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Detection of *Helicobacter pylori* and clarithromycin resistance in gastric biopsies of pediatric patients by using a commercially available real-time polymerase chain reaction after NucliSens semiautomated DNA extraction☆

Sonia Agudo[a,*], Teresa Alarcón[a], Pedro Urruzuno[b], María Josefa Martínez[c], Manuel López-Brea[a]

[a]*Department of Microbiology, Hospital De La Princesa, 28006 Madrid, Spain*
[b]*Department of Gastroenterology, Hospital Doce Octubre, 28041 Madrid, Spain*
[c]*Department of Gastroenterology, Hospital Niño Jesus, 28009 Madrid, Spain*

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Abstract

The aim of this study was to evaluate a commercially available kit, MutaREAL *Helicobacter pylori* (Inmundiagnostik, Bensheim, Germany) real-time polymerase chain reaction (PCR), for detection of *H. pylori* infection and point mutations in the 23S rRNA genes responsible for clarithromycin resistance in gastric biopsies.

Methods: Gastric biopsies were obtained by endoscopy from pediatric patients with gastric symptoms, cultured according to standard microbiologic procedures, and clarithromycin resistance was determined by E-test. DNA extraction was performed by NucliSens platform with the NucliSens magnetic extraction reagents (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions. MutaREAL kit was used according to manufacturer recommendations in a LightCycler (Roche Diagnostics Gmbh, Mannheim, Germany) for the detection of *H. pylori* infection and clarithromycin susceptibility.

Results: Amplification was positive for *H. pylori* in 62 and negative in 44 biopsies out of 106 biopsies. All negative biopsies were positive for human β-globin gene. This real-time PCR assay showed sensitivity of 93.33% (negative predictive value, 90.90%) and specificity of 86.95% (positive predictive value, 90.32%) for *H. pylori* detection. Clarithromycin resistance was detected in 26 cases by PCR with a sensitivity and specificity of 90.62 and 95.83, respectively.

Conclusions: MutaREAL kit was able to detect *H. pylori* and its clarithromycin susceptibility with high efficacy. This method is quicker than culture and is suitable to be done in 1 h after DNA extraction. The new system of automatic extraction will lead to reduction in the total time.

Keywords: Clarithromycin; Biopsies; Real-time PCR; Resistance and easyMAG

1. Introduction

Helicobacter pylori is a spiral microaerophilic Gram-negative bacteria, about 3 μm long with a diameter about 0.5 μm. It has 4 to 6 flagella, and it is found in the gastric mucous layer adherent to the epithelial lining of the stomach. The organism has an abundant urease enzyme production, which is important for colonization, because of the formation of ammonia on the gastric mucosa, increasing the pH of its environment. This enzyme is also important for the detection of the organism.

H. pylori is associated with various digestive diseases, such as peptic ulcer (duodenal and gastric), chronic active gastritis, and mucosa-associated lymphoid tissue, and it is considered a risk factor in the development of the gastric cancer.

At present, several diagnostic assays for *H. pylori* detection are available (Czinn, 2005; Versalovic, 2003). Invasive

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* Corresponding author. Tel.: +34-915202317; fax: +34-915202317.
E-mail address: soniaagu@hotmail.com (S. Agudo).

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methods requiring gastric endoscopy include rapid urease testing, culture, histology, and molecular diagnostics. Non-invasive approaches include fecal antigen detection, serologic testing, and urea breath testing, but no information on resistance against antibiotics can be obtained with these tests.

H. pylori is most frequently acquired during childhood and usually persists throughout life unless a specific treatment is given, causing different digestive diseases in childhood and adulthood (Sinha et al., 2004). Eradication therapy is recommended for patients with peptic ulcer disease and mucosa-associated lymphoid tissue lymphoma, atrophic gastritis, first-degree relatives of gastric cancer patients, unexplained iron deficiency anemia, and chronic idiopathic thrombocytopenic purpura. The Maastricht III consensus report recommended proton pump inhibitor (PPI) or ranitidine bismuth citrate-based triple regimen with clarithromycin and amoxicillin or metronidazole as first-line therapy (Malfertheiner et al., 2007). However, side effects, poor compliance, and resistance to the antibiotics used are common causes of treatment failure (Megraud, 2004; Mégraud and Lehours, 2007). In many cases, the macrolide drug clarithromycin is the key component of these combination therapies.

The prevalence of primary and acquired clarithromycin resistance is increasing worldwide, in some European countries, including France, Portugal, Spain, Poland, Turkey, and Bulgaria, and in the United States. In other northern European countries, this increase was not observed (Francesco et al., 2007). The difference depends on macrolide consumption (Koletzko et al., 2006). Several studies suggest that clarithromycin resistance is higher in strains obtained from children than in those from adults (Alarcón et al., 2003) because children have been more exposed to macrolides. It is very frequent nowadays to treat respiratory infections in young children with this group of antibiotics (López-Brea et al., 2001).

Resistance to this antibiotic is due to point mutations in the peptidyl transferase loop in both copies of 23S rRNA genes. Three major point mutations in 2 positions on the 23S rRNA (equivalent to *Escherichia coli* coordinates 2058 and 2059) have been described in which an adenine residue is replaced by a guanine or a cytosine residue in different positions: A2142C, A2142G, and A2143G. Mutation A2143G is the most frequently reported (53% to 95%), followed by mutation A2142G, and mutation A2142C is less common (Domingo et al., 1998). Other rare mutations such as A2115G, G2141A, T2717C, and T2182C have also been identified (Burucoa et al., 2008).

In routine practice, the detection of clarithromycin resistance is mainly based on phenotypic methods performed after culture: agar diffusion for the E-Test or the agar dilution method, which is considered the reference; however, these methods are time consuming and can take up to 2 weeks. Detection of point mutations conferring resistance to clarithromycin by molecular methods may constitute a more reliable approach. Numerous polymerase chain reaction (PCR)-based techniques have been developed to detect these mutations, such as PCR–restriction fragment length polymorphism, PCR-DNA-enzyme immunoassay, and reverse hybridization line probe assay. More recently, real-time PCR methods were developed that were based on amplification of a fragment of the 23S rRNA gene of *H. pylori* followed by melting curve analysis (López-Brea and Alarcón, 2006; Oleastro et al., 2003). The first attempt was performed by Gibson et al. (1999) on *H. pylori* strains. Extraction of nucleic acids (RNA and DNA) in the clinical samples is an important step prior to molecular amplification.

The aim of this study was to evaluate a commercially available kit, MutaREAL® *H. pylori* real-time PCR, that can be used for both specific detection of *H. pylori* infection and for the determination of point mutations in the 23S rRNA genes responsible for clarithromycin resistance in Spanish *H. pylori* clinical isolates obtained from gastric biopsies from pediatric patients. It was used a semiautomated DNA extraction method (NucliSens easyMAG). *H. pylori* clinical isolates and known *H. pylori*-positive biopsies were tested in previous experiments to check the reliability of the MutaREAL® *H. pylori* real-time PCR.

2. Materials and methods

2.1. Patients

Symptomatic pediatric patients (aged 18 years old and under) attended to the Gastroenterology Unit from 2 children's hospitals (Hospital Infantil Universitario Niño Jesús and Hospital Universitario Doce de Octubre, Madrid, Spain) were included in this study. Both hospitals were *H. pylori* reference centers, and parents signed an informed consent form for the endoscopy. At the same time, samples were taken; patients had not received PPIs or antibiotics for at least 2 weeks.

2.2. Bacterial strains and gastric biopsy specimens

Three different batches of strains and/or specimens were included in this study: 1) 46 *H. pylori* clinical isolates cultured from gastric biopsies and stored at −80 °C until used, (2) 36 biopsies *H. pylori* positive by culture and stored at −20 °C until DNA extracted, and (3) 106 biopsies with unknown *H. pylori* status when PCR was performed (obtained from February 2006 to June 2008).

Biopsies were received at the Department of Microbiology (Hospital Universitario de la Princesa, Madrid, Spain) and processed during 3 h. All samples for culture were placed in sterile saline solution for transport. All biopsies were cultured as follows: Tissue was streaked onto both nonselective (Columbia agar, with 5% sheep blood; bioMérieux, Marcy l'Etoile, France) and selective media (Pylori agar, bioMérieux) incubated 10 days at 37 °C in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2). Isolates were identified as *H. pylori* based on colony