Genotyping of *Helicobacter pylori* cagA Gene from a Patient Who Failed Eradication Therapy: A Case Report and Review of the Literature

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ABSTRACT

***Helicobacter pylori* (*H. pylori*) is a bacterium that causes chronic gastritis, gastric and duodenal ulcers and gastric cancer. Here we report a female patient presenting with dyspepsia. She was tested positive six times by the Rapid Urease test and Urea breath test (UBT). A culture of gastric biopsy was done and the isolate showed resistance to Clarithromycin and Metronidazole while polymerase chain reaction (PCR) revealed the presence of cagA *H. pylori* virulence gene. Presence of cagA might not be a risk factor in development of Metronidazole resistance to antibiotic therapy. In conclusion, we report a female Malaysian Indian with cagA positive H. pylori infection, but experienced Metronidazole resistance to antibiotic therapy.**

KEY WORDS

***Helicobacter pylori*, CagA, antibiotic resistance**

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative microaerophilic bacterium that causes gastrointestinal diseases such as chronic gastritis, gastric and duodenal ulcers and gastric cancer[1-3]. *H. pylori* infection can be diagnosed by invasive (culture, rapid urease test, PCR and histology) and non invasive tests (serology, stool antigen test and ^{13}C-urea breath test (UBT)[4-6]. Invasive test has the advantage of being able to determine antibiotic- susceptibility and *H. pylori* genotypes[7].

In order to eradicate *H. pylori* infection, triple therapy using a proton pump inhibitor (PPI) with Clarithromycin and Amoxicillin or Metronidazole is recommended as the first-line treatment regimen. In case the triple therapy fails bismuth-containing quadruple therapy, which involves the inclusion of additional antibiotics to the first-line treatment regimen is used[8].

In Malaysia, Indians have been found to posses the highest prevalence of infection of about 68.9-75% as compared to Chinese 45.0-60.6% and Malays 8-43.3%[9,10].

A number of *H. pylori* virulence genes, including *cagA*, and *SabA* and have been associated with the most serious clinical outcomes and pathogenic bacteria[11,12].

CASE REPORT

This is 41 years old Malaysian female of Indian origin referred from Melaka Hospital for persistent dyspepsia. The patient was positive for *H. pylori* six times by Rapid urease test and UBT. The endoscopic finding was gastritis. She had a history of antiphosphollipid syndrome, bronchial asthma and dystunation uterine bleeding. Despite several treatment regimens the eradication therapy failed. Later on 20/2/2013 the patient underwent an upper gastrointestinal endoscopy at Hospital Kuala Lumpur and two gastric biopsies were taken for culture and sensitivity and Polymerase chain reaction (PCR).

The biopsy sample was put in brucella broth and immediately cultured onto Columbia agar supplemented with 8% sheep blood and dent

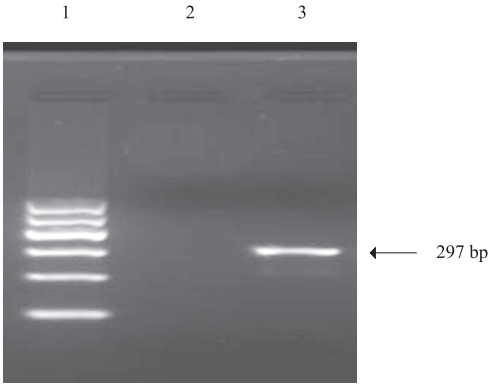

Figure 1. genotyping of cagA gene by PCR: lane 1, 100-bp DNA marker, lane 2 negative control without DNA, lane 3, *H. pylori* cagA positive strain.

Received on November 1, 2014 and accepted on February 18, 2015
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antibiotic. The plate was incubated at 37℃ for seven days under microaerophilic conditions. The isolate was confirmed to be *H. pylori* by Gram's stain and positive urease, catalase, and oxidase tests.

Minimum inhibitory concentration was determined by E-test strips. The isolate showed resistance to clarithromycin and metronidazole but was sensitive to Teicoplanin and Levofloxacin.

Genomic DNA extraction

DNA was extracted from biopsy tissue by use of the QIAamp DNA kit (Qiagen, Germany) according to the manufacturer's recommendations and stored at -20℃ until analysis.

PCR conditions and amplification of cagA gene

PCR amplification of *cagA* was carried out using two primer sets D008 (5'- ATAATGCTAAATTAGACAACTTGAGCGA-3') and R008 (5' TTAGAATAATCAACAAACATCACGCCAT-3')[13]. The amplification product of cagA gene was 297 base pairs (bp) in length.

The PCR reaction mixtures were prepared by using TopTaq Master Mix Kit (Qiagen, Germany) in a final volume of 25 µl containing 1.25 units TopTaq DNA polymerase, 1 X PCR buffer, 1.5 mM MgCl2 and 200 µM of each dNTP, 0.2 µM of each primer, 10 µl of molecular grade water and 2.5 µl of DNA. The mixtures were placed in a PCR thermocycler (Eppendorf, Germany). The PCR conditions included an initial denaturation of target DNA at 94℃ for 1 min, followed by 35 cycles of denaturation at 94℃ for 1 min, primer annealing at 58℃ for 1 min, extension at 72℃ for 1 min and final extension at 72℃ for 15 min.

PCR products were run on 1.5% agarose gels in TBE buffer according to the manufacturer's instructions and it revealed presence of *H. pylori* cagA virulence gene indicating 297 bp (Figure 1).

DISCUSSION

The incidence of antibiotic resistance differs from one geographic area to another. It is high in developing countries compared to developed countries[14]. In Malaysia Metronidazole is used as first-line therapy for *H. pylori* infection with highest resistance of (40%) reported[15]. High prevalence of Metronidazole resistance has also been reported in India (90%)[16].

The relationship between the success or failure of *H. pylori* eradication therapy and cagA status has been explained by the enhanced gastric mucosal inflammation. Patients having severe inflammatory cell infiltrations in the antral mucosa were associated with higher cure rates[17], therefore the presence of cagA might not be a risk factor in development of Metronidazole resistance[18]. In contrast, our case which is cagA positive is resistant to Metronidazole therapy.The continued resistance observed in this patient might be due to combination of bacterial, environmental and genetic factors. Further studies need to be done especially in areas where the resistance rate is high.

ACKNOWLEDGEMENT

This study was supported by University Sains Malaysia (USM) Research University grant 1001 / PPSP / 812108.

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