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The evaluating expression profile of virulence factors in Helicobacter pylori strains by system biology; an example from Colombia

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ABSTRACT

Abstract
Helicobacter pylori is one of the most common bacteria in the stomach, colonizing about one-half of the population in the world, while most of them remain asymptomatic throughout their lives and gastric cancer (GC) occurs in only 1-2% of people. It seems that the final outcomes of Helicobacter pylori infection are dependent on bacterial virulence factors, host genetic characteristics, and the environmental conditions. In this study, we compared the expression of 20 known virulence factors associated with the development of GC in the isolated Helicobacter pylori strains from the Colombian patients belonging to the regions with low and high GC risks. Based on the results of the present study, it was found that the 20 studied virulence factors are closely related with each other and regulate their expressions through the required intermediates. We also showed that the Helicobacter pylori strains belonging to the region with high GC risk were more virulent and have developed into GC by destroying the intercellular bindings, cell skeletal dysregulation, and cell survival and proliferation stimulation, while the H. pylori strains in the region with low GC risk expressed virulence factors related to the chronic inflammation and apoptosis; adhesion factors were also different in both groups.

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Introduction

Helicobacter pylori is a gram-negative, microaerophilic, S-shaped, and motile bacterium due to flagella, which inhabits the human gastric submucosal (1). About half of the world's population is infected with Helicobacter pylori, most of whom are colonized as children. Unfortunately, this bacterium escapes from various immune system responses via different ways and causes chronic inflammation in gastric epithelial cells and the long run causes digestive disorders for its host, as the International Agency for Research on Cancer (IARC) first identified Helicobacter pylori in 1994 as the primary cause of gastric cancer (GC) (2-3). Helicobacter pylori is the etiological cause of gastrointestinal disorders including GC, chronic gastritis, gastric adenocarcinoma, and MALT (mucosa-associated lymphoid tissue) lymphoma (4-5).

According to the review of the literature, Helicobacter pylori encodes about 1,500 different proteins, some of which have been identified as the virulence factors affecting the severity of diseases. Virulence factors include cagA, vacA, urease, NAP,

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effective enzymes in DNA metabolism, oxidative stress, protease, cag-PAI members (cag pathogenicity island), surface factors, and some insertion sequences (6-9). Due to the high heterogeneity and recombination rate among the Helicobacter pylori strains, the expression patterns of virulence factors are different in the Helicobacter pylori populations (10-12).

All the Helicobacter pylori strains are divided into 6 ancestral populations, including hpEroupe, hpEastAsia, hpAsia2, hpAfrica1, hpAfrica2, and hpNEAfrica that each of the strains belonging to the lineages has its specific genetic characteristics and virulence pattern, such that the rate of GC in the people infected with hpEroupe and hpEastAsia is more prevalent than other Helicobacter pylori lineages (13-14).

Since almost all the Helicobacter pylori strains of the Japanese people are in the form of cagA positive (EPIYA motif ABD), the existing strains in East Asia (i.e. Japan, China, South-Korea) appear to be more virulent. However, there have been limited studies comparing the expression pattern and pathogenicity of different populations regarding Helicobacter pylori (2,14-16).

This study aimed was to evaluate and compare the prevalence of virulence in common factors related to GC in the Helicobacter pylori strains belonging to the regions with the high and low incidence rates of GC.

Methods

We reviewed the microarray studies in Gene Expression Omnibus (from http://www.ncbi.nlm.gov/geo/) and ArrayExpress (from http://www.ebi.ac.uk/arrayexpress) to find a study on the expression pattern of mRNA between the H. pylori strains of high and low incidence GC regions. The keywords used to achieve the eligible studies included Helicobacter pylori, GC, and intestinal metaplasia. Our inclusion criteria included microarray studies related to the Helicobacter pylori strains. The strains should have been from high risk and low-risk regions for GC, and the data quality and distribution should have been acceptable, while the studies evaluating the population of Homo Sapiens, Mus Musculus, or the cell lines were excluded. Moreover, studies involving any interventions (such as medications or other chemical compounds), studies conducted by independent investigators under various conditions, and studies that were not of good quality were considered as the exclusion criteria. The quality and consistency assessment of the data was performed using the R package MetaQC (17).

Finally, differentially expressed mRNAs (DEMs) were determined based on the false-discovery rate (FDR) of 0.43% and 1.0-fold change.

A protein-protein interaction network was constructed based on the STRING and KEGG pathway database information by Cytoscape software. Gene ontology was performed using KEGG pathway, eggNOG, Biocyc, and the previous articles, and finally, a molecular signaling network was proposed based on the findings of the present study, to justify the carcinogenesis process of Helicobacter pylori strains in the high-risk areas of GC

Results

A study by Dr. Alexander Sheh and his colleagues with GSE41497 access code was found under the GPL16166 platform. It indicated that the mRNA expression profile of Helicobacter pylori strains included three strains PZ5056, PZ5080, and PZ5086, which belonged to the Colombian region with high GC incidence, as well as three isolates PZ5004, PZ5024, and PZ5026, which belonged to the Colombian region with low GC incidence; all the 6 Helicobacter pylori strains were cagA+ and vacAs1m1. According to Sheh et al. (2013), the strains PZ5026, PZ5056, PZ5080, and PZ5086 originated from hpEroupe, while PZ5004 and PZ5024 stains had African origin. de Sablet et al. (2011) also showed that the Colombian region Helicobacter pylori strains with low GC incidence are often of the hpAfrica type (2). Previous studies have also indicated that hpAsia and hpEroupe strains are more carcinogeneses as compared to the hpAfrica strains [18-19]; the results of this study were consistent with the findings of previous studies.

The expression of the most important virulence factors of Helicobacter pylori affecting GC (according to previous studies) was also calculated as fold change and listed in Table 1.

Table 1: The main virulence factor of H. pylori in hpEroupe strains vs. hpAfrican strains

These virulence factors were related to the processes of acid acclimation, motility and chemotaxis, DNA metabolism, cell envelope, translation and regulation, and type IV secretion system. We constructed our protein-protein interaction network of our 20 considered virulence factors using different database information and showed that they were closely related (Fig. 1).

Based on our analysis, it was found that flgD, jhp0730, BabA, hopM, sabB, cag4, cagA, HopQ, and virB11 increased the expression in the hpEroupe route strains. The flgD and jhp0730 genes are involved in the flagella biosynthesis route and movement toward the gastric submucosa (9). Since motility causes penetration to the mucus and escaping from the acidic condition of the stomach, the Helicobacter pylori motile strains

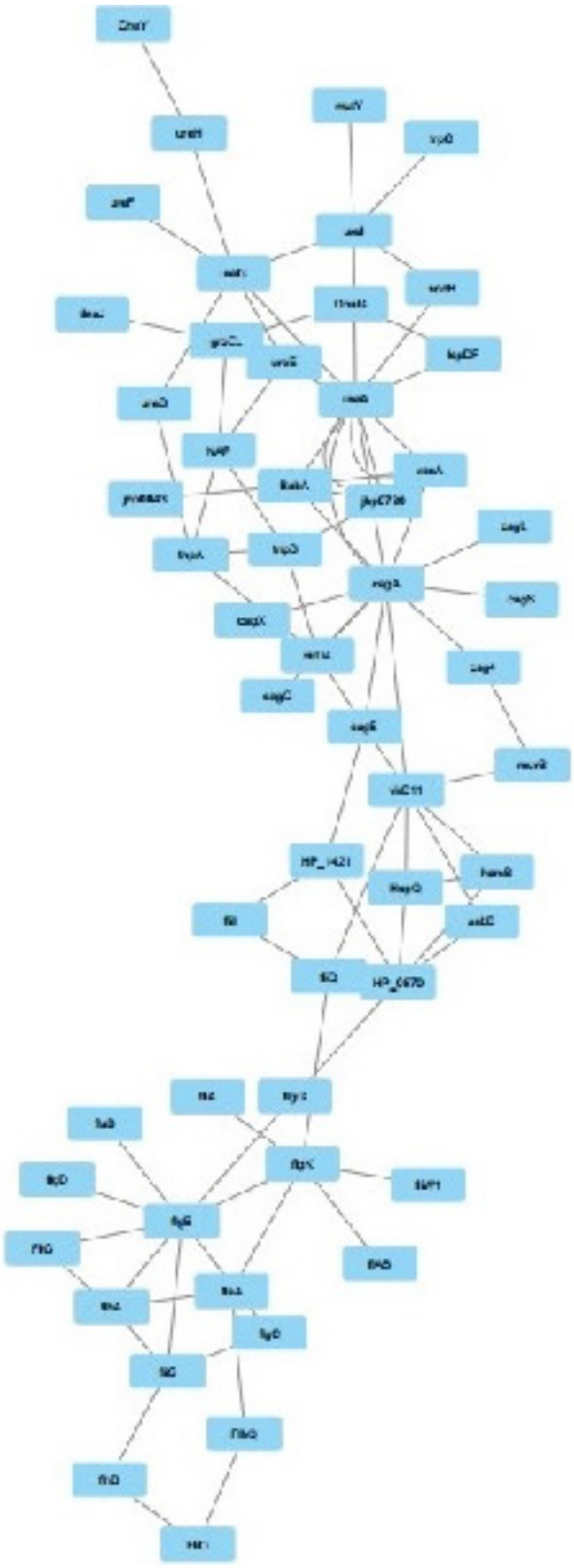

Figure 1: The protein interaction network between DEMs in H. pylori strains from high GC risk

Some also believe that the antibodies against flagella are used as a non-invasive biomarker to assess the GC risk (21).

Clyne et al. (2000) suggested that Helicobacter pylori may also use flagella as an adhesion [22]. However, flgD and jhp0730 can influence and increase the expression of adhesion genes, type IV secretion system (T4SS), and cagA by affecting the intermediates such as fliQ and HP_0870 [9,23-24].

BabA, hopM, sabB, and HopQ belong to the class of adhesion molecules of Helicobacter pylori (5,25). According to the review of the literature, binding to the gastric epithelium is the most important step in the establishment of chronic colonization, protection and absorption of metabolites for the optimal bacterial growth, and thus the Helicobacter pylori strains that have greater ability and attachment capacity to the gastric epithelium are more carcinogenic (9, 26-27). BabA is a 78KDa protein that binds to the Lewis b antigenic glucose group, expressed at the gastric epithelium level (25). Studies in western countries have shown that BabA has a significant relationship with the development of peptic ulcer and GC (28). HopM, HopQ, SabB, and BabA are also external membrane proteins of Helicobacter pylori that increase the risk of peptic ulcer and GC by inducing chronic inflammation, provoking a pro-inflammatory response, and the specific adhesion (25, 29-30). Based on the interaction network, the adhesion genes are the intermediate elements and the bridge between the genes involved in T4SS and the involved genes in motility, as is evident in the pathogenesis of Helicobacter pylori. The outline of Helicobacter pylori pathogenesis processes includes 1- the neutralization of acidic conditions of the stomach, 2- the movement to sub-mucosa, 3- attachment, and 4- secretion of cagA and vacA toxins via T4SS (9,25).Cag4, cagA, and virB11 are the most important virulence factors related to the development of GC [31-32]. Cag4 is a hydrolytic enzyme that causes the formation and expression of Cag-T4SS by destroying the cell-wall (33). CagA has also been shown in previous studies as the most important factor in increasing the risk of GC [34]. Nearly all the Helicobacter pylori isolates isolated from the Japanese population (the country with the highest incidence of GC) contain cagA (harboring cagA) [35-36]. Moreover, virB11 (HP1451), along with cagA, is a part of Helicobacter pylori cag pathogenicity island and its role is the formation of type IV secretion system (37). CagA is induced by T4SS into the gastric epithelial cell. Thus, virB11 plays a prominent role in the development of GC (38). There are several studies on the role of cagA, cagE and, virB11 in the development of GC (37-38). A meta-analysis conducted by Pormohammad et al. (2018) showed that cagA and vacA s1m1 allele had a significant relationship with an increased

risk of GC. This phenomenon was observed in all the Helicobacter pylori strains of this study identified from the Colombian region with high GC incidence (7). However, the expression of some other identified virulence factors including ureG, flhB, flaAB, hsp60, NAP, cagQ, cagX, TnpB, homB, jhp0043 and superoxide dismutase b in the hpEroupe origin strains were downregulated, while increased in the Colombian strains with hpAfrica route. These genes were mostly related to the production processes of urea, flagella, DNA metabolism, outer membrane protein, and cagPAI. Urease complex is used by Helicobacter pylori to neutralize the acidic effect of the stomach. Kao et al. (2016) showed that blocking the production of urease and virulence factors associated with the motility and chemotaxis results in the non-ccolonization of Helicobacter pylori (9,25). Urease protects against damage due to stomach acid. However, some studies have not reported any significant relationship between urease and the developments in the peptic ulcer or GC [39-40]. flhB and flaA are among the components associated with the Helicobacter pylori motility and chemotaxis, which have also been identified as biomarkers in some studies for the diagnosis of GC (9,25). As a part of IS605 transposase, TnpB and TnpA enter the cag-PAI and cause its disruption. TnpB in the hpAfrica route strains increases the expression, while it did not appear to increase the risk of GC (8). cagQ and cagX are parts of the PAI cag, which cause the apoptosis of the gastric epithelial cells; the rate of apoptosis in the AGS cell line also showed an increase in the hpAfrica route strains in this study (2,8,41). NAP, jhp0043, and superoxide dismutase b are also involved in pathways such as DNA repair and oxidative stress resistance. Some studies have suggested that NAP stimulates the pro-inflammatory response that is associated with the development of peptic ulcer (9,25,42). According to previous reports, DNA damage occurs as a result of recombination between the Helicobacter pylori strains, which is recovered by the SOS response, jhp0043, and NAP. Also, after to colonization with H. Pylori, we have the PMN cells infiltration, due to which NAP and superoxide dismutase b protect H. pylori against the free radicals of oxygen (42-44). HomB is expressed along with cagA and is a risk factor for GC together with it (8). Also, hsp60, which undergoes upregulation in acidic conditions and stimulates proliferation and severity of diseases by stimulating the NF_κB pathway, and is associated with gastric adenocarcinoma (9,25,40). Generally, Helicobacter pylori strains use multiple virulence factors and the final clinical outcomes are determined depending on bacterial and host interaction. Due to heterogeneity and continuous recombination between the Helicobacter pylori strains, each lineage has its phenotype (2,36,40). We showed in this study that each of the Helicobacter pylori strains of the populations of hpEroupe and hpAfrica have their virulence factors. Finally, we proposed a gene network to justify the carcinogenic processes of Helicobacter pylori strains (Fig. 2).

Discussion

While half of the world's population is infected with Helicobacter pylori. The H. pylori-related gastrointestinal disease is seen in about 25% of people, and 10-20% of people with peptic ulcer and gastric adenocarcinoma are seen in only 1-2% of people and about 75% of the Helicobacter pylori-infected populations are asymptomatic (25,45). Helicobacter pylori pathogenesis has several important steps: 1- neutralizing the acidic pH of the stomach, 2- moving and progressing to the gastric sub-mucus, 3- Colonization and attachment to the gastric epithelial cell, 4- inducing the chronic inflammation, and producing vacA and cagA toxins (9,25,46). The phenomenal reason why GC occurs in a limited population has roots in the interaction of the bacteria and the host [1]. Final clinical outcomes of infection with H. pylori depends on the genetic characteristics and virulence factors of the Helicobacter pylori strains, genetics, and host polymorphism, nutrition and environmental conditions of the individuals (1,9,25).

Colombia is one of the northwestern Latin American countries with an area of 1,141,000 square kilometers, making it the second most populated Latin American country after Brazil (47). Colombia is also one of the developing countries where most of its population is infected with Helicobacter pylori at childhood, but the rate of GC varies in different parts of this country. For instance, there are two areas in Southwestern Colombia, including Andean with a GC frequency of about 150 per 100,000 populations, and the coastal region with a GC frequency of about 6 per 100,000 populations, which has become a puzzle (48-51). It is while both regions are Spanish and African ancestral, and the frequency of the H. pylori cagA positive strains in the Andean region is about 9% higher than in the coastal region (51). A similar phenomenon can be observed in the African population, which is called "African Enigma" (52). Studies have shown that even though that nearly 100% of the African population is infected with H. pylori, the frequency of GC in these areas is very low (52-53). Since each family of Helicobacter pylori has its unique genetic characteris

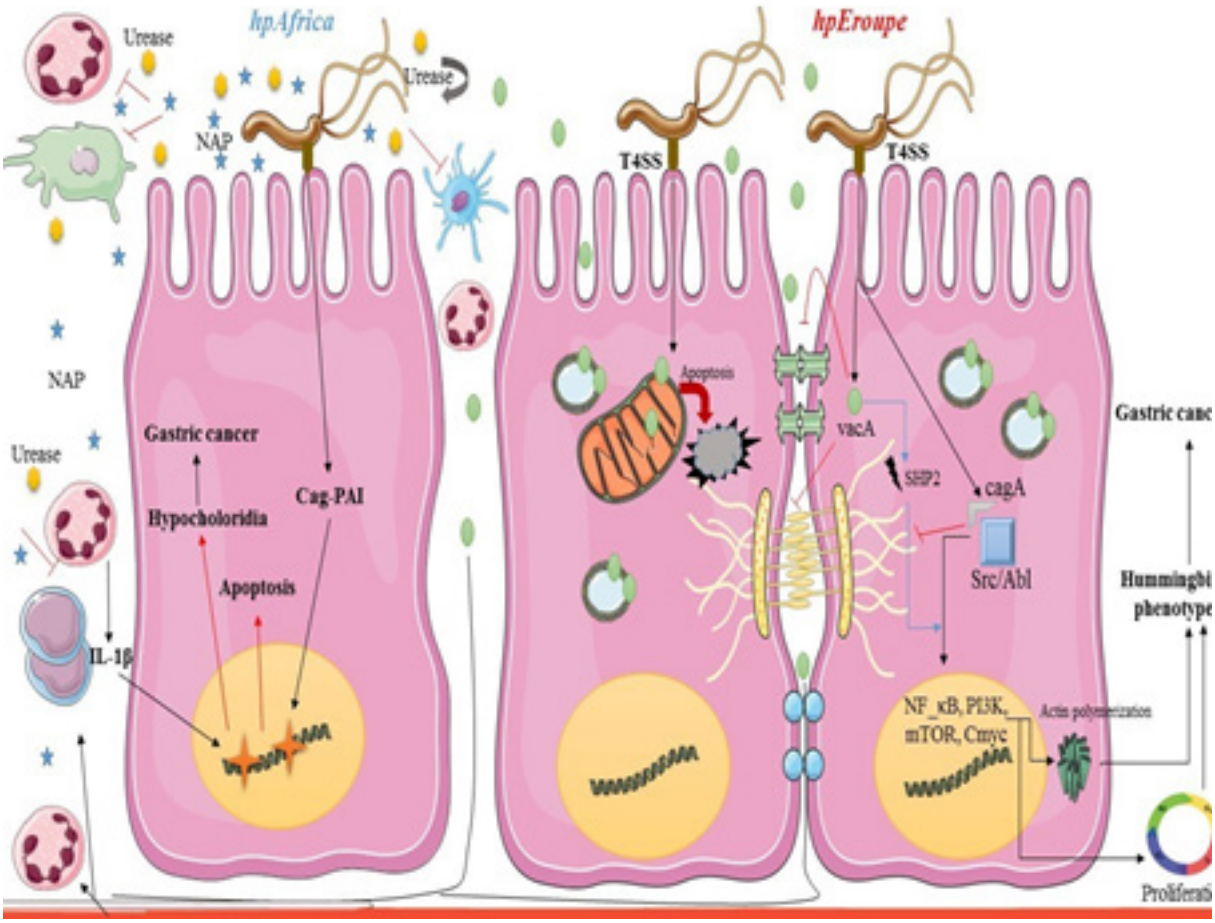

Figure 2: The proposed schematic diagram to represent pathogenesis of Helicobacter pylori strains compression between hpEroupe and hpAfrica lineages

lence factor, these paradoxes may be due to the Helicobacter pylori strains (54-55). Studies have shown that the population of the Andean mountains has a Spanish ancestor, while the population living in the coastal region is a mixture of Spaniards and Africans (48,51). Shiota et al. (2014) showed that the Helicobacter pylori strains of the Andean region are of the hpEroup origin type, while the coastal region population strains are of hpEroupe and hpAfrica origin (56). Sablet et al. (2014) also showed that the frequency of hpEroupe origin is higher in pre-cancerous patients of the Colombian population living in the coastal region (57). Hence, we decided to do a study on the changes in the virulence expression pattern of the Helicobacter pylori strains belonging to the Andean and coastal region populations.

The H. pylori strains of the Colombian region-with high GC incidence were all of hpEroupe ori gion with low GC incidence was of hpEroupe and the rest was of HpAfrica route, which was consistent with the findings of previous studies. We have demonstrated that the expression pattern of virulence factors differs between hpEroupe and hpAfrica strains. The rate of cagA expression in the hpEroupe origin strains was much higher than the hpAfrica origin strains, which justifies the high frequency of GC in the Andean patient population.

According to our results, the Colombian H. pylori strains related to hpEroupe origin mainly express the virulence factors related to the proliferation and cell survival, actin rearrangement, and cell junction degradation, while the strains belonging to the hpAfrica family express the virulence factors associated with gastric acid neutralization, escaping from the immune system, chronic inflammation, and apoptosis. Moreover, the adhesion molecule expression pattern was also different in the hpEroupe and hpAfrica strains. The strains belonging to the hpEroupe family appear to be more virulent than the hpAfrica lineages, because the frequency of GC in the colonized populations with hpEroup members differs significantly from that of hpNEAfrica, hpAfrica1, and hpAfrica2 (56-57).

Although both groups of the studied Helicobacter pylori were cagA+ and vacA s1m1, the rate of cagA expression in areas with high GC risk strains was much higher than that in the low GC risk areas. Loh et al. (2011) showed that the rate of cagA ex-

pression in the isolated strains from Colombia's high GC risk areas was higher than in low GC risk areas (51). Also, Bravo et al. (2002) showed that the frequency of vacA s1m1 in the high GC risk isolates was higher than in the low GC risk areas (14). Moreover, it has been considered that the sequence of cagA gene in the H. pylori strains of the Colombian patients of the high GC incidence regions has differences with the population of the regions with low GC incidence, which has led to the changes in the frequency of the GC rate in these two areas. For instance, Sicinschi et al. (2010) showed that the number of copies of the EPIYA-C motif and CagA multimerization (CM) motif in the H. pylori strains of high GC risk areas is significantly higher than the strains of low GC risk areas [48]. Loh et al. (2011) also showed that the cagA sequence in the strings of Colombia's high GC risk areas contains a unique nucleotide sequence "AATAAGATA motif" that increases cagA expression and thus increases the GC cases in this area of Colombia (51). Also, based on our results, the Helicobacter pylori adhesions, including BabA and sabB, were increased in the strains belonging to the high GC risk areas. The blood-antigen binding protein A (BabA) and Sialic acid-binding adhesion (sab) bind to the molecules in the ABO and Lewis antigens, respectively (9,25,58).

Previous studies of Colombia's high GC risk population have also shown that gastrointestinal disorders often occur in the people with blood antigens A and Lewis b, and it has been shown nowadays that these blood antigens play the role of binding molecules for adhesion molecules, i.e. BabA and SabA / B of Helicobacter pylori and cause the establishment of persistent infection [58-60]. In a study of Colombia's high GC risk population, Quiroga et al. (2005) found that high populations of Helicobacter pylori expressed the factors of virulence of BabA, oipA, and cagE (61). Also, genetics and host characteristics also play a role in the GC. For instance, factors such as low serum selenium levels in the Colombian high GC risk population, intestinal helminthiasis in the low GC risk population or Single-nucleotide polymorphism (PMN), high GC risk populations (Túquerres in the Colombian Andes) and low GC risk (coastal) town Tumaco) plays a 25-fold difference in GC in the Colombian populations (62-64). Recently, in their study of Helicobacter pylori strains of the high GC risk of Colombia population, Gutiérrez Escobar et al. (2017) suggested the presence of a new subtype called "hspColombia" in this geographical area (65).

Conclusion

We evaluated and compared the virulence expression pattern of GC-related factors in the Helicobacter pylori strains isolated from the high GC risk and low GC risk population in Colombia. We showed that the pattern of expression of virulence factors in these two groups was different; we also showed that the expression of cagA, cag PAI elements, and T4SS were higher in the H. pylori strains of Colombian high GC risk population. This phenomenon justifies the increase in the number of GC cases in this area.

Conflict of Interest

The authors declare no conflict of interest.

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