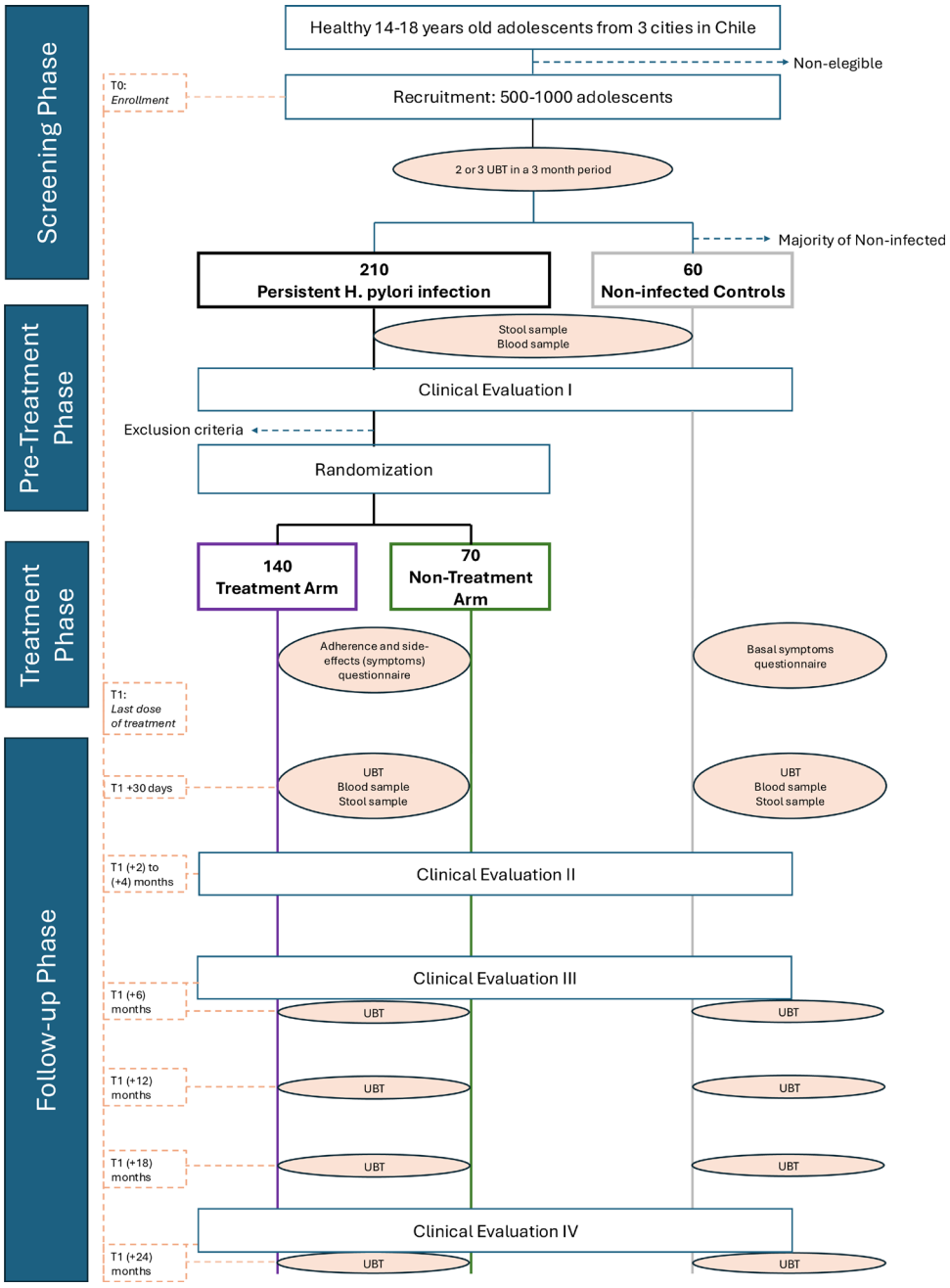

Figure 1 Flowchart: trial phases and activities. *H. pylori*, *Helicobacter pylori;* UBT, urea breath test.

subject will provide two breath samples: a baseline and a postdose sample 30 min after ingesting the ^{13}C-urea

Fresh stool samples (within 1 hour of emission) will be collected and handled by a responsible family member following specific instructions (storage in a refrigerator at 4 °C). Within 24 hours, study personnel will collect the sample, transport it to the laboratory and store it at −70 °C. A swab sample collected in Cary Blair transport medium will be used for stool culture on the collection day.

Study personnel will obtain 10 mL of blood using routine procedures.

Sample testing

- *UBT*: samples will be analysed using the IR-Force Infrared Spectrometer. A delta-over-baseline ≥4.0 ± 0.4 will be considered positive.
- *Biomarker analysis*: PG I/PG II/gastrin-17 will be assessed in plasma using GastroPanel (Biohit Oyj, Helsinki, Finland). VCAM-1 and CXCL13 will be measured using ELISA commercial kits.

Antimicrobial resistance

- *Escherichia coli culture in stool samples*: a swab of each stool sample kept in Cary Blair medium will be streaked on MacConkey agar and grown overnight at 35–37 °C. Suspicious colonies will be selected, inoculated in differential chromogenic agar (Oxoid Brilliance UTI

agar) and grown overnight at 37 °C. *Escherichia coli* will be defined according to the growth of dark pink colonies in chromogenic agar. The selected strains will be cultured in TSA agar and grown overnight at 35–37 °C. Then, they will be frozen in cryogenic tubes with 10% glycerol brucella medium. Finally, susceptibility to antibiotics (ampicillin, ampicillin–sulbactam, cefazolin, ceftazidime, levofloxacin and gentamicin) will be performed by Kirby–Bauer disk diffusion method on Muller Hinton agar.

- *Enterococci culture in stool samples*: faecal samples in CaryBlair swabs will be cultured on Chromocult Enterococci broth, incubated at 37 °C for 24 hours or at room temperature for 48 hours and checked for change in broth colour from yellow to blue. Then, 10 µl of this broth will be inoculated in Bile aesculin agar, incubated at 37 °C for 24 hours and checked for growth of small black coloured colonies. The presence of enterococci will be confirmed by further tests, such as negative catalase reaction and growth of light turquoise colonies on chromogenic agar (Oxoid Brilliance urinary tract infections (UTI) agar). The selected strains will be cultured in Tryptic soy agar (TSA), grown overnight at 35–37 °C, and then frozen in cryogenic tubes with 10% glycerol brucella medium. Antimicrobial susceptibility testing (erythromycin, ampicillin, penicillin and vancomycin) will be performed using the Kirby–Bauer disk diffusion method on Muller Hinton agar.
- *H. pylori clarithromycin resistance gene.*

DNA purification from stools: DNA will be extracted from 200 mg of faecal samples using the DNeasy Power Soil Pro Kit (QIAGEN), according to the manufacturer's protocol.

ureC amplification by nested-quantitative polymerase chain reaction (qPCR): to confirm the detection of *H. pylori* in stools, amplification of *ureC* gene will be performed using nested-qPCR; first reaction in a MiniAmp Plus thermal cycler (ThermoFisher) and second reaction (qPCR) in a QuantStudio3 Real-Time PCR (ThermoFisher) using Synergy Brands (SYBR) Green kit 2x (KAPA SYBR FAST qPCR) as previously published.[38]

Detection of clarithromycin-resistant strain by nested-TaqMan qPCR: a first round of conventional PCR using primers for the 23S rRNA gene will be made from DNA isolated from stools. Later, a product of this first PCR will be submitted to nested-qPCR using probe AG/GA (FAM/BHQ) and probe AA (HEX/BHQ) as previously published.[39]

Microbiome studies

The composition of the bacterial faecal community will be characterised by amplification and sequencing of the 16S ribosomal subunit gene. Purified genomic DNA will be obtained using the DNeasy Power Soil Pro Kit (QIAGEN) for subsequent amplification and sequencing of the 16S ribosomal subunit gene V3–V4 variable region by Illumina MiSeq platform.

Sequenced amplicons will be analysed using DADA2 pipeline[40] in RStudio.[41] The filtered sequences will be aligned with 100% similarity to obtain an amplicon sequence variant (ASV). Taxonomic levels will be identified from ASVs using the SILVA132 database,[42] and then alpha- and beta-diversity analysis will be performed. Microbiome taxa abundance will be expressed as a percentage of the total reads.

Data communication to subjects and parents

Any clinically relevant findings from clinical evaluations will be communicated to the subjects and their parents, along with medical counselling on how to proceed. If necessary, they will be referred to the appropriate health professionals. The results of tests with potential clinical significance (such as UBT and gastropanel) will be communicated to the subjects and their parents through written reports sent via email.

Data management

Epidemiological, clinical and laboratory data will be collected using electronic forms on password-protected tablets and notebooks. The electronic forms are hosted on the REDCap platform, and access to each form is restricted to personnel responsible for entering the corresponding data. Each user has an individual, non-transferable password-protected account and receives prior training on using the platform. The system includes an audit trail to track changes made to the entered data and identify the user responsible for those changes. Data entered into REDCap are encrypted and stored on servers at the Faculty of Medicine, University of Chile. This information is backed up daily. To ensure data security and access to new tools, the REDCap platform is updated at least two times a year.

Statistical considerations

Sample size

In a previous pilot study, the rate of upper gastric symptoms postintervention appeared lower in the treatment group than in the non-treatment group, although statistically non-significant (10%). A sample size of 189, randomised in a 2:1 ratio across both groups, would yield 80% power under a one-sided type I error of 5% to detect this difference (primary outcome 1). Regarding PG II levels, this sample size enables us to detect a difference of 6.3 ng/dL, favouring the treatment group (8.9 vs 15.2) with 99% power under a two-sided type I error of 2.5% (primary outcome 2). Assuming a 10% dropout rate, this necessitates recruiting 210 infected individuals for this trial (140 in the treatment and 70 in the non-treated group).

Statistical methods and data analysis

The prevalence of persistent *H. pylori* infection, eradication and reinfection rates will be presented with 95% CIs. Associations between eradication and changes in clinical findings indicating gastric disease and biomarkers will be examined by comparing pretreatment and

follow-up evaluations/samples. Statistical differences will be assessed using Pearson's χ^2 test or Fisher's exact test for categorical variables (based on sample size), an independent sample t-test for normally distributed continuous variables or the Mann–Whitney U-test for non-normally distributed continuous variables. Changes in AMR following treatment (at timepoints 1 and 6–12 months) will be compared using Pearson's χ^2 test. Microbiome taxa relative abundance data distribution will be scrutinised using the Shapiro–Wilk normality test. A multidimensional scaling analysis will be conducted, and group comparisons will be performed using the Kruskal–Wallis test. Correlation networks between microbial taxa abundance and clinical parameters will be established by Spearman correlation test. Statistical analyses will be executed in R[43] and RStudio[41] (R Foundation for Statistical Computing, Vienna, Austria).

Adjusted analysis

Analysis of primary outcomes will be adjusted for gender and city (basis of stratification) and age. For efficacy endpoint (binary: successful eradication vs no eradication), a logistic regression model will be used. For clinical endpoint (change of proportions) and biomarkers endpoint (longitudinal and continuous), a mixed-effects linear model will be used.

Subgroup analysis

Subgroup analysis will be conducted for those children with non-complete eradication therapy (defined as one or more missing doses before completing 14 days of treatment). In those cases, a UBT will be performed 1 month after the last day of treatment to confirm successful eradication or non-eradication. Subjects with non-complete therapy but successful eradication will be included for the analysis of primary and secondary endpoints in a subgroup analysis. Subjects with non-complete therapy and non-eradication will only be included for analysis in secondary outcomes as a subgroup. Based on our previous pilot study, we expect to find a high rate of successful eradication in the treated group, even if they do not complete the 14 days of treatment.

Analysis of population and missing data

Primary clinical and biomarkers endpoints will be evaluated 1-month posteradication therapy, and only subjects from intervention arm with successful eradication and non-treated subjects will be included in the analysis. Therefore, subjects who withdraw from the study before 1-month post-treatment or who do not achieve successful eradication will not be included in the analysis of these outcomes (per-protocol analysis). Based on our previous studies, we expect a low dropout rate at 1-month post-treatment (<10%) and a high eradication rate (>90%), and therefore, we will perform a complete case analysis only. If higher dropout rates are observed, missing data will be addressed with inverse probability of attrition weighting or other methods depending on the potential causes of dropout.

Study major dates

Recruitment for screening phase started on 2 August 2022. Primary completion date, defined as the date on which data collection is completed for all the primary outcome measures, is projected for 31 October 2025; Study completion date, defined as the date on which the last participant is examined for completion of all the outcomes, is projected for 31 March 2027.

Patient and public involvement

There is no patient and public involvement in the design and execution of this study.

ETHICS AND DISSEMINATION

The trial and informed consents/assents received approval from the Human Research Ethics Committee at the Faculty of Medicine, University of Chile, with the assigned approval number 073-2022. Participation in the study is entirely voluntary, and strict confidentiality of all information is assured. All participants in this study will be asked to provide written informed consent (and assent for those under 18 years of age) (online supplemental file 2).

For those subjects and their parents who express interest in participating, informed consent and assent forms are provided either in person or via email, according to their preference. An in-person or telephone visit is then scheduled with a study nurse or physician. During this visit, all questions are addressed, and the written informed consent or assent process is conducted either in person or remotely via the REDCap platform. The person responsible for obtaining informed consent and assent will be a trained study physician, nurse or other healthcare professional who verbally provides the study information in addition to the written document and addresses the participants' questions and concerns.

Dissemination will include presentations at national and international conferences and publication in peer-reviewed journals.

During the trial, only study personnel will have access to data. After trial ending, in the case of data sharing, only deidentified participant data will be shared with other investigators on request and with prior authorisation from the corresponding ethics committee. The data sharing plan is available in the trial registry and in the online supplemental table 3.

Any modifications to the protocol that may impact the conduct of the study, potential benefit of the patient or may affect patient safety, including changes in study objectives, study design, patient population, sample sizes, study procedures or significant administrative aspects will require a formal amendment to the protocol to be approved by the Ethics Committee.

All the participants information is stored securely in the REDCap platform, administered by the Faculty of Medicine, University of Chile.

This is a low-risk study, as it involves the prescription of a well-standardised and safe antibiotic eradication therapy in a population with no greater risk of adverse events than the general population. For any health condition identified during study activities that require medical attention, the study team will refer the participant to the appropriate healthcare system.

Independent and random audits performed by the Ethics Committee can take place during the conduct of the study.

Trial registration and status

Recruitment for the screening phase of study began on 2 August 2022. Clinical trial registration (NCT05926804) was finalised on 1 June 2023, after the first patient was screened for *H. pylori* infection, but before any subjects were randomised to receive treatment or not. The first randomisation occurred on 1 September 2023, qualifying as a prospective registration. A total of 142 persistently infected subjects have been recruited, and 106 subjects have been randomised by the time of the final revision of this article.

Any future changes in the protocol design or interventions will be prospectively registered in ClinicalTrials.gov.

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Contributors MO conceived the idea for the study and is the principal investigator. SG, YL and MO designed and wrote the original protocol. CC and NM designed and are responsible for the laboratory assessments. SG and YL are responsible for clinical trial execution in Colina, BZT in Coyhaique and LF in Temuco. AL is the trial manager and responsible for statistics and data management. XA significantly contributed to protocol writing and editing. YL and MO have contributed equally to the protocol design and the current execution of the study; therefore, they both can be considered as corresponding authors. Guarantor is MO.

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REFERENCES

1 Arnold M, Abnet CC, Neale RE, *et al*. Global Burden of 5 Major Types of Gastrointestinal Cancer. *Gastroenterology* 2020;159:335–49.
2 Ministerio de Salud, Departamento de Epidemiología. Defunciones y mortalidad por cáncer. Available: https://deis.minsal.cl/ [Accessed 15 Nov 2024].
3 Sung H, Ferlay J, Siegel RL, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA A Cancer J Clinicians* 2021;71:209–49.
4 Hooi JKY, Lai WY, Ng WK, *et al*. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 2017;153:420–9.
5 Correa P, Piazuelo MB. The gastric precancerous cascade. *J Dig Dis* 2012;13:2–9.
6 Ford AC, Gurusamy KS, Delaney B, *et al*. Eradication therapy for peptic ulcer disease in Helicobacter pylori-positive people. *Cochrane Database Syst Rev* 2016;4:CD003840.
7 Lee Y-C, Chiang T-H, Chou C-K, *et al*. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. *Gastroenterology* 2016;150:1113–24.
8 Wong BC-Y, Lam SK, Wong WM, *et al*. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004;291:187–94.
9 Fock KM, Talley N, Moayyedi P, *et al*. Asia-Pacific consensus guidelines on gastric cancer prevention. *J Gastroenterol Hepatol* 2008;23:351–65.
10 O'Connor A, O'Morain CA, Ford AC. Population screening and treatment of Helicobacter pylori infection. *Nat Rev Gastroenterol Hepatol* 2017;14:230–40.
11 Ford AC, Forman D, Hunt RH, *et al*. Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2014;348:g3174.
12 Lee YC, Lin JT. Screening and treating Helicobacter pylori infection for gastric cancer prevention on the population level. *J Gastroenterol Hepatol* 2017;32:1160–9.
13 Tao ZH, Han JX, Fang JY. Helicobacter pylori infection and eradication: Exploring their impacts on the gastrointestinal microbiota. *Helicobacter* 2020;25:e12754.
14 Yap TW-C, Gan H-M, Lee Y-P, *et al*. Helicobacter pylori Eradication Causes Perturbation of the Human Gut Microbiome in Young Adults. *PLoS ONE* 2016;11:e0151893.
15 Liou J-M, Chen C-C, Chang C-M, *et al*. Long-term changes of gut microbiota, antibiotic resistance, and metabolic parameters after Helicobacter pylori eradication: a multicentre, open-label, randomised trial. *Lancet Infect Dis* 2019;19:1109–20.

16 Ye Q, Shao X, Shen R, *et al*. Changes in the human gut microbiota composition caused by Helicobacter pylori eradication therapy: A systematic review and meta-analysis. *Helicobacter* 2020;25:e12713.
17 Jakobsson H, Wreiber K, Fall K, *et al*. Macrolide resistance in the normal microbiota after Helicobacter pylori treatment. *Scand J Infect Dis* 2007;39:757–63.
18 Sjölund M, Wreiber K, Andersson DI, *et al*. Long-term persistence of resistant Enterococcus species after antibiotics to eradicate Helicobacter pylori. *Ann Intern Med* 2003;139:483–7.
19 O'Ryan ML, Lucero Y, Rabello M, *et al*. Persistent and transient Helicobacter pylori infections in early childhood. *Clin Infect Dis* 2015;61:211–8.
20 O'Ryan ML, Rabello M, Cortés H, *et al*. Dynamics of Helicobacter pylori detection in stools during the first 5 years of life in Chile, a rapidly developing country. *Pediatr Infect Dis J* 2013;32:99–103.
21 Kienesberger S, Perez-Perez GI, Olivares AZ, *et al*. When is Helicobacter pylori acquired in populations in developing countries? A birth-cohort study in Bangladeshi children. *Gut Microbes* 2018;9:252–63.
22 Rowland M, Clyne M, Daly L, *et al*. Long-term follow-up of the incidence of Helicobacter pylori. *Clin Microbiol Infect* 2018;24:980–4.
23 Chobot A, Porębska J, Krzywicka A, *et al*. No association between Helicobacter pylori infection and gastrointestinal complaints in a large cohort of symptomatic children. *Acta Paediatr* 2019;108:1535–40.
24 Asgeirsdottir GA, Kjartansdottir I, Olafsdottir AS, *et al*. Helicobacter pylori infection in Icelandic children. *Scand J Gastroenterol* 2017;52:686–90.
25 Szaflarska-Popławska A, Soroczyńska-Wrzyszcz A. Prevalence of Helicobacter pylori infection among junior high school students in Grudziadz, Poland. *Helicobacter* 2019;24:e12552.
26 Burgard M, Kotilea K, Mekhael J, *et al*. Evolution of Helicobacter pylori associated with gastroduodenal ulcers or erosions in children over the past 23 years: Decline or steady state? *Helicobacter* 2019;24:e12629.
27 Boyanova L, Hadzhiyski P, Markovska R, *et al*. Prevalence of *Helicobacter pylori* is still high among symptomatic Bulgarian children. *Acta Microbiol Immunol Hung* 2019;66:255–60.
28 Tang Z, Shi J, Ji M, *et al*. The characteristics of 83 giant peptic ulcers in Chinese children: Evaluation and follow-up. *Saudi J Gastroenterol* 2018;24:360–4.
29 George S, Lucero Y, Torres JP, *et al*. Gastric Damage and Cancer-Associated Biomarkers in *Helicobacter pylori*-Infected Children. *Front Microbiol* 2020;11:90.
30 Jones NL, Koletzko S, Goodman K, *et al*. Joint ESPGHAN/NASPGHAN Guidelines for the Management of Helicobacter pylori in Children and Adolescents (Update 2016). *J Pediatr Gastroenterol Nutr* 2017;64:991–1003.
31 Homan M, Jones NL, Bontems P, *et al*. Updated joint ESPGHAN/NASPGHAN guidelines for management of Helicobacter pylori infection in children and adolescents (2023). *J Pediatr Gastroenterol Nutr* 2024;79:758–85.
32 Akamatsu T, Ichikawa S, Okudaira S, *et al*. Introduction of an examination and treatment for Helicobacter pylori infection in high school health screening. *J Gastroenterol* 2011;46:1353–60.
33 Akamatsu T, Okamura T, Iwaya Y, *et al*. Screening to Identify and Eradicate Helicobacter pylori Infection in Teenagers in Japan. *Gastroenterol Clin North Am* 2015;44:667–76.
34 Lucero Y, Lagomarcino AJ, Torres JP, *et al*. Helicobacter pylori, clinical, laboratory, and noninvasive biomarkers suggestive of gastric damage in healthy school-aged children: A case-control study. *Int J Infect Dis* 2021;103:423–30.
35 Lucero Y, Lagomarcino AJ, Torres JP, *et al*. Effect of Helicobacter pylori eradication therapy on clinical and laboratory biomarkers associated with gastric damage in healthy school-aged children: A randomized non-blinded trial. *Helicobacter* 2021;26:e12853.
36 Harris PA, Taylor R, Thielke R, *et al*. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–81.
37 Harris PA, Taylor R, Minor BL, *et al*. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
38 Taborda MI, Aquea G, Nilo Y, *et al*. Non-invasive Diagnostic of Helicobacter pylori in Stools by Nested-qPCR. *Pol J Microbiol* 2018;67:11–8.
39 Gonzalez-Hormazabal P, Musleh M, Escandar S, *et al*. Prevalence of clarithromycin resistance in Helicobacter pylori in Santiago, Chile, estimated by real-time PCR directly from gastric mucosa. *BMC Gastroenterol* 2018;18:91.
40 Callahan BJ, McMurdie PJ, Rosen MJ, *et al*. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;13:581–3.
41 RStudio Team. RStudio: integrated development for R. 2020.
42 Quast C, Pruesse E, Yilmaz P, *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;41:D590–6.
43 R Core Team. R: a language and environment for statistical computing. 2021.