ARTÍCULO ORIGINAL

Antimicrobial resistance profile in *Helicobacter pylori* isolates from Costa Rica

Perfil de resistencia antimicrobiana en aislamientos de Helicobacter pylori en Costa Rica

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ABSTRACT
Objectives: *Helicobacter pylori* infection relates to multiple gastric diseases, and its eradication is becoming more challenging due to antimicrobial resistance to commonly used antibiotics, especially clarithromycin. The aim of this study is to describe the antimicrobial susceptibility profiles of *H. pylori* isolates from Costa Rican patients. **Materials and methods:** Antimicrobial susceptibility was assessed for 14 *H. pylori* isolates using the E-test method for amoxicillin, clarithromycin, levofloxacin, metronidazole, tetracycline, and rifampicin. Due to its importance, the point mutations A2143G and A2144G related to clarithromycin resistance were also studied using PCR-RFLP. **Results:** Resistance to clarithromycin (29%), levofloxacin (29%), metronidazole (29%), and rifampicin (7%) was found in the studied isolates. These included three isolates with multidrug resistance. A2143G mutation was detected in one isolate and A2144G was detected in two isolates, and the presence of mutations correlated significantly with phenotypic resistance to clarithromycin (r=0.8321, p=0.0071). **Conclusions:** Antibiotic resistance was high in the studied isolates from Costa Rica. The number of isolates is small, but the results highlight the need for further studies to assess the antibiotic resistance as a growing problem in the country. *H. pylori* culture and phenotypic tests are difficult, but molecular testing can be used as a quick and cheap option to monitor clarithromycin resistance.
Keywords: *Resistance, Antibiotic; Clarithromycin; 23S rRNA (source: MeSH NLM).*

RESUMEN
Objetivos: La infección por *Helicobacter pylori* se asocia con múltiples patologías gástricas, y su erradicación es un reto debido a la resistencia a los antimicrobianos más comúnmente utilizados, especialmente la claritromicina. El objetivo de este estudio es describir los perfiles de susceptibilidad antimicrobiana de aislamientos de *H. pylori* obtenidos de pacientes costarricenses. **Materiales y métodos:** La susceptibilidad antimicrobiana se evaluó en 14 aislamientos de *H. pylori* mediante el método E-test para amoxicilina, claritromicina, levofloxacina, metronidazol, tetraciclina y rifampicina. Debido a su relevancia, también se analizaron las mutaciones puntuales A2143G y A2144G asociadas con resistencia a claritromicina, utilizando la técnica de PCR-RFLP. **Resultados:** Se observó resistencia a claritromicina (29%), levofloxacina (29%), metronidazol (29%) y rifampicina (7%) en los aislamientos estudiados, incluyendo tres aislamientos con resistencia múltiple a los antimicrobianos. La mutación A2143G se detectó en un aislamiento y la mutación A2144G en dos aislamientos, encontrándose una correlación significativa entre la presencia de mutaciones y la resistencia fenotípica a claritromicina (r=0,8321, p=0,0071). **Conclusiones:** La resistencia a los antibióticos fue elevada en los aislamientos de *H. pylori* analizados en Costa Rica. El número de aislamientos es limitado, pero indica la necesidad de realizar más estudios para abordar el creciente problema de la resistencia antimicrobiana. El cultivo y las pruebas fenotípicas de *H. pylori* son difíciles de realizar, pero las pruebas moleculares pueden emplearse como una alternativa rápida y económica para el monitoreo de la resistencia a claritromicina.
Palabras clave: *Resistencia a Antibióticos; Claritromicina; ARNr 23S (fuente: DeCS BIREME).*

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INTRODUCTION

Helicobacter pylori is a Gram-negative bacillus that colonizes the human gastric mucosa [1]. It is considered a pathobiont, associated with gastritis, peptic ulcer disease (PUD), B-cell MALT lymphoma and gastric adenocarcinoma (GAC) [2]. *H. pylori* is difficult to culture due to its microaerophilic metabolism and demanding nutritional requirements, resulting in poor growth under standard laboratory conditions [3].

H. pylori prevalence varies amongst regions. A recent meta-analysis reported a global crude prevalence of 45.6% among adults during 2010-2022, with the highest rates in Africa and the Middle East (88.6% in Jordan), and the lowest in Europe (9.1% in Finland) [4]. In the Americas, prevalence was estimated at 42.5%, with the highest rates in Guatemala (86.6%), Ecuador (85.7%), and Nicaragua (83.3%) [4]. Similarly, a study conducted in six Latin American countries reported an overall prevalence of 79.4% [5]. Both studies highlight the importance of the infection in this region. In Costa Rica, prevalence is higher among dyspeptic patients (70-90%) [6] than in the general population (57% in urban adults and 14% in children under 7 years of age) (Romero-Carpio, unpublished data).

H. pylori-associated gastritis is considered an infection, regardless of symptomatology [7]. The Maastricht VI/ Florence Consensus recommends eradication therapy for all infected individuals [8]. Under the test-and-treat strategy, the American College of Gastroenterology (ACG) recommends testing in patients with PUD, MALT lymphoma, and for primary and secondary prevention of gastric cancer [9]. Eradication treatment must completely eliminate *H. pylori* to stop the development of pathologies [10]. Eradication of *H. pylori* completely cured PUD in more than 90% of cases, prevented the recurrence of bleeding [11], and reduced GAC risk [7]. The therapeutic goal is to achieve eradication rates above 90% while minimizing the development of antimicrobial resistance [8,12].

Current eradication regimens combine proton-pump inhibitors (PPIs) with antibiotics such as amoxicillin, clarithromycin, metronidazole, tetracycline, rifabutin, and bismuth compounds [13]. PPIs enhance antimicrobial efficacy by increasing gastric pH. Most antimicrobials fail as monotherapy *in vivo*. Clarithromycin is the most effective, with an eradication rate of 40% in a twice-a-day administration for 14 days [14]. It is an acid-stable, bacteriostatic macrolide that inhibits protein synthesis by binding to the peptidyl-transferase region of the 23S rRNA. Clarithromycin is well absorbed orally and achieves higher tissue than plasma concentrations [15].

The Maastricht VI/Florence Consensus stratifies treatment according to local clarithromycin resistance (CLR; MIC ≥1 µg/mL)[8]. In regions with CLR <15%, first-line therapy includes bismuth quadruple therapy (BQ) or clarithromycin-based triple therapy. In areas with CLR ≥15% or unknown prevalence, BQ is recommended, with non-bismuth quadruple (concomitant) therapy as an alternative. Levofloxacin-containing regimens are reserved for second- or third-line therapy, and rifabutin for rescue treatment. Acid suppression is achieved using PPIs or potassium-competitive acid blockers (P-CABs), such as vonoprazan. The ACG recommends an optimized BQ for both naïve and treatment-experienced patients in the US, where high CLR (22.2-31.5%) and levofloxacin resistance (37.6%) are reported [9]. Eradication failure occurs in at least 20% of cases and is mainly attributed to poor adherence, inadequate acid suppression, and antimicrobial resistance [16].

In Costa Rica, several studies have evaluated eradication rates of H. pylori therapies. A triple regimen consisting of amoxicillin, bismuth subsalicylate, and metronidazole achieved an eradication rate of 10.9% [17]. In contrast, a 10-day clarithromycin-based triple therapy (amoxicillin, clarithromycin, and a proton pump inhibitor [PPI]) yielded eradication rates of 84.7% and 90.5% in different clinical settings [18,19]. However, rising clarithromycin resistance (CLR) substantially compromises treatment efficacy and contributes to eradication failure. Consequently, routine clarithromycin susceptibility testing using molecular or culture-based methods is recommended, when available, before prescribing clarithromycin-containing regimens [8]. Clarithromycin resistance in H. pylori is primarily mediated by point mutations in the peptidyl-transferase region of the 23S rRNA gene, which reduce antibiotic binding to the ribosome [1]. The most prevalent mutations are A2142G, A2143G, and A2144G. Additionally, other mutations in the 23S rRNA gene, such as T2289C, C2245T, G2224A, T2182C, and T2717C, have occasionally been reported in macrolide-resistant *H. pylori isolates*.

In Costa Rica, the current prevalence of antimicrobial resistance in *H. pylori* remains unknown, but an increase can be inferred due to global data on augmented frequency of clarithromycin and levofloxacin resistance. In the absence of local epidemiological data, it is not possible to determine whether empirically selected treatment regimens are appropriate. Antimicrobial susceptibility testing for *H. pylori* in Costa Rica is limited, primarily due to the organism's fastidious nature and the high contamination rates associated with primary cultures. Implementing molecular approaches in both isolates and biopsy samples could enhance detection rates and provide preliminary data on CLR, thereby improving therapeutic decision-making. In this context, the objective of this study was to characterize phenotypic resistance profiles to clarithromycin, metronidazole, levofloxacin, tetracycline, amoxicillin and rifampicin of *H. pylori* isolates obtained from Costa Rican individuals. Then, we aim to determine the presence of A2143G and A2144G mutations in the 23S rRNA gene in order to assess the correlation between phenotypic and genotypic methods and highlight the value of molecular testing in the characterization of CLR in *H. pylori* in Costa Rica.

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MATERIALS AND METHODS

Selection of isolates and strains

14 *H. pylori* isolates were cultured from upper gastrointestinal endoscopic biopsies from Costa Rican patients, following previously defined inclusion and exclusion criteria [20]. Two reference strains: 26695 and 43504 were used as controls.

Bacterial culture

Frozen pure *H. pylori* stocks were thawed and inoculated onto Helicobacter-selective agar [20] and incubated for 48-120 h at 37°C under microaerophilic conditions (CampyGen sachets, Oxoid, UK). Identification was confirmed by morphology, motility, Gram staining, and biochemical testing (urease, catalase, oxidase). Confirmed isolates were subcultured for 48h to obtain sufficient biomass for downstream assays.

Phenotypic antimicrobial resistance testing

Minimal inhibitory concentrations (MICs) were determined using E-test strips (bioMérieux, France). Bacterial suspensions in Brucella broth (Oxoid, UK) were adjusted to 3 McFarland. One milliliter was plated onto 150 mm Mueller-Hinton agar supplemented with 5 % sheep blood and Vitox supplement (Oxoid, UK). E-test strips for clarithromycin, metronidazole, levofloxacin, tetracycline, amoxicillin and rifampicin were placed on dry plates and incubated at 37°C under microaerophilic conditions for 72h.

DNA extraction and quantification

DNA was extracted from subcultures using the PureLink Genomic DNA MiniKit (ThermoScientific, USA) according to manufacturer's instructions. DNA concentration was measured using a NanoDrop™ 2000 spectrophotometer.

Polymerase chain reaction (PCR) and Restriction fragment length polymorphism (RFLP) analysis

A 425-bp fragment of domain V of the 23S rRNA gene was amplified using a previously described protocol by Occhialini *et al* [21]. Amplicons were visualized by electrophoresis on a 1% TopVision agarose gel containing GelRed (0.5 µL) under UV light. Each amplicon was digested separately with FastDigest (ThermoScientific, USA) enzymes *Eco31I (BsaI)* to detect A2143G, or *BpiI (BbsI)* to detect A2144G following manufacturer's instructions. Digestion products were resolved on TopVision agarose gels under the same conditions as PCR amplicons.

Statistical analysis

All analyses were performed using GraphPad Prism 9.4.0 for Windows. Correlation analysis was performed with Spearman's correlation coefficient.

Ethical considerations

Ethical approval was obtained by local (CEC-UCR) and national (CONIS, Ministry of Health) Committees, and informed consent at sample collection included the future use of specimens. Bacterial isolates were coded, anonymized, and unlinked to personal patient information.

RESULTS

Phenotypic resistance was detected for clarithromycin, levofloxacin, metronidazole and rifampicin in *H. pylori* isolates from Costa Rican patients

According to antimicrobial resistance profiles, isolates were classified as resistant, intermediate or susceptible according to CLSI and EUCAST breakpoints (Table 1). One isolate was resistant to clarithromycin, and one isolate had intermediate resistance to this antibiotic. Two isolates were resistant to metronidazole and two were resistant to levofloxacin. Two isolates demonstrated resistance to three antibiotics-clarithromycin, metronidazole, and levofloxacin (Table 2). Among these, one isolate also exhibited resistance to rifampicin. Summarized results for all the tested antibiotics are shown in Table 1. Overall, 50% of the clinical isolates showed resistance to at least one of the primary antibiotics commonly used in *H. pylori* eradication therapy. The complete MIC profiles for all isolates and reference strains are presented in Table 2.

Table 1. Minimal inhibitory concentration breakpoints used in this study and summarized antimicrobial susceptibility profiles for 14 *H. pylori* isolates from Costa Rican patients.

Antibiotic	MIC breakpoints (ug/mL)[1]			Number of isolates (%)		
	S	I	R	S	I	R
Amoxicillin	≤ 0.125		≥ 0.125	14 (100%)		0 (0%)
Clarithromycin	≤ 0.25	0.5	≥ 1	10 (71%)	1 (7%)	3 (22%)
Levofloxacin	≤ 1		≥ 1	10 (71%)		4 (29%)
Metronidazole	≤ 8		≥ 8	10 (71%)		4 (29%)
Rifampicin	≤ 1		≥ 1	13 (93%)		1 (7%)
Tetracycline	≤ 1		≥ 1	14 (100%)		0 (0%)

[1] According to CLSI and EUCAST guidelines. S: Susceptible, I: Intermediate, R: Resistant.

Table 2. Minimal inhibitory concentration for 14 *H. pylori* isolates from Costa Rican patients.

Isolate	MIC (ug/mL)					
	AMX	CLA	LEV	MTZ	RIF	TET
PR-001	0.032	0.047	0.064	0.38	0.023	0.064
PR-006	≤ 0.016	0.047	0.064	0.38	0.047	0.023
PR-009	≤ 0.016	0.032	0.064	0.125	0.032	<0.016
PR-014	≤ 0.016	0.25	0.094	0.19	0.25	0.064
PR-016	≤ 0.016	0.064	3	0.25	0.094	0.032
PR-017	0.032	0.125	0.125	1	1	0.016
PR-018	≤ 0.016	0.016	0.094	48	0.5	0.125
PR-021	0.064	≥ 256	≥ 32	32	32	0.25
PR-026	≤ 0.016	0.047	0.125	4	0.5	0.016
PR-029	≤ 0.016	≥ 256	0.064	0.25	0.125	0.016
PR-032	0.032	0.016	0.38	32	0.023	0.25
PR-033	0.032	0.064	>32	0.5	0.19	0.032
PR-039	0.047	0.5	0.125	2	0.25	0.047
PR-046	0.032	≥ 256	≥ 2	128	0.012	0.047

MICs above resistance value are in bold font.
AMX: Amoxicillin, CLA: Clarithromycin, LEV: Levofloxacin, MTZ: Metronidazole, RIF: Rifampicin, TET: Tetracycline.

Mutations Associated with CLR were detected in *H. pylori* isolates from Costa Rican patients

Following PCR amplification of 23S rRNA gene (Figure 1A) and enzymatic digestion of the resulting amplicons, one isolate had the A2143G mutation. Digestion with the restriction enzyme *BbsI* produced two distinct fragments of 332 bp and 93 bp, consistent with the presence of the mutation (Figure 1B). In the case of the A2144G mutation, digestion with *BsaI* identified two isolates carrying this mutation, producing fragments of 333 bp and 92 bp, respectively (Figure 1C). The specific mutation identified in each isolate is summarized in Table 3.

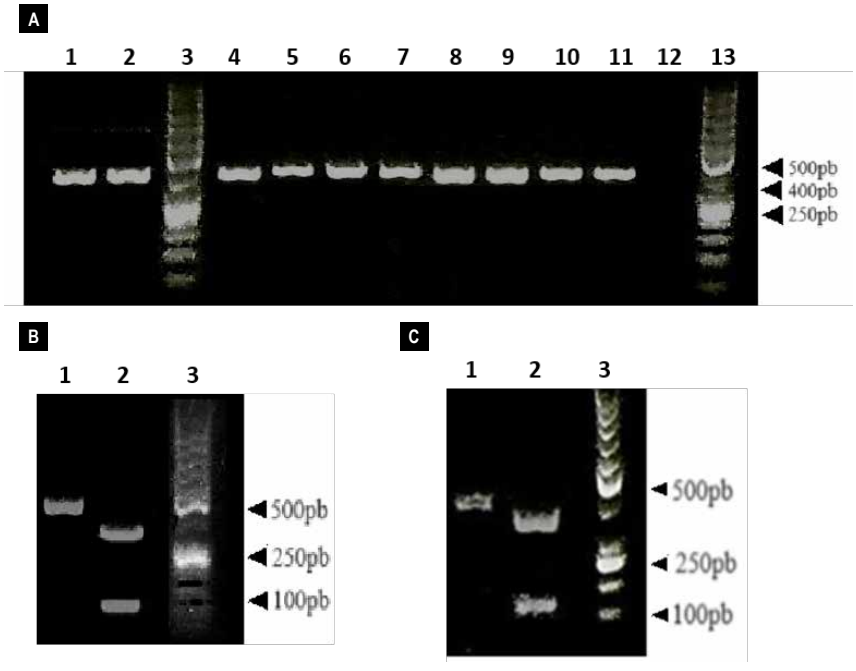

Figure 1. Detection of CLR-associated mutations in *H. pylori* by RFLP-PCR. (A) PCR products showing the 23S rRNA gene 425bp amplicon from selected *H. pylori* isolates. Lanes: 3, 13: 50bp DNA ladder (GeneRuler™, Thermo Scientific™); 1,2 : *H. pylori* 26695 and 60190; 4–11: clinical isolates PR-014, PR-021, PR-029, PR-046, PR-006, PR-017, PR-018, PR-033; 12: reagent control. **(B)** RFLP products digested with *BbsI*. Lanes: 1: PR-029 (one band, no A2143G mutation), 2: PR-046 (two bands, A2143G mutation present), 3: 50bp DNA ladder. **(C)** RFLP products digested with *BsaI*. Bands of 333bp and 92bp correspond to the presence of the A2144G mutation. Lanes: 1: PR-021 (one band, no A2144G mutation), 2: PR-046 (two bands, A2144G mutation present), 3: 50bp DNA ladder.

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Table 3. CLR-associated mutations detected in 14 *H. pylori* isolates from Costa Rican patients.

Isolate	MIC for CLA (ug/mL)	23S rRNA gene mutation
PR-001	0.047	ND
PR-006	0.047	ND
PR-009	0.032	ND
PR-014	0.25	ND
PR-016	0.064	ND
PR-017	0.125	ND
PR-018	0.016	ND
PR-021	≥ 256	ND
PR-026	0.047	ND
PR-029	≥ 256	A2144G
PR-032	0.016	ND
PR-033	0.064	ND
PR-039	0.5	A2144G
PR-046	≥ 256	A2143G

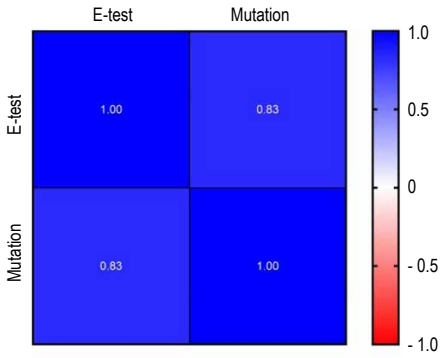

Figure 2. Correlation matrix between phenotypic CLR and point mutations in the 23S rRNA gene. Non-parametric Spearman correlation test revealed a strong positive correlation (Spearman's r=0.8321; 95% CI [0.5616-0.9419]; *p*=0.0071).

Phenotypic RCL and 23S rRNA mutations were correlated in *H. pylori* isolates

Among the 14 *H. pylori* isolates analyzed, 10 (71.43%) were phenotypically susceptible to clarithromycin, 3 (21.43%) were resistant, and 1 isolate (7.14%) exhibited an intermediate resistance phenotype. The A2143G mutation was detected in isolate PR-046 (resistant), while the A2144G mutation was identified in isolates PR-029 (resistant) and PR-039 (intermediate). Notably, isolate PR-021, which was phenotypically resistant, did not show either of these mutations. A statistically significant correlation was observed between the presence of these mutations and CLR phenotype, as determined by Spearman's correlation coefficient (r=0.8321; 95% confidence interval [CI]: 0.5616-0.9419; p=0.0071). The correlation matrix is presented in Figure 2.

DISCUSSION

H. pylori association with severe pathologies and the high infection prevalence highlight the importance of studying antibiotic susceptibility patterns to improve detection methods and optimize treatment strategies. Increased *H. pylori* resistance to different antibiotics has been reported globally, and this resistance is the main factor impairing the efficacy of current therapeutic regimes [22–24]. In Costa Rica, data on *H. pylori* antimicrobial resistance are limited and largely outdated. This study provides an updated assessment of antimicrobial susceptibility in a small set of *H. pylori* isolates collected in 2019.

Phenotypic testing revealed CLR in 22% of isolates, with one additional isolate showing an intermediate MIC. Earlier studies in Costa Rica reported substantially lower CLR rates: 5.3% in isolates collected between 1998 and 2000, alongside high metronidazole resistance (40.4%) [25], and 4.2% CLR in 250 isolates collected in 2000 (unpublished data). Collectively, these findings may suggest a potential increase in CLR over the past two decades, warranting larger-scale studies to reassess the suitability of clarithromycin-based triple therapy as first-line treatment in Costa Rica. This concern is clinically relevant, as eradication rates with clarithromycin-based triple therapy reach 80-92% in macrolide-susceptible infections but decline sharply to 12-38% in the presence of macrolide resistance [26].

Even if the sample size is small, the resistance rates observed in this study are comparable to those reported internationally. In the United States, CLR increased from 9.1% in 2009-2010 to 24.2% in 2011-2013, with an overall prevalence of 16.4% between 2008 and 2012 [27]. In another study conducted across 18 European countries, using samples collected between 2008 and 2009, CLR was reported at 17.5%, with higher rates (>20%) in Central and Southern Europe [28]. In 15 medical centers in Korea between 2017 and 2018, CLR was 17.8% [29], while in China a multicenter analysis across the country found an average primary CLR of 28.9% [30]. Very high CLR has been reported in Chile, with a rate of 40% in isolates from both public and private healthcare centers [31]. A study that included 189 patients in Honduras in 2013, reported the resistance rates for levofloxacin (20.9%), metronidazole (67%), amoxicillin (10.7%), and clarithromycin (11.2%). Whilst the CLR is not very high, the authors suggest that the high levofloxacin resistance rate may be associated with intense quinolone use in the country, much more common than in other countries with related characteristics [32]. A similar situation was described in a study from Dominican Republic, where metronidazole and levofloxacin showed high resistance rates (82.9 and 35.9%, respectively) and CLR was low (3.1%) [33]. These data suggest that clarithromycin-based regimens may remain viable first-line options in some countries, depending on local resistance patterns.

In regions lacking routine surveillance and reliable resistance data-including Costa Rica—the Toronto Consensus proposes local eradication rates as a surrogate marker for resistance, discouraging clarithromycin-based

triple therapy when eradication rates fall below 85% [34]. In Costa Rica, studies report eradication rates ranging from 84.7% [18] to 90.5% [19], hence this therapy could still be used. The apparent increase in CLR may compromise eradication success in empirically treated patients, as treatment outcomes strongly depend on clarithromycin susceptibility [34]. These findings highlight the need for continuous monitoring.

Efforts to improve eradication therapy are ongoing. Antimicrobial susceptibility testing to guide treatment failure in *H. pylori* is not widespread and unavailable in most clinical settings [35], and clarithromycin-based empirical schemes are generally employed, with reported success rates ranging from 60% to 100%. Culture-based susceptibility testing from gastric biopsies is limited by low sensitivity due to cultivation difficulties, prior antibiotic exposure, reduced bacterial load, and contamination [36]. Molecular methods, such as PCR-restriction fragment length polymorphism (PCR-RFLP), offer an alternative approach by detecting point mutations in the 23S rRNA gene associated with CLR. Three mutations-A2144G, A2143G, and A2142C-account for approximately 90% of primary clarithromycin resistance in Western countries [36]. In this study, PCR-RFLP identified a CLR rate of 21.4%, with concordance between genotypic and phenotypic results in three of four resistant or intermediate isolates.

To date, no molecular studies assessing CLR-associated 23S rRNA mutations have been conducted in Costa Rica. Nevertheless, our findings are consistent with reports from Latin America, including Colombia (18.8% CLR with A2144G or A2143G) [1], Brazil (16.9% CLR, predominantly A2143G) [37], and Peru (52.3% CLR, with A2142G/A2143G detected in 43.5% of isolates) [38]. A major limitation of this study is the restriction to two mutations (A2143G and A2144G) due to limited resources, which may underestimate true CLR prevalence. Future studies should include additional mutations, such as A2142G, or employ sequencing-based approaches.

The absence of detectable mutations in PR-021, despite a CLR-resistant phenotype, sug-gests alternate resistance mechanisms. This isolate had multidrug resistance (clarithro-mycin, levofloxacin, metronidazole, rifampicin), implying that non–23S rRNA mechanisms may contribute. Intrinsic mechanisms such as efflux pumps have been implicated. Efflux pump inhibitors reduce MICs of clarithromycin, metronidazole, amoxicillin, and furazolidone in multidrug-resistant strains [39]. Hirata *et al* demonstrated universal efflux pump gene expression in *H. pylori* isolates; CLR resistance decreased upon efflux inhibitor exposure, suggesting efflux systems as promising targets to reverse drug resistance [40]. Moreover, point mutations in other *H. pylori* genes have been linked to CLR. Lyu *et al* identified five mutational positions across four genes; notably, *fliJ* and *clpX* were impli-cated. *fliJ* encodes a flagellar export chaperone essential to the type III secretion system [41], while *clpX* encodes an ATP-binding subunit of the ClpXP protease, crucial for protein remodeling and degradation [42]. The presence of 23S rRNA mutations not included in this study cannot be ruled out.

Eradication strategies should be guided by local evidence to maximize efficacy and reduce treatment failure, with an emphasis on susceptibility-guided therapy. Although sequencing remains the gold standard for resistance detection, its cost and complexity limit routine use. PCR-RFLP provides a rapid and cost-effective alternative by exploiting restriction site alterations caused by point mutations [1]. Detection of A2143G and A2144G mutations by PCR-RFLP correlates well with phenotypic resistance and demonstrates high specificity (88%) and sensitivity (85%), with a negative predictive value of 97% relative to culture [1]. This method therefore represents a practical solution in resource-limited settings.

This study has several limitations, including a small sample size (n=14), limited geographic and socioeconomic representativeness, potential selection bias from private clinic patients, reliance on cultured isolates rather than direct biopsies, and restricted mutation analysis. Despite these constraints, the results align with regional trends. Future studies should incorporate larger, more diverse populations and noninvasive sampling approaches. Clinicians are encouraged to monitor treatment outcomes and antimicrobial use patterns, and clinical laboratories should consider implementing accessible phenotypic and molecular resistance testing methods [7].

Excessive and inappropriate antimicrobial use poses a major threat to *H. pylori* eradication and infectious disease management. The use of regimens with suboptimal efficacy may promote the selection of resistant strains and increase secondary resistance. These findings emphasize the urgent need for national surveillance of *H. pylori* resistance in Costa Rica to guide empirical therapy, reduce healthcare costs, and minimize adverse effects on the gut microbiota [43]. The data generated in this study could positively impact the development of clinical guidelines. Until now, the absence of recent local clinical data on antimicrobial resistance has led to the continued use of clarithromycin-containing regimens as first-line eradication therapy, as well as levofloxacin-based regimens for rescue treatment. Incorporation of the present findings highlights the need to expand current knowledge on antimicrobial resistance in *H. pylori* in Costa Rica through additional studies and supports the need of more data for the adjustment of therapeutic strategies. In particular, these data justify increased caution when prescribing eradication regimens containing clarithromycin or levofloxacin and favor the inclusion of bismuth salts and the preferential use of quadruple therapies, as suggested by Otero *et al* [44], in order to optimize treatment efficacy.

In conclusion, resistance to clarithromycin and levofloxacin in *H. pylori* may be increasing in Costa Rica, supporting the need for routine resistance detection in clinical practice and further studies in antibiotic resistance prevalence. While culture-based testing remains the

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reference method, its limitations restrict widespread implementation. The strong concordance observed between phenotypic resistance and A2143G/A2144G mutations supports PCR-RFLP as a rapid, cost-effective, and reliable tool for detecting CLR in *H. pylori* isolates and gastric biopsies. Adoption of this approach may improve treatment selection and contribute to more effective control of *H. pylori infection*.

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