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ORIGINAL ARTICLE

Point mutations in the glycosyltransferase domain of the *pbp1a* gene in amoxicillin-resistant *Helicobacter pylori* isolates[☆]



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Abstract

Introduction: *Helicobacter pylori* (*H. pylori*) eradication treatment includes a proton pump inhibitor and two antibiotics: amoxicillin and clarithromycin. The goal of that treatment is to eradicate the infection in at least 90% of the patients. Failure to eradicate the infection can have multiple causes, among which is the presence of point mutations in the antimicrobial target genes.

Objective: To characterize the mutations present in the *pbp1a* gene and their possible association with resistance to amoxicillin *in vitro*.

Methodology: Susceptibility to amoxicillin was evaluated in 147 isolates of *H. pylori* from the Colombian municipality of Túquerres. PCR amplification and sequencing of the glycosyltransferase domain of the *pbp1a* gene were carried out on Túquerres isolates, and the association between mutations and resistance was evaluated.

Results: A total of 5.4% (8/147) Túquerres isolates were resistant to amoxicillin *in vitro*. PCR amplification of the glycosyltransferase domain of the *pbp1A* gene was performed on 87.5% of the amoxicillin-resistant isolates *in vitro*, and in the DNA sequencing analysis, a total of 2 changes of amino acids from 3 DNA mutations that encoded the PBP1A-1 protein were observed.

Conclusion: The present study is the first report on *pbp1a* gene mutations in *H. pylori* isolates coming from a population in Túquerres. Mutations that have not been reported in previous studies were found.

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PALABRAS CLAVE

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 Transpeptidasas

Mutaciones puntuales en el dominio transglicosilasa del gen *pbp1a* en aislados de *Helicobacter pylori* resistentes a amoxicilina

Resumen

Introducción: El tratamiento para la erradicación de *Helicobacter pylori* (*H. pylori*) incluye un inhibidor de la bomba de protones y 2 antibióticos: amoxicilina y claritromicina; este tratamiento tiene como objetivo erradicar la infección en al menos el 90% de los pacientes. El fracaso del tratamiento de erradicación de la infección puede tener múltiples causas, entre ellas la presencia de mutaciones puntuales en los genes diana de los antimicrobianos.

Objetivo: Caracterizar las mutaciones presentes en el gen *pbp1a* y su posible asociación con la resistencia a la amoxicilina *in vitro*.

Metodología: Se evaluó la susceptibilidad a amoxicilina en 147 aislados de *H. pylori* de Túquerres, se amplificó por PCR y secuenció los dominios transglicosilasa del gen *pbp1a* en aislados de Túquerres, y se evaluó la asociación entre las mutaciones y la resistencia.

Resultados: Se observó que el 5.4% (8/147) de aislados de Túquerres fueron resistentes a amoxicilina *in vitro*. La amplificación por PCR de los dominios transglicosilasa del gen *pbp1a* se realizó en el 87.5% de los aislados resistentes *in vitro* a amoxicilina, y en el análisis de la secuenciación del ADN se observó un total de 2 cambios de aminoácidos a partir de 3 mutaciones en el ADN que codifica para la proteína PBP1A-1.

Conclusión: Esta investigación constituye el primer reporte sobre las mutaciones en el gen *pbp1a* en aislados de *H. pylori* provenientes de las poblaciones de Túquerres; se encontraron mutaciones no reportadas en investigaciones previas.

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Introduction and aim

Helicobacter pylori (*H. pylori*) is a Gram-negative, spiral-shaped, microaerophilic bacterium, with flagellates that enable its motility toward and adherence to the gastric epithelium.¹ The bacterium is associated with gastritis and peptic ulcer disease and is considered a risk factor for gastric cancer.¹

H. pylori has virulence factors that enable it to colonize the gastric mucosa and develop the disease, inducing a higher incidence and prevalence of its pathogenicity. The most important virulence factors are: urease, the flagellum, the *cagA* gene, the *vacA* cytotoxin, and the BabA protein.²

H. pylori colonizes the gastric mucosa of over 50% of the world population. It is generally acquired in childhood and persists for a lifetime. Notably, the clinical result of the infection is influenced by environmental factors, the genetic diversity of the strain, and the genetic diversity of the host.³

Regarding treatment, the first-line regimen for *H. pylori* eradication is the triple therapy that includes a proton pump inhibitor and 2 antibiotics, such as amoxicillin and clarithromycin, and its aim is to eradicate the infection in at least 90% of patients.⁴ Eradication treatment failure can be due to inadequate doses, a lack of treatment adherence, the presence of efflux pumps, and mutations in the genes targeted by the antimicrobials.

Antimicrobial resistance is a natural phenomenon that is accelerated by the selective pressure on microorganisms produced by exposure to antibiotics. As a result, conventional antibiotic treatments become inefficacious,

infections persist, and the risk of their propagation increases.⁵

One of the most widely used antibiotics in eradication therapy is amoxicillin, a semi-synthetic derivative of ampicillin. It inhibits cell wall biosynthesis, binding to the penicillin-binding proteins (PBPs)⁶ in the cytoplasmic membrane. PBP1A has glycosyltransferase/transpeptidase activity, and its function is to elongate the peptidoglycan strands and form the cross-linking between the peptidoglycan peptides. Thus, its inhibition interferes with peptidoglycan synthesis and cell growth.⁷

The prevalence of *H. pylori* resistance to amoxicillin varies geographically, with values of 2.2%, 65.6%, 11.6%, and 0.5% for America, Africa, Asia, and Europe, respectively.^{8,9} Even though information on the prevalence of resistance in Colombia is limited, it has been reported at 7% in Bogotá,¹⁰ 3.8% in the coffee-growing region,¹¹ and 20.5% in Tumaco.¹² None of those studies evaluated the *pbp1a* gene and its association with resistance to amoxicillin. Mutations in the *pbp1a* gene and their association with resistance to amoxicillin is unknown, with respect to *H. pylori* isolates from a population in Túquerres, located in the Colombian Andes; with the prevalence of *H. pylori* (> 80%) and high risk for the development of gastric cancer;¹³ as well as the possible association between the mutations in that gene and *H. pylori* eradication failure. Such information is useful for the development and implementation of methodologies for the rapid detection of resistance and the application of eradication regimens. Therefore, the aim of the present study was to characterize the mutations present in the *pbp1a* gene and their possible association with resistance to amoxicillin *in vitro*.

Materials and methods

An observational study was conducted that included 149 samples of gastric mucosa taken from patients from the municipality of Túquerres, in the department of Nariño, Colombia, that had symptoms of dyspepsia, for the purpose of evaluating antimicrobial resistance to amoxicillin *in vitro*, through the agar dilution method. To achieve the proposed aims, gastric mucosa biopsy samples were taken, after which cultures were made and biochemical tests were carried out, to identify pure *H. pylori* colonies, confirmed through PCR molecular identification of the structural gene, *ureA*. Antimicrobial susceptibility to amoxicillin *in vitro* was then evaluated, and finally, the glycosyltransferase domains of the *pbp1a* gene were amplified and sequenced for the identification of point mutations. A detailed description of the methods utilized follows below.

Gastric mucosa biopsy

The sample was selected from adults of both sexes that were referred to the medical service for clinical evaluation. Gastroduodenoscopy was ordered as part of their evaluation due to symptoms that included abdominal pain, gastroesophageal reflux, vomiting, respiratory symptoms, dyspepsia, and diarrhea, reported by the patients during the clinical consultation. Table 1 describes the biopsy protocol.

In accordance with the guidelines of the Colombian Department of Health, the samples were stored in media and then transported to the pathology laboratory of the *Universidad del Valle*, where the corresponding analyses were performed.

Gastric biopsy culture

The initial culture, from which *H. pylori* from the gastric biopsies was isolated, was made by placing the biopsy sample in 200 μ L of sterile NaCl at 0.89% and macerating it in a sterile macerator. The resulting maceration was then taken with a disposable inoculating loop (Fisherbrand[®], calibrated at 10 μ L/drop) and streaked in Columbia agar (Oxoid[®]) with 10% defibrinated sheep blood plus Dent supplement (Oxoid[®]), containing vancomycin, cefsulodin, trimethoprim lactate, and amphotericin B. After the culture was made, the rest of the gastric mucosa maceration was preserved in a thioglycolate solution (Merck[®]) with 20% glycerol (Promega[®]) and stored at -70°C .

The Petri dishes were incubated in a CO₂ incubator (Shel Lab[®]) at 37°C , with an atmosphere of 10% CO₂ and 90% humidity. Growth was evaluated at 72 h and then periodically, for 7 to 10 days, searching for small, translucent colonies in the shape of dew drops, consistent with the morphology of *H. pylori*. The quality of growth and fungal or bacterial contamination in the primary isolate were evaluated, as was the antibiogram.

Culturing the preserved samples

To evaluate the susceptibility to amoxicillin in the 147 preserved and viable isolates of *H. pylori* from a Túquerres

population, the bacterium had to be isolated again from the preserved samples. To do so, a fraction of the preserved maceration was taken (-70°C), utilizing a sterile lancet, and deposited in Columbia agar (Oxoid[®]), with 10% defibrinated sheep blood and no supplement. It was spread *en masse* and incubated at 37°C in a microaerophilic environment (10% CO₂ and 90% humidity). Growth was evaluated at 72 h and then periodically, for 7 to 10 days. The remaining content of the preserved maceration was stored at -70°C .

Purification of the *Helicobacter pylori* isolates

The previously obtained colonies that had a phenotype consistent with *H. pylori* were streaked in triplicate, utilizing a sterile loop (10 μ L), in Columbia agar (Oxoid[®]), plus 10% defibrinated sheep blood, with the Dent supplement (Oxoid[®]), containing vancomycin, cefsulodin, trimethoprim lactate, amoxicillin, and amphotericin B. The Petri dishes were incubated (Shel Lab[®] incubator) at 37°C in a microaerophilic environment (10% CO₂ and 90% humidity). Growth was initially evaluated at 48 to 72 h, followed by periodic evaluations every 24 h for 7 to 10 days. Phenotype identification tests and *ureA* gene amplification were performed on the resulting colonies.

Helicobacter pylori phenotype identification

Small, punctate, translucent colonies, with well-defined edges were macroscopically searched for, and the following identification tests were carried out on them: the oxidase test, catalase test, urea test (host test), and Gram stain test. Lastly, for their correct identification, they were compared with the results presented in *Bergey's Manual of Systematic Bacteriology* for the identification of *H. pylori*. In addition, molecular identification was performed through PCR amplification of *ureA*, the structural gene of *H. pylori*.

Evaluation of *H. pylori* isolate susceptibility to amoxicillin through the agar dilution method

Antimicrobial susceptibility was determined by applying the agar dilution method to *H. pylori* isolates from the patients, in which the cutoff point for considering a strain resistant to amoxicillin was defined, with a minimum inhibitory capacity (MIC) greater than or equal to 1.0 μ g/mL (Figueroa et al., 2012).

That was carried out on the 147 *H. pylori* isolates obtained from the initial Columbia agar culture. An inoculate of approximately 6×10^8 CFUs/mL (McFarland 2) was spread, in triplicate and by depletion, in Mueller Hinton (Merck[®]) agar, supplemented with 10% defibrinated sheep blood, at doubled concentrations (0.25, 0.5, 1, 2, and 4.0 μ g/mL) of amoxicillin, and incubated (Shel Lab[®] incubator) at 37°C in a microaerophilic environment (10% CO₂ and 90% humidity). Bacterial growth was evaluated after 72 h. The *H. pylori* ATCC 43504 was used as the quality control strain for monitoring the accuracy of the MIC of *H. pylori*, when using the agar dilution method.

Table 1 Endoscopic biopsy protocol.

Specimen	Storage medium	Procedure
Two samples of the gastric mucosa were taken from the antrum, the greater curvature, and the lesser curvature	Thioglycolate and glycerol	<i>H. pylori</i> isolation and virulence marker detection through PCR
Four samples: 2 were taken from the antrum and 2 from the gastric body	Formaldehyde at 10%	Histopathologic analysis
Two samples of the gastric mucosa were taken from the body, the greater curvature, and the lesser curvature	Thioglycolate and glycerol	<i>H. pylori</i> isolation and virulence marker detection through PCR
One sample of the gastric mucosa was taken from the body, mid anterior wall, and the lesser curvature of the antrum	Thioglycolate and glycerol	Preservation at -70°C

DNA extraction

For bacterial DNA extraction, 1 ml of the *H. pylori* isolates preserved (80% thioglycolate and 20% glycerol) at -70°C was taken and deposited in a 1.5 ml Eppendorf tube. It was then centrifuged (Forma Scientific® refrigerated centrifuge) at 13,000 rpm for 2 min. The supernatant was discarded and 300 μl of lysis buffer (Proteinase K 100 $\mu\text{g}/\text{mL}$, SDS 10%, EDTA 0.5 M and Tris-HCl pH 8) was added. The solution was incubated at 56°C for 18 h, followed by 10 min at 72°C . After incubation, 120 μl of NaCl 5 M was added and centrifuged at 13,000 rpm for 5 min. The supernatant was transferred, and the precipitate was discarded. A total of 840 μl of absolute ethanol (Mallinckrodt®) was added and centrifuged at 13,000 rpm at 4°C for 20 min. The supernatant was discarded and 300 μl of 70% ethanol was added and centrifuged at 13,000 rpm for 5 min, discarding the supernatant. The solution was dried for 2 h, then resuspended in 100 μl of TE buffer, and stored at -20°C . The quantity and purity of bacterial DNA was determined by an optic density reading of 260/280 nm in a spectrophotometer (Gene Quant II® Pharmacia Biotech), according to the manufacturer's instructions.

UreA gene amplification

The molecular identification of *H. pylori* was performed through PCR amplification of the *ureA* gene. The reaction was carried out in a thermocycler (Swift MiniPro™, Esco), adding the following reagents to a 0.2 ml tube: 1 X of PCR buffer (Promega® 5X Green Buffer), 1 μM of MgCl_2 (Promega®), 0.25 mM of dNTPs (Promega®), 50 pmol/ μl of each primer (sense 3'-AAGACATCACTATCAACG-5'/ antisense 5'-CCCGCTCGCAATGTCTAA-3'), 0.5 units of GoTaq DNA polymerase (Promega®); and 25 ng of *H. pylori* genomic DNA at a final volume of 25 μl . Amplification was carried out through an initial denaturation at $95^{\circ}\text{C}/2$ min, followed by 35 cycles ($95^{\circ}\text{C}/1$ min, $54^{\circ}\text{C}/1$ min, and $72^{\circ}\text{C}/1$ min) and a final extension at $72^{\circ}\text{C}/15$ min. The amplification products were obtained by electrophoresis at 80 volts for 1 h in a horizontal chamber (Spectroline Bi-O-Vision®). They were visualized

through fluorescence in UV light (260/280 nm) in a transilluminator (Spectroline Bi-O-Vision®), in 2% agarose gels (Sigma®) stained with 1 μl of ethidium bromide (0.5 $\mu\text{g}/\text{mL}$). To determine the amplified products of all the genes, a 100 bp molecular weight marker (Fermentas®) was used. The size of the amplicon corresponded to approximately 167 bp (expected fragment through an *in silico* analysis, with respect to the migration distance of the base pair marker).

Amplification of the *pbp1a* gene glycosyltransferase domains in *Helicobacter pylori*

PCR amplification of the glycosyltransferase domains (*pbp1a*) was carried out using a thermocycler (Swift MiniPro™, Esco) and the following reagents were added to a 0.2 ml tube: 1 X PCR buffer (Promega 5X Green Buffer®), MgCl_2 1 mM (Promega®), 10% DMSO, dNTPs 0.288 mM (Promega®), 50 pmol/ μl of each *pbp1a-1* primer [R-GCCATTCTTATCGCTCAAGTT and F-TCTCGTGTGAGCACCATG], 0.5 units of GoTaq DNA polymerase (Promega®); and 25 ng of *H. pylori* genomic DNA, at a final volume of 50 μl . The amplification thermal cycle consisted of an initial denaturation at $95^{\circ}\text{C}/2$ min, followed by 35 cycles ($95^{\circ}\text{C}/1$ min, $54^{\circ}\text{C}/1$ min, $53^{\circ}\text{C}/1$ min) for *pbp1a-1* (glycosyltransferase domain), then at $72^{\circ}\text{C}/1$ min, and a final extension at $72^{\circ}\text{C}/5$ min.

The PCR amplification products were obtained through electrophoresis at 80 volts for 1 h in a horizontal chamber (Spectroline Bi-O-Vision®). They were viewed through fluorescence in UV light (260/280 nm) in a transilluminator (Spectroline Bi-O-Vision®) in 2% agarose gel (Sigma®), stained with 1 μl of ethidium bromide (0.5 $\mu\text{g}/\text{mL}$). To determine the size of the amplified products of all the genes, a 100 bp molecular weight marker (Fermentas®) was used. The amplicon size was 340 bp for the *pbp1a-1* glycosyltransferase domain. The primers were designed using the sequence for the GenBank *pbp1a* gene code: AE000511.1 in the Primer3 V 0.4.0 software.¹⁴

Table 2 Patient grouping by age intervals.

Age (years)	Men	Women
18-34	25 (16.8 %)	29 (19.5 %)
36-47	21 (14.1 %)	32 (21.5 %)
48-68	23 (15.5 %)	19 (12.8 %)
Total	69 (46.3 %)	80 (53.7 %)

Mutation sequencing and identification

The purified amplified products of the *pbp1a* gene were sequenced in both senses (sense and antisense), employing the genetic analyzer (ABI 3130 Applied Biosystem®) and the Big Dye Terminator (Applied Biosystem®) methodology, according to the standardized conditions at the Human Molecular Genetics laboratory of the *Universidad de Valle*. BioEdit v 7.1.11® software was utilized in the edition, alignment, and translation of the sequences. The changes in the sequences were collated through local alignment, with GenBank accession: AE000511.1, for the *pbp1a* gene of *H. pylori* strain 26695.¹⁵

The alignment was exported in the FASTA format and opened in the notepad. The information was copied and pasted onto a book in Excel, adding the logical formula (Datum X = Datum Y)*1, which presented values of 0 for different data between the aligned sequences and 1 for equal data. The changes were determined for each sample. For the *pbp1a* samples, the DNA was additionally translated to protein in the BioEdit software and the same analysis was performed to determine the changes.

Statistical analysis

The frequency and proportion of the resistant isolates from a Túquerres population were calculated. The performance of the amplification and sequencing of the *pbp1a* gene fragments was evaluated through descriptive analyses. The frequency of the mutations in the genes was calculated. Only descriptive analyses were carried out for the mutation variables in the *pbp1a* gene due to the low number of samples, upon comparing the resistance *in vivo* with the resistance *in vitro* (between 0 and 3 data).

Ethical considerations

The present study was approved by the *Comité Institucional de Revisión de Ética Humana (CIREH)* of the Faculty of Health of the *Universidad del Valle*, regulated by Resolution 008430 from October 4/1993, issued by the Colombian Department of Health.

Results

Of the 149 participants, 53.7% (80/149) were females, 29 of whom were between 18-34 years of age. Of the males, 25 were between 18-34 years of age (Table 2).

The bacterium was isolated from 147 of the 149 participants. Upon evaluating the antimicrobial resistance to amoxicillin *in vitro* in the *H. pylori* isolates, through the

Table 3 Characteristics of the resistant isolates after evaluation of susceptibility to amoxicillin *in vitro*.

	n = 147
Susceptible	139 (94.6%)
Resistant	8 (5.4%)
Total	147 (100%)

Table 4 Characteristics of the patients with resistant isolates.

Age (years)	n = 8
19-35	2 (20.0)
36-47	4 (60.0)
48-68	2 (20.0)
Sex	
Male	4 (50.0)
Female	4 (50.0)

agar dilution method, 139/147 (94.6%) of the isolates were susceptible and 8/147 (5.4%) were resistant to amoxicillin *in vitro* (Table 3). Distribution by sex was 50% in the resistant isolates and they were obtained from patients between 19-35, 36-47, and 48-65 years of age (Table 4).

PCR amplification of the *ureA* gene was positive for the 147 isolates obtained and PCR amplification of the glycosyltransferase domains (PBP1A) of the *pbp1a* gene of *H. pylori* was performed on 87.5% of the isolates resistant to amoxicillin *in vitro*. In the DNA sequencing analysis of the 8 resistant isolates, there was a total of 2 changes of amino acids. Those changes occurred from 3 mutations in the DNA that encoded for the PBP1A-1 protein. The changes were evaluated in relation to the sequence of *H. pylori* strain 26695 that was susceptible to amoxicillin. The F125 L mutation was observed in 6 of the isolates, whereas the I79 V mutation was observed in 4 of the isolates (Table 5).

Discussion and conclusions

Amoxicillin is a commonly used antibiotic in *H. pylori* eradication therapy. Even though the majority of *H. pylori* isolates are still susceptible to amoxicillin, resistance to that antibiotic is emerging in clinical isolations, especially in countries where the drug is widely distributed without a prescription.¹⁶ At present, the underlying molecular mechanism to resistance to amoxicillin has only been identified in a few *H. pylori* isolates. In those isolations, resistance was mediated by mutations in the gene that encodes PBP1A.¹⁶

The PBPs are a group of proteins that are characterized by their affinity for and binding to penicillin. They are enzymes that participate in the synthesis and maintenance of the peptidoglycan layer of the bacterial cell wall.¹⁶ It is important to keep in mind that there are 2 types: PBP1A and PBP2A.⁷ Resistance is produced through modifications in the PBPs, which leads to a decrease in the affinity for the medication. Those modifications include mutations and/or

Table 5 Description of the mutations in DNA in the *pbp1a-1* sequences in the *H. pylori* isolates.

<i>H. pylori</i> 26695		Change					
Codon-i	AAi	DNA	Codon-f	AAf	Mutation	R	%
TTT	F	T373C/T375C	CTC	L	F125L	6	60
ATT	I	A235G	GTT	V	I79V	4	40
Total						10	100

AAf: amino acid encoding for codon-f; AAi: original amino acid encoding for codon-i; Codon-f: sequence of the mutated codon in the resistant *H. pylori* strains; Codon-i: sequence of the original codon in *H. pylori* strain 26695; R: number of mutations observed in the sample.

mosaics in PBP2X and PBP2B, as well as in PBP1A, for the highly resistant isolates.¹⁴

There are few Colombian studies directed at evaluating *H. pylori* susceptibility to amoxicillin and they differ in the susceptibility analysis methodology and the prevalence of resistance. In our study, the prevalence of resistance to amoxicillin in *H. pylori* isolates from a Túquerres population was 5.4% (8/147), which is very similar to values reported in previous studies. In Bogotá, the proportion of resistance was reported at 7% (6/84), through the disk diffusion technique,¹⁰ and at 3.8% (3/79), through the E-test technique,¹¹ and contrasted with that reported in *H. pylori* isolates from the coffee-growing region (Armenia and Pereira), where resistance to amoxicillin did not occur.¹⁵ Those registers illustrate the geographic variation in the prevalence of resistance to amoxicillin in Colombia. The contrasts in said prevalence could be explained by greater exposure to the antimicrobial.^{17,18}

Variants and changes were found in the uncharged polar amino acids, F125L and I79V. The association of those mutations could be explained by their close presence to the enzymatic activity sites of the PBP protein that are located in the C-terminal region (SKN368-371, SNN433-435, and KTG555-557). That is where mutations cause conformational changes in the PBPs, decreasing the binding affinity to amoxicillin and resulting in resistant phenotypes.¹⁹ On the other hand, the association could also be explained by the presence of mutations, such as A296V, A494H, A541M, and Q572G, in the *pbp2* gene, and A499V and Q536L in the *pbp3* gene, which are found in regions not evaluated in the present study.^{20,21}

Despite the fact that the mutations in the *pbp1a* gene are associated with resistance to amoxicillin in *H. pylori*, there are other genes that could be responsible for the resistant phenotype, such as mutations in the *hopC* gene and the deletion in the porin-encoding *hopB* gene of *H. pylori*, whose presence enables the bacterium to survive at amoxicillin concentrations of 125 mg/l and 250 mg/l, respectively.²² In addition, if the changes are simultaneous to mutations in the *pbp1a* gene, the bacterium can grow in amoxicillin concentrations up to 400 mg/l, suggesting an additive effect.²³ Beta-lactamases are among the factors associated with resistance, as described in a study on *H. pylori* strain 3778, in which a product identical to beta-lactamase TEM-1 (GenBank accession: EU726527) was found. It enabled the growth of that strain in media with amoxicillin concentrations above 256 mg/l.²⁴ Efflux pumps are not likely involved in resistance to amoxicillin, given that the

antibiotic has very low hydrophobicity, which is an indispensable requisite in drugs that are substrates of those types of structures.²⁵

The present study is the first report on mutations in the *pbp1a* gene in *H. pylori* isolates from a Túquerres population. Mutations were found that have not been reported in previous analyses. Despite our study results, we suggest evaluating the effect of the mutations on the designs of directed mutation or modeling, to identify their true contribution to resistance to amoxicillin.

Study strengths and limitations

The aims of the present study were to characterize the mutations present in the *pbp1a* gene and to determine their possible association with resistance to amoxicillin *in vitro*, in a patient population from Túquerres, located in the Colombian Andes; the prevalence of *H. pylori* (>80%); and the high risk for the development of gastric cancer. The use of microbiologic and molecular techniques to identify *H. pylori* provided more reliable results, but studies are needed to evaluate isolates from different regions of Colombia and the world, to identify mutations in the strains circulating in the environment and their association, not only with resistance to amoxicillin, but to other drugs used in *H. pylori* eradication, to reduce treatment failure.

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Conflict of interest

This project was financed by the *Departamento Administrativo de Ciencia Tecnología e Innovación de la República de Colombia* - COLCIENCIAS, Grant No. RC: 1902002; and by the *Registro Poblacional de Cáncer de Cali, Universidad del Valle*, Cali, Colombia.

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