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Applicability of the 229 bp 16S RRNA Domain for Detecting *Helicobacter pylori* among Gastritis Patients by PCR Technique

Amin A. Al-Sulami*, Saad Shaheen Hamadi and Gaidaa Kadhum Baqir

College of Education, Basrah University, Iraq.

*Corresponding author. E-mail: aminabdulah@yahoo.com.

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The aim of this study was to detect the presence of *Helicobacter pylori* in patients with various gastrointestinal symptoms using modified Columbia urea agar (MCUA) and evaluating the 229-bp domain of the 16S rRNA sequence to determine an optimum method for the detection of H. pylori. This study was carried out in the Teaching Hospital in Basrah during the period from March 2011 to October 2012. A total of 200 patients, 110 males and 90 females, whose age ranged from 18-75 years were included in this study. Gastric biopsy samples were taken from all patients and cultured. The genomic DNA was extracted from all bacterial cultures and used for polymerase chain reaction (PCR). The identification of the isolates was done using the primer for the 229 bp of the 16S rRNA sequence. The isolation rate of *H. pylori* on MCUA medium was 142 (71%) out of 200 patients investigated. Of the142 presumptively identified *H. pylori* isolates, 133 (93.7%) isolates showed positive results at 229 bp domain for 16S rRNA gene by PCR. The bacterium was successfully isolated using this method, also PCR proved to be a sensitive and specific test for detecting *H. pylori* DNA in clinical samples cultured on modified Columbia Urea Agar (MCUA).

Key words: *Helicobacter pylori,* gastrointestinal, PCR technique, 229 bp 16S RRNA Domain.

INTRODUCTION

Helicobacter pylori has been recognized as the major etiological agent of chronic gastritis and plays an important role in the pathogenesis of gastric and duodenal ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma (Ernst and Gold, 2000; Compare et al., 2010). Approximately two third of the world population are infected with *H.pylori* (Lee et al., 1994). The eradication of *H. pylori* has become an accepted therapy in order to prevent the relapse of the disease. Accurate diagnosis is essential for the effective treatment and management of infections that are caused by this organism (Shiotani et al., 2000).

There are several methods described for the diagnosis of *H. pylori, these* includes culture, histological sections, rapid urease test (RUT), serology, urea breath test (UBT), and stool antigen test (Monteiro et al., 2001; Nakamura, 2001; Tanih et al., 2008). A reliable test to detect this infection is crucial, but none of the tests available is suitable for all clinical situations, each has its own drawback and pitfalls (Monteiro et al., 2001; Pandya et al., 2009).

On the other hand, molecular methods in particular polymerase chain reaction (PCR) have the potential to detect more cases of infection because of its highly sensitive, specific and fast approach (Ho and Windsor, 2000; Zsikla et al., 2006; Cirak et al., 2007). A large genetic heterogeneity with *H. pylori* strains has been reported among various populations worldwide (Gottke et al., 2000; Caroll et al., 2003). Assays based on PCR technology have been developed to detect the presence of *H. pylori* DNA, using various primers for several gene targets from clinical specimens which vary in sensitivity and specificity (Lu et al., 1999; Liu et al., 2008). The targets of these PCR methods include the 16S rRNA gene (AL-Jobori et al., 2011), the 26-kDa species-specific antigen (SSA) gene (Hammer et al., 1992), the urease A (ureA) gene (Espinoza et al., 2011), and the urease C (ureC) gene (Brooks et al., 2004).

Liu et al. (2008) reported a specific and sensitive method to detect *H. pylori* in biologic media. They also discovered the 546-bp domain of the 16S rRNA sequence which is conserved among *H. pylori* strains, and defined a 229-bp domain within this conserved sequence. This sub-domain is considered to be 100% homologous in most *H. pylori* strains available in Gen Bank and also specific for *H. pylori*. It is important to develop specific and sensitive diagnosis for the detection and identification of this microorganism in biological specimens due to the extensive polymorphism of many *H. pylori* genes and the absence of particular genes in some strains (Camorlinga-Ponce et al., 2004).

The aim of this study therefore, was to detect the presence of *H. pylori* in patients with various gastrointestinal symptoms using modified Columbia urea agar (MCUA) and evaluating the 229-bp domain of the 16S rRNA sequence in identifying the bacteria.

MATERIALS AND METHODS

Sample Collection

Two hundred patients with various gastrointestinal symptoms, who attended endoscopy unit at the Teaching Hospital in Basrah during the period from March, 2011 to October, 2012 were studied. These include 110 males and 90 females whose age ranged from 18-76 years. They were diagnosed as having gastritis, gastric and duodenal ulcers and gastric cancer using endoscopic examination under the supervision of a gastroenterologist. All these patients had symptoms and signs like epigastric discomfort, bloating, nausea, vomiting or upper abdominal pain. Patients who had received antibiotics, bismuth, proton pump inhibitors within the last four weeks were excluded from the study.

Isolation of bacteria

Gastric biopsy samples were taken from the antrum of the stomach of all patients, which was then transported to the laboratory in 2 ml brain heart infusion broth (BHIB) for bacterial culture. The samples were homogenized and cultured on modified Columbia Urea Agar (MCUA) slant tube which was then incubated under microaerophilic condition at 37°C for 1-2 day(s). Growth of H. pylor*i* resulted in color changes from yellow to pink indicating urease activity. The resulting system is a simple monophasic-diphasic culture setup (MDCS); a diphasic solid liquid environment at the lower part of the test tube and a monophasic solid environment above it. A portion of the bottom and the upper part of the slanted MCUA tube subcultures were cultured on plates of MCUA for purification (Al-Sulami et al., 2008). *H. pylori* isolates were identified by Gram staining, cultural characteristics and biochemical tests (oxidase, catalase, and urease positivity).

DNA extraction and PCR

Genomic DNA was extracted from all presumptive *H. pylori* cultures using genomic DNA mini kit (Geneaid, UK) according to the manufacturer instruction. The extracted DNA was stored at -20°C until used, and a gel of 0.8% agarose was used for electrophoresis and then confirmed by using primers specifically designed for the identification of *H. pylori* based on 16SrRNA sequence. The specific primers were supplied from Bionner Corporation (USA) and used for PCR of all isolates. The identification of the isolates was based on the use of the

primer of 229 bp of the 16S rRNA sequence (Liu et al,2008) composed of forward 5′- TCG GAA TCA CTG GGC GTA A -3′ and reverse 5′-TTC TAT GGT TAA GCC ATA GGA TTT CAC -3′. For detection of *H. pylori* by PCR, amplification conditions were carried out in a 20 µl of reaction mixture containing 5 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl DNA samples and 8 µl free deionized water. PCR conditions include: denaturation at 95°C for 5 min, followed by 30 cycles at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for45 s, and an additional extension step at 72°C for 10 min. After completing PCR cycles, the amplified PCR products were electrophoresed in 2% agarose and 2000bp ladder was used as DNA molecular weight standard. The product was detected and examined under ultraviolet transilluminator. A 229-bp band was considered a positive PCR result**.**

RESULTS AND DISCUSSION

The culture of all specimens on modified Columbia urea agar using MDCS method (AL-Sulami et al., 2002) revealed the presence of *H. pylori* by the change in the color of the slant MCUA tube from yellow to pink as shown in Figure 1.When the isolates were subcultured on MCUA plates, the positive *H. pylori* also showed change in color from yellow to pink as shown in Figure 2. *H. pylori* isolates were confirmed by Gram staining and cultural characteristics as gram-negative spiral shaped bacterium with colonies creamy in color and small to medium in size. Also these isolates showed positivity for catalase, oxidase and urease tests (Gomes and Martinism , 2004 and Samie et al., 2007).

The isolation rate of *H. pylori* on MCUA medium was 71%, this account for 142 patients out of 200 patients investigated while 58 patients (29%) were negative for *H. pylori*.

Of these 142 isolates, 133 (93.7 %) isolates showed positive results in the 229 bp domain for 16SrRNA gene by PCR While 9 (6.33%) were negative. PCR products for 16SrRNA based primers gave bands on agarose gel corresponding to a 229 base pair product when compared to the molecular ladder, thus identifying the isolates as *H. pylori* as shown in Figure 3. The culture method is highly specific for identifying active *H. pylori* infection (Ndip et al., 2008). Although it is being considered a successful method for detecting *H. pylori* in gastric biopsy specimens, it requires several days for culturing and isolating this bacterium thus delaying the result that is needed for proper treatment to be established (Kisa et al., 2002). A faster, more sensitive and more specific method should be sought.

Figure 1. Culture of biopsy sample on MCUA slant: **a-** MCUA slant positive for *H.pylori*. **b-**MCUA slant negative for *H. pylori*.

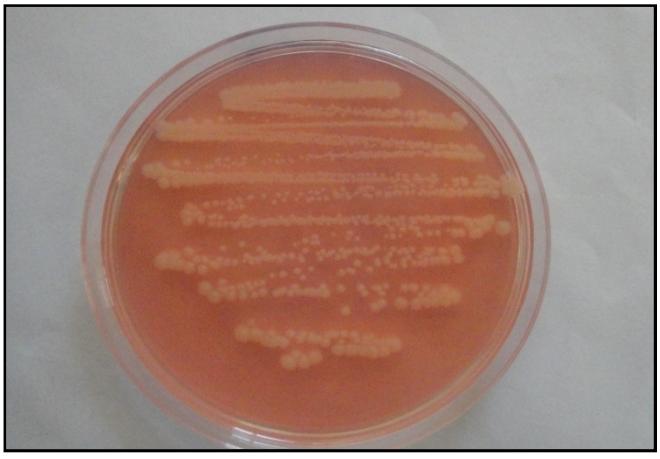

Figure 2. *H. pylori* colonies after subculture on MCUA plates.

Molecular methods like polymerase chain reaction

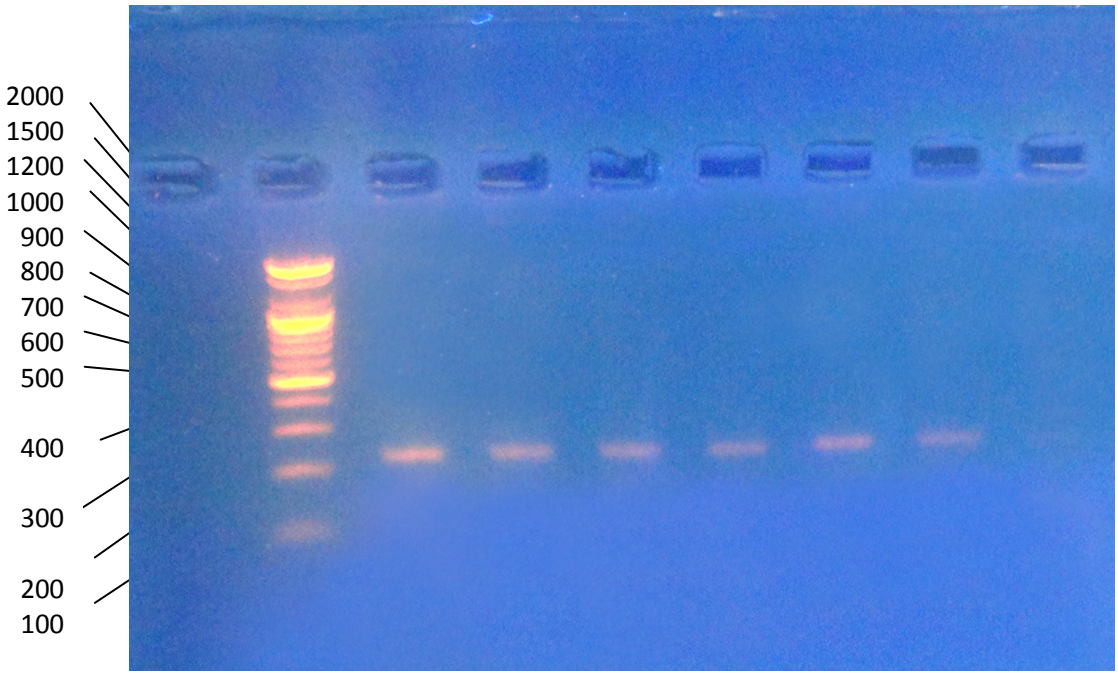

Figure 3. PCR products for 229pb of 16S rRNA on agarose gel gave band at 229 bp when compared with molecular ladder (2000-100).

(PCR) have the potential to accurately determine both the presence of infection and the genotype of bacteria, and have marked sensitivity and specificity (Minami et al., 2006; Zsikla et al., 2006). 16S rRNA was chosen for detection of *H.pylori* because it exhibits a high degree of functional and evolutionary homology within all bacteria and these sequences have been used for phylogenetic comparisons and classifications of microbial organisms (Drancourt et al., 2000; Gorkiewicz et al., 2003).

This research succeeded in the direct isolation of this bacterium which can be further refined by the above PCR technique.

In conclusion, the 229 bp 16Sr RNA sequence PCR is a highly sensitive and specific method for confirming the presence of *H. pylori* in human gastric tissues. Detection of *H. pylori* DNA by this approach may improve the clinical diagnosis and molecular epidemiological research of *H. pylori* infection.

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