Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 21, No. (4) : 2019 : 941-944

ISSN-0972-3005

DETECTION OF *HELICOBACTER PYLORI* BY POLYMERASE CHAIN REACTION FROM FECES OF ASYMPTOMATIC PATIENTS

JHOAN GUAMÁN[1,2], FAVIAN BAYAS-MOREJÓN[2]*, RIVELIÑO RAMÓN-CURAY[2], JAGGER SEGURA-OCHOA[2], IVAN GARCÍA-CÁCERES[2] AND DARWIN NUÑEZ[1]

[1]Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación, Facultad de Ciencias de la Salud y del Ser Humano, Universidad Estatal de Bolívar, CP: 020150; Guaranda- Ecuador
[2]Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación, Facultad de Ciencias Agropecuarias, Recursos Naturales y del Ambiente, Universidad Estatal de Bolívar; CP: 020150, Guaranda- Ecuador

(Recieved 15 April, 2019; accepted 2 June, 2019)

Key words : Detection, H. pylori, PCR, Feces

Abstract – The objective of this work was to make a diagnosis of the presence of *Helicobacter pylori* in human biological samples (feces). To this end, the samples were selected from 50 outpatients of the Hospital Alfredo Noboa Montenegro General (ANMH) from Guaranda and María Auxiliadora private laboratory from San Miguel de Bolívar in Ecuador with apparent symptomatology to the presence of *H. pylori*. The samples were processed and analyzed by polymerase chain reaction (PCR). After the data collection through the clinical record, the average age was 30 years (range 5 - 65 years). Through molecular analysis, 11 samples: 4 of 16 from men and 7 of 34 from women were positive for *H. pylori*, showing in the gel the characteristic 394-bp band. In this work, the PCR technique has proved to be an effective methodology for the detection of *H. pylori* in biological samples.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral bacterium, Gram negative, which colonizes the human stomach and is prevalent throughout the world. It is considered a pathogen of global interest because it is a microorganism easily contagious among hosts or hosts (Smolka and Backert, 2012; Cava and Cobas, 2003). This bacterium has been associated with peptic ulcer disease, gastric adenocarcinoma and B lymphoma of lower grade associated with the mucosa (Hasni, 2012). In addition, since 1994 *H. pylori* has been associated with gastric cancer (Palomino and Tomé, 2016). It is usually found in the deeper portions of the mucus gel that covers the gastric mucosa and the gastric epithelium (Harrison, 2012).

Although almost 50% of the world population is infected with *H. pylori*, the highest prevalence, incidence and age distribution are in developing countries (Palomino and Tomé, 2016). Among the most important routes of transmission of the pathogen are the oral-oral, oral and fecal-oral routes, there being two possible vectors for the transmission of *H. pylori*, these being: water and food (Moreno-Mesonero *et al*., 2017; Bayas *et al*., 2016).

The diagnostic methods of *H. pylori* infection are direct and indirect, the first based on the study of samples obtained by gastric biopsy; the indirect ones based on the detection of certain characteristics of the bacterium such as: ability to hydrolyze through the urea, breath test or the measurement of specific antibodies (Gisbert, 2016). There are other techniques such as histology, culture, serological techniques, enzyme-linked immunosorbent assay (ELISA), and antigenic screening (Gisbert, 2016; Forbes *et al*., 2009; Secretaria De Salud, 2006).

However, molecular methods are laboratory techniques that are used to isolate high purity DNA or amplify a specific region of molecules, usually through a polymerase chain reaction (PCR). Nowadays, PCR is considered an essential tool in molecular biology, the main explanation for this development is due to the difficulty of detecting pathogens through classical microbiology (Herráez, 2012; Farfan, 2015).

There are several methods of DNA extraction that adjust to the needs of each type of sample, these include physical, chemical, enzymatic treatments or a combination of these (Green and Sambrook, 2012). Considering the previous, the objective of this study was to detect *H. pylori* by PCR from human biological samples.

MATERIALS AND METHODS

Materials

By a clinical data sheet considering the symptoms, 50 patients were selected from the Alfredo Noboa Montenegro Hospital (ANMH) in the city of Guaranda and from the María Auxiliadora private laboratory in San Miguel de Bolívar, Ecuador. The age criteria are shown in Table 1. The samples obtained from the selected patients were taken to analysis in the laboratory of microbiology and molecular biology of the Research Department of the State University of Bolivar.

Samples preparation

To analysis of 5 to 10 g of stool samples were processed, for which, each sample was homogenized in 90 mL of sterile buffered peptone water (BPW), was mixed vigorously and incubated at 37 ° C for 24 hours, after this time, 300 µL of each homogenate was placed in 1.5 mL eppendorf micro tubes for subsequent molecular analysis.

Extraction of genetic material

For the bacterial DNA extraction, the Chelex Method was applied (Suenaga and Nakamura, 2005). The technique was carried out as follows: the re-suspended was centrifuged at 13000 xg for 15 minutes at 4 °C, after this step the supernatant was removed and the pellet was preserved. The pellet was washed for 3 times suspending it in 1.5 mL of acetone.

Each preparation was centrifuged at 13000 xg for 10 min at 4 °C. Then the supernatant was removed and the pellet was preserved for further processing with the Chelex resin. For which, 200 µL of chelex-100 (5%) and 0.2 mg of proteinase K were added to the pellets and then incubated at 75 °C (in a bath) for 40 minutes. Each preparation was homogenized in a vortex and centrifuged at 100000 xg for 5 minutes. Finally, about 100 µL of the supernatant was transferred to sterile tubes and stored at -20 °C for subsequent PCR amplification.

Identification of *Helicobacter* isolates by molecular analysis

From the DNA extracted to the molecular analysis at the species level was development, for which, was amplified a fragment of 394-bp of the 16S gene of the vacuolizing protein Vac, specific for *H. pylori*, using the primers VacAF: 5'-GGCACACTGGATTTGTGGCA-3 and VacAR: 5'-CGCTCGCTTGATTGGACAGA-3', the reagents and conditions used in this study were previously described by Santiago *et al.* (2015).

For PCR amplification, the final reaction volume was 50 µL, containing 45 µL of the mixture and 5 µL of template DNA. After PCR, 10 µL of amplified product, previously mixed with 2 µL of loading buffer Blue / orange 6X, loading Dye (Promega, G190A, EE-UU), was analyzed by electrophoresis in 1.5% agarose gel prepared previously in 1 X TAE buffer, with the addition of 10 µL of Red Safe (Invitrogen, 247110-030, EE-UU) was run at 100 volts for 45 minutes. A 50-bp molecular weight marker (Invitrogen, 10488-043, EE-UU) was included in each gel. Finally, the fragments were visualized in a transilluminator under UV light.

RESULTS AND DISCUSSION

Population and extraction of genetic material

After the data collection through the clinical sheet, the following results were obtained. The median age of the population studied was 30 years (range 5 - 65 years), in comparison with the study carried out after a selection of subjects that went to the medical unit of the ANMH and to the private laboratory María Auxiliadora.

The extraction method with Chelex resin is fast and effective, given that, it is the first step for the amplification of template DNA, the technique is characterized especially by the quality of DNA to be obtained, without contamination or inhibitors (Butler, 2007).

Identification of the isolates obtained from *Helicobacter* by PCR

In the present study, of the 50 samples analyzed by PCR with the specific primers (VacAF and VacAR), 11 samples: 4 of 16 (8%) from men and 7 of 34 (14 %) from women were positive for *H. pylori*, in these samples the characteristic band of 394-pb was obtained (Fig. 1).

The diagnosis by PCR of *H. pylori* has a sensitivity

Table 1. Identification of sex and age of sampled patients

AGE	5- 10 years	11- 20 years	21- 30 years	31- 40 years	41 years and up	TOTAL
Men	0	7	2	4	3	16
Women	3	9	9	7	6	34
Total	3	16	11	11	9	50

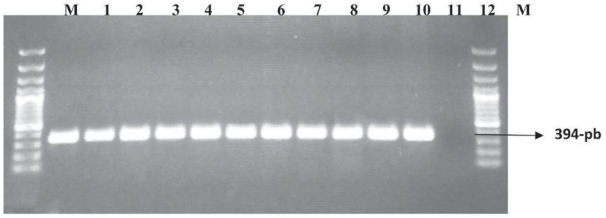

Fig. 1. Electrophoresis analysis of the amplified DNA, Lanes: 1 = Hp3; 2 = Hp5, 3 = Hp6; 4 = Hp10; 5 = Hp14; 6 = Hp17; 7 = Hp23; 8 = Hp26; 9 = Hp27; 10 = Hp28; 11 = Hp34; 12 = Negative control, M = Molecular weight marker (100 bp).

and specificity of 95% and its main advantage is that, the microorganism can be detected regardless of the viability of the bacteria in the samples (Premoli *et al.,* 2004). In studies carried out in Latin America highlights one in pediatric patients with celiac disease where fecal samples were collected for the detection of *H. pylori* by PCR in 31 children from the province of Chaco - Argentina presenting the following results: 2 (14,3%) samples were positive for PCR a low indicator in relation to the biopsy where 12 patients tested positive (Medina *et al.*, 2009).

In Ecuador in the last decade there are no data from studies of detection of *Helicobacter pylori* in feces by PCR, apart from the present work in the which we detected 11 positive samples out of a total of 32 isolated by PCR. Due to the frequency that is relatively low by the conditions in which it was isolated, It is important to highlight that there are no studies carried out on human feces in Latin America and European countries because they are bacteria that are very difficult to isolate in this type of samples.

CONCLUSION

The conventional PCR technique is an effective methodology for the detection of *H. pylori* in biological samples, reducing considerably the time of analysis and showing certain sensitivity in particular compared with culture methods, although in rare cases false negatives may occur, probably due in the presence of PCR inhibiting substances. However, the results of this work demonstrate the need to continue improving the detection methods of the pathogen.

ACKNOWLEDGEMENT

We thank the State University of Bolivar, the Research Department and the debt exchange project Ecuador-Spain for having made it possible for this investigation to be carried out.

REFERENCES

Bayas, I.F., González, A., Moreno-Mesonero, L., Moreno, Y. and Ferrús, M. 2016. Detection of *Helicobacter pylori* in vegetables, XXIXth International Workshop on *Helicobacter* and Microbiota in Inflamation on & Cancer, Magdeburg-Germany, Helicobacter. 2016. Doi: 10.1111/hel.12344/abstract.

Butler, J.M. 2007. DNA extraction from forensic samples using chelex. CSH. *Protocols.* 1. Doi: 10.1101/pdb.prot5229.

Cava, F.C. and Cobas, G. Dos décadas de *Helicobacter pylori*. *Vaccimonitor.* 12 (1) : 1-10. ISSN 1025-0298.

Farfan, B.M. 2015. Biologia Molecular Aplicada Al Diagnostico Clinico. *Rev. Méd. Clín.* 2015; Las Condes. 2.

Forbes, B., Sahm, D. and Weissfeld, A. 2009. *Diagnóstico Microbiológico* (12ª Edición). Bailey & Scott. Buenos Aires - Argentina: Panamericana. 2009. 1160 paginas. ISBN 9789500682435.

Gisbert, J. and Molina-Infante, J. 2017. Tratamiento actual de la infección por *Helicobacter pylori*. *Med. Clin.* (Barc.). 148 : 20-22.

Harrison, 2012. Principios de medicina interna. España: mcgraw-hill interamericana editores, S.A. 2012; de C. V.

Hasni, S.A. 2012. Role of *Helicobacter pylori* infection in autoimmune diseases. *Curr. Opin. Rheumatol.* 24 (4) : 429-434.

Herráez, Á. Biologia Moleular e Ingenieria Genetica. Conceptos, Técnicas y Aplicaciones en ciencias de la salud (2nd Edicion). Editorial Harcourt, Elsevier, 2012. Barcelona España: ELSEVIER. ISBN GDSK-HPQ-3D7Y.

Medina, M.G., Medina, M.L., Martín, G., Dikstein, B., Picón, S., Gorodner, J. and Merino, L. 2009. Presencia de *Helicobacter pylori* en biopsias gástricas y heces de pacientes pediátricos con enfermedad celiaca. *Rev. Gastroenterol. Mex.* 74 (2): 94-98.

Moreno-Mesonero, L., Moreno, Y., Alonso, J.L. and Ferrús, M.A. 2017. Detection of viable *Helicobacter pylori* inside free-living *amoebae* in wastewater and drinking water samples from Eastern Spain. *Envir. Microbiol.* 19 (10). doi: 10.1111/1462-2920.1385.

Palomino, E. and Tomé, E. 2012. *Helicobacter pylori*: Rol del agua y los alimentos en su transmisión. *An. Venez. Nutr.* 25 (2): 85-93.

Green, M. and Sambrook, J. 2012. *Molecular Cloning: A Laboratory Manual* (Fourth Edition).Cold Spring Harbor Laboratory Press. 2028 pages. ISBN 978-1-936113-42-2.

Premoli, G., González, A., Milán Mendoza, B., Percoco, T. and Vielma, A. 2004. Diagnóstico de *Helicobacter pylori* mediante Reacción en Cadena de la Polimerasa. *Rev. Cubana Med. Trop.* 56 (2): 85-90.

Santiago, P., Moreno, Y. and Ferrús, M.A. 2015. Identification of viable *Helicobacter pylori* in drinking water supplies by cultural and molecular techniques. *Helicobacter.* 20 (4) : 252-259.

Secretaria de Salud. 2006. Guia para la aplicacion de pruebas rapidas. Mexico: Printed and Made in Mexico. ISBN. 970-721-385-X.

Smolka, A.J. and Backert, S.J. 2012. How *Helicobacter pylori* infection controls gastric acid secretion. *Gastroenterol*. 47(6) : 609-618.

Suenaga, E. and Nakamura, H. 2005. Evaluation of three methods for effective extraction of DNA from human hair. *J. Cromatography B.* 820 : 137-141.