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Correlation between virulence markers of *Helicobacter pylori* in the oral cavity and gastric biopsies

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ABSTRACT – Background – The clinical outcome of *Helicobacter pylori* infection has been associated with virulence factors. The presence of these factors is useful as molecular markers in the identification of the high risk for developing severe gastric pathologies. **Objective** – To correlate the presence of virulence markers *cagA* and *bab2A* of *H. pylori* in oral and gastric biopsy samples. **Methods** – An observational, prospective, descriptive, and cross-sectional study was carried out between September 2011 and September 2012. Patients suffering dyspepsia with indication for upper gastrointestinal video endoscopy who attended the Gastroenterology Service of the Hospital *Dr. Julio C. Perrando* were included. Epidemiological investigation was completed. To detect the bacteria and their virulence genes, samples of saliva, dental plaque and gastric biopsy were taken and processed by PCR. **Results** – Sixty-one patients were selected for this study (30 women and 31 men). *H. pylori* was detected in 31 gastric biopsies and 31 oral samples. Significant difference between oral and gastric samples was found in *cagA* genotype. Agreement between oral and gastric genotypes was found in 38.7% of samples from the same patient. **Conclusion** – This study is the first in provide information about the genotypes of the Argentinean Northeast *H. pylori* strains. Despite the high prevalence of *H. pylori* infection, the most of patients had less virulent genotypes in oral cavity and gastric tissue. The *cagA*/*babA2* combination was not frequent in the samples studied. There was not a statistical correlation between the virulence genes and gastroduodenal or oral diseases. Although in some patients the same genotype was found both in oral and gastric samples, it cannot be ensure that they corresponding to the same strain because a DNA sequencing was not performed.

HEADINGS – *Helicobacter pylori*, genetics. *Helicobacter* infections, diagnosis. Gastric mucosa, microbiology. Saliva, microbiology.

INTRODUCTION

At least half the world's population is infected by *Helicobacter pylori*, making it the most widespread infection in the world. Actual infection rates vary from nation to nation, the people in under developed countries has much higher infection rates than the developed countries[20].

The clinical course of *H. pylori* infection is highly variable depending on bacterial and host (genetic and immune) factors[9].

It has been established that some genes differentially expressed between strains could be used as virulence markers in *H. pylori*[7].

All identified H. pylori strains possess the *vacA* gene which codifies for the VacA toxin; this toxin has a vast array of functions that span induction of apoptosis to modulation of the immune system[13,26].

Additionally, disruption of epithelial cell polarity by CagA protein, codified by the *cagA* gene, is thought to be an indispensable role in the development of gastric carcinoma[23] and the strains *cagA*+ are more associated with severe inflammation that those *cagA*- strains[15].

The first identified and probably the best characterized adhesin of *H. pylori* is a 78 KDa protein termed BabA (blood group antigen binding adhesion)[14]. Carriage of the *babA2*+ strains was associated with more intense chronic inflammation, and presence of glandular gastric atrophy and intestinal metaplasia in the gastric antrum[40].

The presence of *H. pylori* in the oral cavity of patients suffering digestive pathologies has been published and it is more frequent in those patients harboring a periodontal disease[19,35].

Some authors indicate that gastric reflux is not the only route by which *H. pylori* reaches the mouth and its detection and the genotyping in mouth and in stomach are complementary tests to understand some epidemiological issues[21].

Therefore, any information about *cagA* and *babA2* genotypes prevalence among different *H. pylori*-infected clinical groups in the country can help public health authorities to plan preventive policies to reduce the prevalence of diseases associated with *H. pylori* infection[32].

The aim of this work was to detect and correlate the virulence markers *cagA* and *babaA2* of *H. pylori* in gastric biopsies and oral cavity samples.

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METHODS

An observational, descriptive, prospective, and cross-sectional study was carried out between September 2010 and September 2012. Patients with dyspeptic symptomatology and indication for upper gastrointestinal video endoscopy (UGVE) were studied.

We included all patients of both sexes, aged between 18 and 80 years attended to the Service of Gastroenterology of the Hospital *Dr. Julio C. Perrando* in Resistencia, Argentina.

Patients that denied participating, with a history of gastric endoscopy, who had received antibiotics, proton pump inhibitors, histamine receptors antagonists or bismuth compounds in the last four weeks, were excluded.

The selection of patients was non-probabilistic and intentional type. The size of sample was defined on base to the total number of patients annually attending to the Service of Gastroenterology and the prevalence of *H. pylori* in patients suffering dyspepsia according data from literature.

The institutional Bioethical Committee approved all procedures and those patients that accepted to participate provided a written informed consent prior to sampling. Demographic, epidemiological, and clinical data were recorded.

Without oral hygiene, dental plaque and saliva, were sampled. All samples were stored at -20ºC until their further processing by molecular methods.

After oral clinical examination and collection of the oral samples, the patients were subjected to UGVE examination. UGVE has carried out using an endoscope OlimpusRCV-100 GIF-130. Two gastric samples were taken at 2 or 3 cm from the pylorus and were stored at -20ºC to further processing.

DNA was isolated from all samples using the CTAB method and was immediately subjected to conventional PCR.

Purified DNA was carried out for detection of *H. pylori* by conventional PCR using primers derived from the *ureA* gene[18].

In the samples positives for *H. pylori*, the virulence markers were studied, amplifying the *cagA* and *babA2* genes according to the protocols previously published[8].

In all protocols, positive and negative controls supplied by a colleague from the University of Concepción (Chile) were included.

RESULTS

During the period of study, 61 dyspeptic patients with digestive diseases and indication for UGVE were selected, 30 females and 31 males. Patients ranged in age from 18 to 69 years (Average 45 years).

H. pylori was detected in 31/61 gastric samples and in 31/61 oral samples (Figure 1), indicating a prevalence of 50.8% in both body sites.

Due to insufficient quantity or bad quality of DNA obtained, genotyping was performed in 31 positive gastric samples but only in 16 dental plaques and 1 saliva sample (Figure 2).

Table 1 shows the correlation between different genotypes of *H. pylori* in the studied samples. As it was expected, the *vacA* gene was detected in 100% of oral and gastric samples. The *cagA*+ was significantly more frequent in gastric biopsies tan in oral cavity.

In 12/31 (38.7%) patients concordance between the genotypes found in their oral and gastric samples was found.

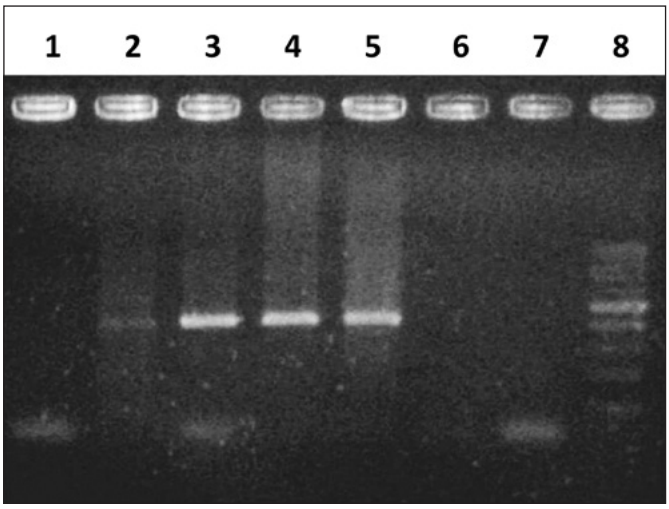

FIGURE 1. Agarose gel electrophoresis of *H. pylori* detection in gastric samples. Lines 2 to 5: positive samples. The molecular weight of the amplicon corresponding to the *ureA* gene is 411 bp. Line 8: 100 bp weight marker.

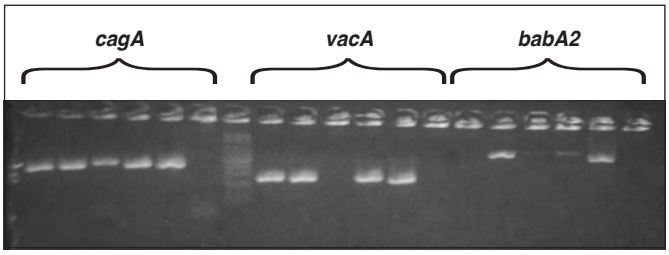

FIGURE 2. Agarose gel electrophoresis of *H. pylori* genotyping products in gastric samples. The molecular weights of the different amplicons are: *cagA* 400 bp, *vacA* 259 and *babA2* 831 bp. Line 7: 100 bp weight marker.

TABLE 1. Distribution of genotypes among different samples

Genotypes	Biopsies (n=31)	Oral (n=17)
vacA	31 (100%)	17 (100%)
cagA	22 (71%)	5 (29%)
babA2	3 (9.7%)	3 (17.6%)

Table 2 and Table 3 show the distribution of genotypes according the different oral and gastroduodenal diseases. No significant differences were found among pathological groups regarding the genotypes studied (*P* value >0.05).

TABLE 2. Relationship between genotypes and digestive diseases

Genotypes	Gastroduodenal diseases				*P* value
	ACG n=18	ACGFM n=8	CGND n=5	Total n=31	
vacA	18	8	5	31	
cagA	13	4	5	22	0.25
babA2	2	0	1	3	0.60

ACG: *H. pylori* associated active chronic gastritis; ACGFM: *H. pylori* associated active chronic gastritis with focus of metaplasia; CGND: chronic gastritis with non-specific duodenitis.

TABLE 3. Relationship between genotypes and oral status

Genotypes	Oral status				
	Gingivitis n=10	Periodontitis n=5	Normal n=2	Total n=17	*P* value
vacA	10	5	2	17	-
cagA	2	0	2	5	0.25
babA2	0	2	1	3	0.60

DISCUSSION

It has become increasingly clear that populations in humans are highly diverse and this heterogeneity can be analyzed at two different levels: genotypic variation among strains and variations in *H. pylori* populations within an individual host[5].

The presence of multiple organisms within a host may occur as a result of recombination events leading to genetic shift, whereas ongoing mutation within a strain can lead to the formation of quasispecies by genetic drift[5].

In the oral cavity there exists a live *H. pylori* that has negative influences on the eradication of stomach infection and as long as physicians agree with the idea of a second colonized site within the oral cavity, the rate for successful eradication of *H. pylori* will increase[39].

From 1989 to date, many researchers worldwide have identified *H. pylori* in plaque and saliva with varying results[1].

H. pylori was found in saliva of 33 (42.3%) patients and in dental plaque samples of 37 (47.4%) patients[38]. In dental plaque 60% of the patients with chronic periodontitis were found to be positive for *H. pylori*[2]. In the present work, *H. pylori* was detected in 50.8% of oral samples.

The *H. pylori* DNA was found with variable frequencies in gastric samples from patients suffering gastroduodenal disorders, ranging from 48 to 63%[17,25,27].

The percentages of positivity for *H. pylori* in gastric samples were lower than previously found in symptomatic patients in Argentina. Medina et al. reported 88.3% of positivity in gastric samples in patients suffering digestive pathologies[19], meanwhile Jimenez et al. published 91% of *H. pylori*- positive gastric samples[11].

Not always *H. pylori* is found simultaneously in oral cavity and in gastric samples. Román-Román et al. found *H. pylori* DNA in saliva and in biopsy in 24% of patients,52.5% were saliva negative/biopsy positive and 6.6% were saliva positive/biopsy negative[31].

Berroteran et al. investigated *H. pylori* presence in dental plaque from dyspeptic patients[4]. They found that 75% of patients presented *H. pylori*-positive gastric pathology, and 38% presented *H. pylori* in the dental plaque, assuming this organism in the dental plaque could be a risk factor for gastrointestinal reinfection.

As published for Trevizani Rasmussen et al., of the 66 patients who were *H. pylori* positive in their gastric biopsies, 19 (28.8%) were found not to have *H. pylori* in the oral cavity. In other hand, of the 12 patients whose gastric biopsies were negative for *H. pylori*, six (50%) were found not to have *H. pylori* in the oral cavity[38].

In gastric biopsies, it was found a higher prevalence than reported by other authors.

The prevalence of *cagA* gene was 48.7% among the positive samples and was not significantly associated with the gastroduodenal diseases[17]. Among patients with chronic gastritis, 39.2% were *cagA*+[27]. The *cagA* gene was detected in 42 (56.0%) strains in Cuban patients with upper gastrointestinal diseases[10].

In one study the prevalence of *cagA* gene was 48.7%, lower than other reports in African countries[17] and was present in 73.3% of isolates[6].

In patient positives for *H. pylori* in gastric biopsies, the *cagA* gene was detected in 43.3% of gastric biopsies, in 43.8% of saliva samples, and in 27.3% of dental plaque samples, noting that dental plaque and saliva can serve as temporary storage for the *cagA* variant *H. pylori* in individuals with gastric disease[34]. In other study, the *cagA* gene was present in gastric biopsies from 84% of patients with gastroduodenal disorders[25].

In saliva samples and in dental plaque the prevalence of *cagA* gene found in the present work was like that reported previously. The *cagA* gene was found in 27 (45%) of the 60 samples of *H. pylori*-positive saliva samples[28]. In a previous study, 14 from 18 patients harbored the *cagA*+ genotype of*H. pylori*, but only 9 of them presented this genotype in stomach[33]. These studies suggest that the genotyping must be performed simultaneously in oral cavity and in stomach.

Some authors, in agreement with the findings here presented, reported about a low correlation between the gastric infection and the presence of *cagA*+ genotype in oral cavity[31].

Although there are several genes associated with adhesion of the bacteria, the *babA2* gene is associated with successful colonization[21].

In the present work, the *babA2*+ genotype was the less frequent, in agreement with previous reports. In patients with chronic gastritis[12]. Nevertheless, a recent study reported that *babA2* prevalence was significantly higher in gastric biopsies obtained from chronic gastritis patients (95%) when compared with duodenal ulcer patients (18.1%) and non-ulcer dyspepsia subjects (26.1%)[30].

Arévalo et al. found that 57% of the gastric isolates were *babA2*+[3], which coincides with other South American studies that reported gene frequencies ranging from 40.4% to 82.3% in stomach samples[16,27,29,37].

When *babA2* and *cagA* are coexpressed in the same *H. pylori* strain, they work synergistically in worsening inflammation and may be a potential risk of intestinal metaplasia[30].

Taking into account the genes association, it was found the *cagA*+/*babA2*+ combination in 9.7% of gastric biopsies and in 17.6% of oral samples. Similarly, this association was observed in 13.3% of gastric samples from patients suffering chronic gastritis as reported by Paniagua et al.[27]. Regarding the coincidence between the same genotype in oral cavity and gastric mucosa, the genotypes found in saliva and biopsy of the same patient had 51.1% agreement[31]. Other studies show that people can be infected simultaneously by two or more genotypes of *H. pylori*[22], due to coinfection or genetic variation[24]. In the present work the same genotypes were found simultaneously in oral and gastric samples from the same patient in 38.7% of them. However, complete genomes of the detected strains should be sequenced, since this is the only way to demonstrate genetic identity.

Nevertheless, as published previously, we did not find a statistical correlation between the virulence genes and the gastroduodenal or oral diseases[17,36]. That could be due to the small number of patients with *H. pylori* harboring each virulence marker.

Additionally, further studies may be performed to correlate different digestive disorders with the presence of various virulence factors, including the iceA protein and the different alleles of *vacA* gen[10].

CONCLUSION

This study is the first in provide information about the genotypes of the Argentinean Northeast *H. pylori* strains. Despite the high prevalence of *H. pylori* infection, the most of patients had less virulent genotypes in oral cavity and gastric tissue. The *cagA*/*babA2* combination was not frequent in the samples studied. There was not a statistical correlation between the virulence genes and gastroduodenal or oral diseases.

Although in some patients the same genotype was found both in oral and gastric samples, it cannot be ensure that they corresponding to the same strain because a DNA sequencing was not performed.

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Authors' contributions

Medina ML: samples and data collection, survey execution, revision of text. Medina MG: survey execution, statistical analysis, revision of text. Merino LA: samples processing, survey execution, writing of text.

Medina ML, Medina MG, Merino LA. Correlação entre marcadores de virulência de *Helicobacter pylori* na cavidade oral e em biópsias gástricas. Arq Gastroenterol. 2017;54(3):217-21.

RESUMO – **Contexto** – O resultado clínico da infecção por *Helicobacter pylori* tem sido associado com fatores de virulência. A presença desses fatores como marcadores moleculares é útil na identificação do risco elevado para o desenvolvimento de graves patologias gástricas. **Objetivos** – Correlacionar a presença de marcadores de virulência *cagA* e *bab2A* do *H. pylori* em amostras de biópsias gástricas e orais. **Métodos** – Um estudo observacional, prospectivo, descritivo e transversal foi realizado entre setembro de 2011 e setembro de 2012. Foram incluídos pacientes com sintomas de dispepsia com indicação de endoscopia gastrointestinal que compareceram ao Serviço de Gastroenterologia do Hospital *Dr. Julio C. Perrando*. Investigação epidemiológica foi concluída. Para detectar a bactéria e seus genes de virulência, amostras de saliva, placa dentária e biópsia gástrica foram tomadas e processadas pelo PCR. **Resultados** – Sessenta e um pacientes foram selecionados para este estudo (30 mulheres e 31 homens). *H. pylori* foi detectado em 31 biópsias gástricas e 31 amostras orais. Foi encontrada diferença significativa entre as amostras orais e gástricas no genótipo *cagA*. A ocorrência simultânea entre genótipos orais e gástricos do mesmo paciente foi encontrada em 38,7% das amostras. **Conclusão** – Este é o primeiro estudo a fornecer informações sobre os genótipos das cepas do *H. pylori* no Nordeste Argentino. Apesar da alta prevalência da infecção pelo *H. pylori*, a maioria dos pacientes tinha genótipos menos virulentos na cavidade oral e tecido gástrico. A combinação *cagA*/*babA2* não foi frequente nas amostras estudadas. Não houve correlação estatística entre os genes de virulência e doenças gastroduodenais ou orais. Embora em alguns pacientes o mesmo genótipo tenha sido encontrado tanto nas amostras orais quanto gástricas, não se pode garantir que correspondam à mesma variação, pois um sequenciamento de DNA não foi realizado.

DESCRITORES – *Helicobacter pylori*, genética. Infecções por *Helicobacter*, diagnóstico. Mucosa gástrica, microbiologia. Saliva, microbiologia.

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