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ORIGINAL ARTICLE

Prevalence of *Helicobacter pylori* infection and the incidence of *ureA* and clarithromycin resistance gene *23S rRNA* genotypes status in Saudi Arabia

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KEYWORD

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Abstract *Objectives:* To determine the prevalence of *Helicobacter pylori* ureA and clarithromycin resistance gene *23S rRNA* genotypes among *H*. pylori in Saudi Arabia.

Methods: A total of 100 serum and fecal samples from 70 patients and 30 healthy volunteers, from patients who presented with symptoms suggestive of gastritis or peptic ulcer disease, were taken from the main hospital in the Southern region of Saudi Arabia from September 2010 C.E. to March 2011 C.E. corresponding to Shawwal 1431 A.H. to Rabi Al-Thani 1432 A.H. We cultured the samples for *H. pylori* and a polymerase chain reaction was carried out to check for the presence or absence of ureA gene and clarithromycin resistance gene 23S rRNA genotypes.

Results: Among the 70 suspected patients, the suspected bacteria isolated from the fecal samples of 60, (85.7%) were positive using the culture techniques. The presence of ureA gene and clarithromycin resistance gene 23S rRNA was determined by using the polymerase chain reaction, Among the 100 fecal specimens, 65 fecal specimens from 70% patients showed positive results to clarithromycin resistance gene 23S rRNA (sensitivity, 93%; Specificity, 100% and Accuracy, 95%), Only 60 fecal specimens were positive with ureA gene (sensitivity, 86%; Specificity, 100% and Accuracy, 90%).

Conclusion: 23S rRNA gene was associated with clarithromycin resistance in *H. pylori*. There was a high prevalence of *H. pylori* resistance to clarithromycin in Saudi Arabia. *H. pylori* is a neutrophilic bacteria that has been able to colonize the human stomach by using a variety of acid-adaptive mechanisms as Urease activity that hydrolyzed the Urea producing 2 NH_3 and H_2CO_3.

1. Introduction

Helicobacter pylori (*H. pylori*) is a gram negative curved rod with a tuft of 4–7 polar flagella. It induces gastric mucosal inflammation, which may progress into peptic ulcers (Dunn et al., 1997). The *H. pylori,* has now been associated with gastritis, peptic ulcers, gastric adenocarcinoma, and gastric

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mucosa-associated lymphoid type (MALT) B-cell lymphomas. *H. pylori*'s helix shape was thought to have evolved to penetrate the mucoid lining of the stomach, more than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection was more prevalent in developing countries, and incidence decreased in Western countries (Yamaoka and Yoshio, 2008 and Brown, 2000). *H. pylori* bacterium is a spiral urease producing organism that lies in the interface between gastric epithelial cell surface and the overlying mucus gel. A variety of host factors and bacterial factors contribute to the pathogenesis of gastrointestinal diseases resulting from *H. pylori* infection. It was intensely antigenic and secretes various factors like urease, catalase, mucinase, lipase, hemolysin and alkaline phosphatase that decreases viscosity of the mucus. The production of catalase protects the bacteria against the toxic effects of reactive oxygen metabolites formed in neutrophils from hydrogen peroxide. The multiple polar flagella permit them to penetrate the mucous layer. Adherence of *H. pylori* to gastric epithelial cells and vacuolating cytotoxin are the virulence factors, as they were associated with degenerative changes in the epithelial cells (Aroori, 2001).

2. Methods

Serum and fecal samples were collected from 100 individuals, 70 of them suffered from clinical symptoms relating to the gastrointestinal tract (severe dyspeptic symptoms such as discomfort or pain or both, centered in the upper abdomen). Cases were taken from the clinic of Gastroenterology Surgery Department, Department of Internal Medicine, Department of Tropical Medicine and Department of Pediatric Gastroenterology of King Fahd Central Hospital in Jazan region in Saudi Arabia. A total of 70 patients (50 adults with a median age of 45 years [ranging from 22 to 68 years] and 20 children with a median age of 5 years [ranging from 4 to 12 years]) and 30 asymptomatic healthy volunteers with a median age of 45 years [ranging from 22 to 68 years] were included in the study. For culture and isolation of the organism, all samples were analyzed within 2 h of collection; otherwise they were held at −20 °C until processing.

To successfully isolate the organism from stool samples, the stool was diluted to a 20% w/v solution in phosphate-buffered saline (PBS) and the suspension sieved through a 250 μm strainer before plating onto selective media (Parsonnet et al., 1999). A fresh fecal sample was suspended in 0.1 mol/L sodium phosphate buffer to a final fecal slurry concentration of 20% w/v. The suspension was centrifuged at 15,000 g for 30 min and then resuspended in the buffer and a second centrifugation performed before plating onto selective growth media. Each fecal specimen was smeared on chocolate agar selective media.

Stool specimens were stored at −70 °C until they were used for DNA extraction. 220 mg of fecal samples was used as starting material. Following lysis, 100 μl of eluate was obtained from each sample. Each eluate was then purified, eliminating any RNA residue, by the addition of 5 μl of RNase A (10 mg/ml) followed by incubation at 60 °C for an hour (Dubois, 1995). Then 5 μl of eluate was used for the seminested PCR, while the remainder was stored at −20 °C.

For DNA extraction, one gram of stool from each patient was dissolved in 100% ethanol and chloroform and then centrifuged at 2,135×*g* and rinsed with acetone. The sample was then mixed with 8 M urea containing 1% sodium dodecyl sulfate, 20 mM Tris–HCl (pH 8.0), 100 mg of Chelex (Bio-Rad, Hercules, Calif.) and 50 mg of polyvinylpyrrolidone with subsequent incubation at 60 °C. The samples were then boiled and centrifuged at $469 \times g$. The supernatant was organically extracted, precipitated with alcohol, and redissolved with 0.7 M sodium chloride and 1% hexadecyltrimethylammoniumbromide (CTAB) (Sigma) for incubation at 65 °C. Organic extraction and alcohol precipitation were performed for subsequent RNase A (1 mg/ml; Sigma) and proteinase K (0.5 mg/ml; Bio-Rad) incubation at 58 °C for 2 h. Another round of organic extraction and alcohol precipitation was preformed with reconstitution in a solution of 3 mM Tris–HCl (pH 7.5) and 0.2 mM EDTA.

Stool DNA extracts were subjected to real-time PCR, which was performed in a LightCycler apparatus (Roche Diagnostics) using primers shown in (Table 1). Samples were run in duplicate and were considered positive if at least one of the reactions was positive.

3. Results

The suspected bacteria isolated from the fecal samples of 60 from 70 patients with symptoms and signs suggesting gastritis were cultured on chocolate agar plates and incubated for 7 days at 37 °C under microaerophilic conditions. The culture showed the typical morphology of *H. pylori*, it grows slowly,

Table 2 The result for *H. pylori* detection from 100 specimens by PCR technique.

	No	No. positive by PCR	
		23S rRNA	*ureA*
Symptomatic	70	65	60
Asymptomatic	30	0	0
Total	100	65	60
Sensitivity (%)		93	86
Specificity (%)		100	100
Accuracy (%)		95	90

Table 1 Oligonucleotide primers specific for *H. pylori* (23S rRNA, ureA) used for detection of *H. pylori*.

Primer	Sequence 5′–3′	Length (bp)	Position	References
ureA–F	CGTGGCAAGCATGATCCAT	77	2877–2895	GenBank accession No. M60398
ureA–R	GGGTATGCACGGTTACGAGTTT	77	2953–2932	GenBank accession No. M60398
23S-F	AGATGGGAGCTGTCTCAACCAG	121	2437–2458	GenBank accession No. U27270
23S-R	TCCTGCGCATGATATTCCC	121	2573–2555	GenBank accession No. U27270

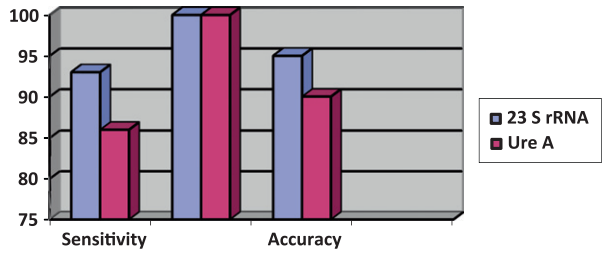

Figure 1 The relationship between Sensitivity, Specificity and Accuracy of urease gene and 23S rRNA sequencing using PCR analysis.

forming gray translucent colonies that look like spreading fluid droplets.

Among the 100 fecal specimens, 65 fecal specimens from 70 individuals showed positive results for clarithromycin resistance gene 23S rRNA (sensitivity, 93%; Specificity, 100%; and Accuracy, 95%), only 60 fecal specimens were positive with ureA (sensitivity, 86%; Specificity, 100%; and Accuracy, 90%), (Table 2 & Fig. 1).

4. Discussion

Spirals, gram negative bacilli resembling Campylobacter were found in patients with type B gastritis (Warren and Marshall, 1983). The organisms were originally classified as *Campylobacter* but were subsequently reclassified as a new genus, *Helicobacter*. The *Helicobacter pylori (H. pylori)*, has now been associated with gastritis, peptic ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid type (MALT) B-cell lymphomas. *H. pylori*'s helix shape was thought to have evolved to penetrate the mucoid lining of the stomach, more than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract, infection were more prevalent in developing countries, and incidence was decreased in Western countries (Yamaoka and Yoshio, 2008 and Brown, 2000). Urease production was a consistent finding in the *Helicobacter* species of humans that colonize the stomach but was uncommon in species found in the intestines (Murray et al., 2002). *H. pylori* bacterium is a spiral urease producing organism that lies in the interface between gastric epithelial cell surface and the overlying mucus gel. A variety of host factors and bacterial factors contribute to the pathogenesis of gastrointestinal diseases resulting from *H. pylori* infection. It was intensely antigenic and secretes various factors like urease, catalase, mucinase, lipase, hemolysin and alkaline phosphatase that decrease the viscosity of mucus. The production of catalase protects the bacteria against the toxic effects of reactive oxygen metabolites formed in neutrophils from hydrogen peroxide. The multiple polar flagella permit them to penetrate the mucous layer. Adherence of *H. pylori* to gastric epithelial cells and vacuolating cytotoxin are the virulence factors, as they are associated with degenerative changes in the epithelial cells (Aroori, 2001). 23S rRNA gene was associated with clarithromycin resistance in *H. pylori*. There was a high prevalence of *H. pylori* resistance to clarithromycin in Saudi Arabia (Mohamed et al., 1994).Countries in the Middle East such as Saudi Arabia have high prevalence of *H. pylori* and this could be the cause of high resistance to metronidazole and clarithromycin. High prevalence of *H. pylori* was reported by many authors previously. It was reported that 40% of the Saudi population in the age group of 5–10 years and 70% of people $\geqslant$ 20 years of age had *H. pylori*, which makes it one of the highest endemic areas in the world (Al-Moagel et al., 1990). The other reason of high prevalence of *H. pylori* resistance to metronidazole and clarithromycin was that Saudi Arabia has a large community of expatriates from the Far East and the Indian subcontinent where metronidazole and clarithromycin resistance were prevalent due to frequent usage of the drug for parasitic diseases and for common ailments such as diarrhea (Ahmad et al., 1997). The clarithromycin resistance was a prime concern for physicians who are using clarithromycin-based triple therapy as a primary regimen for ulcer patients infected with *H. pylori* (Dore et al., 2000).

In the present study, results of PCR were compared with *H. pylori* status. Sixty five fecal specimens from 70 individuals showed positive results for clarithromycin resistance gene 23S rRNA (Sensitivity, Specificity and Accuracy were 93%, 100% and 95% respectively), (Table 2 & Fig. 1). Only 60 fecal specimens were positive with ureA (Sensitivity, Specificity and Accuracy were, 86%, 100% and 90% respectively), (Table 2& Fig. 1).

PCR technique was excellent in sensitivity and specificity and allows determination of antibiotic sensitivities. PCR also provides a means of identifying mutations associated with antimicrobial resistance (De Francesco et al., 2006 and Lawson et al., 2005). RT-PCR with *H. pylori ureA* primers was less sensitive than RT-PCR with 23S rRNA primers in detecting *H. pylori*, possibly because of the lower quantities of *ureA* rRNA within each bacterial cell. These findings were consistent with the hypothesis that the amount of *H. pylori* urease production in vivo may be low (Blaser, 1993).

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