Direct detection of *Helicobacter pylori* from biopsies of patients in Lagos, Nigeria using real-time PCR—a pilot study

A. Ajayi¹, T. Jolaiya² and S. I. Smith¹, ³*

**Abstract**

**Objective:** Prompt diagnosis of *Helicobacter pylori* infection is essential for proper treatment and eradication of the pathogen because prolonged infection could lead to gastric cancer. Sensitive and cost effective diagnostic methods are key to guiding treatment options that will reduce mortality. This study was aimed at detecting *H. pylori* from biopsies of peptic ulcer patients. Real-time PCR using TaqMan and EvaGreen assays targeting 16S rRNA and *ureA* genes were used to detect *H. pylori* DNA extracted from 40 biopsy samples comprising 20 biopsies obtained from the antrum and 20 from the corpus of 20 patients undergoing endoscopy for duodenal ulcer investigation in Lagos, Nigeria.

**Results:** *H. pylori* was detected in 80% of the biopsy samples by combined cycle threshold (*Ct*) and melting temperature (*Tm*) values. Mean *Ct* value for *ureA* gene ranged from 21.40 to 37.53 and 22.71 to 35.44 for 16S rRNA gene. Average melting temperatures (*Tm*) of 81.57 and 82.90 °C among amplicons of *ureA* and 16S rRNA were observed respectively. *H. pylori* DNA was generally detected in biopsies collected from antrum and corpus. Real-time PCR in the diagnosis of *H. pylori* can be considered a simple, low cost and efficient alternative or addition to the gold standard.

**Keywords:** *Helicobacter pylori*, Diagnosis, Real-time PCR, Biopsy, Ulcer

**Introduction**

Gastritis, duodenal ulcer, gastric ulcer and in some cases gastric cancers are hallmarks of *Helicobacter pylori* infection. *H. pylori* is a Gram negative bacterium that colonize 50% of the stomach of humans globally [1, 2]. *H. pylori* possess several virulence factors including the production of urease that enable it successfully colonize the stomach where it can persist for a long period of time. The pathogen has been classified as a type 1 carcinogen hence, its persistence in infection without eradication may lead to chronic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer [3–5]. *H. pylori* is a fastidious bacterium making it very delicate to culture. Although non-invasive methods of detecting *H. pylori* exist [6], culture remains the gold standard which requires competence and a lot of materials making it expensive. Detection of *H. pylori* DNA directly from biopsies by molecular methods especially PCR have been reported by several workers [7, 8] with excellent sensitivity and specificity. In Nigeria, the use of conventional PCR in the detection of *H. pylori* DNA from biopsies have been demonstrated [9, 10]. However, the use of real-time PCR in the detection of *H. pylori* in biopsies have not been explored in the country. The purpose of this study was to detect *H. pylori* DNA isolated from gastric biopsies (corpus and antrum) obtained from patients in Lagos Nigeria.

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Main text
Samples used for this study were biopsies obtained from 20 patients undergoing endoscopy for duodenal ulcer investigation in Lagos, Nigeria. A total of 40 biopsies comprising 20 obtained from the corpus and 20 from the antrum were analysed.

Methodology
DNA was extracted from gastric biopsies (corpus and antrum) and H. pylori reference strain J99 (NCBI:txid85963) with QIAamp DNA Mini Kit (Qia-gen, Hilden, Germany) according to manufacturer’s instructions.

Detection of H. pylori was done by singleplex real-time PCR amplifying fragments of 16S rRNA and ureA genes using specific primers and probes listed in Additional file 1: Table S1 with slight modification (quencher of probes was Black Hole Quencher (BHQ) instead of TAMARA). Detection of H. pylori targeting the two set of primers was first validated by using H. pylori DNA extracted from reference H. pylori strain J99 (NCBI:txid85963) and DNA extracted from Salmonella Typhimurium ATCC 14028, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 29522. A standard curve of the assay was determined as serial dilution of DNA from H. pylori J99 positive control which was prepared with final concentration of 10¹–10⁵; reactions were ran in triplicate. The curve was determined by plotting threshold cycle (Ct) value against count of log DNA copies from which positive samples were considered as amplification plot with Ct values <40. Real-time PCR was carried out in a StepOne Real-Time PCR system (Applied Biosystem, Singapore). A 20 µL reaction was used in both the TaqMan and EvaGreen assays which contained 11.2 µL nuclease free water, 4 µL of Solis Biodyne 5 × HOT FIREPol® Probe qPCR Mix Plus (for TaqMan assay) and 5X HOT FIREPol® EvaGreen Supermix (Solis Biodyne Tartu, Estonia), 0.4 µL (10 µM) each of both forward and reverse primers and 4 µL of (20 ng/µL) DNA template. PCR cycling parameters for the EvaGreen assay were an initial denaturation at 95 °C for 12 min and 40 cycles of denaturation at 95 °C for 15 s, annealing for 1 min at 54 °C for 16S rRNA gene, 55 °C annealing temperature for ureA gene, extension at 72 °C for 20 s and a melting step. While PCR cycling parameters for the TaqMan assay were initial denaturation at 95 °C for 12 min, denaturation at 95 °C for 15 s, annealing/elongation at 54 °C for 16S rRNA gene and 55 °C for ureA gene both for 1 min. After which amplification results were analysed with StepOne Software v.23 and GraphPad Prism software version 5 (GraphPad Software, LA Jolla, CA, USA).

Results
Real-time PCR assay for the detection of H. pylori from genomic DNA extracted from biopsies and extracted DNA from H. pylori reference strain J99 (NCBI:txid85963) showed amplification curves for all targeted genes (ureA and 16S rRNA). However, there was no amplification with DNA extracted from other bacterial pathogens (Salmonella Typhimurium ATCC 14028, Staphylococcus aureus ATCC 29,213, Pseudomonas aeruginosa ATCC and Escherichia coli ATCC 29522) as shown in Fig. 1 indicating specificity of primers and probes used.

Eighty percent of the samples were positive for H. pylori. However, H. pylori was detected in the antrum of two patients but was not detected in the corpus. The reverse was observed in a third patient in which H. pylori was detected in the corpus but absent in the antrum. The logarithm of the number of H. pylori DNA copies in the samples correlated largely well with the ureA and 16S rRNA threshold cycle (Ct) values which is a representation of the DNA copy number in the PCR reaction. Mean Cₜ value for ureA gene ranged from Mean±SD: 21.40±15.14 to 37.53±0.89 and for 16S rRNA gene Mean±SD: 22.71±0.12 to 35.44±0.87 as shown in Figs. 2 and 3. Correlation coefficient (R²) and amplification efficiency (E) was 0.994/90.35%, 0.997/97.94% for ureA and 16S rRNA respectively.

The intercalating chemistry EvaGreen used showed smooth melting curve (Additional file 2: Figure S1) and average melting temperatures (Tₘ) of 81.57 and 82.90 °C among amplicons of ureA and 16S rRNA respectively.

Discussion
Helicobacter pylori infection remains a major public health issue around the world. The prevalence of H. pylori infection in Nigeria is estimated at about 87.7% which indicates a high infection burden [1] making prompt, accurate and efficient diagnosis imperative. The PCR results for the detection of H. pylori from biopsies and other bacterial pathogens indicated the reliability of using the H. pylori ureA and 16S rRNA primer set in this study. Eighty percent of the samples were positive for H. pylori with the assay showing great efficiency in detecting small quantities of H. pylori DNA. Correlation coefficient (R²) and amplification efficiency (E) of both ureA (0.994/90.35%) and 16S rRNA (0.997/97.94%) proved to be good. Hence it could be inferred that these targets had great specificity since all H. pylori strains harbour the gene that encodes urease. In the study of Ramírez-Lázaro et al. [11], they suggested the combination of both 16S rRNA and ureA genes in the diagnosis of H. pylori from biopsies for better sensitivity. However, in the
study by Beer-Davidson et al. [12] in which they reported the detection of *H. pylori* in stool samples of children in Israel using real-time PCR targeting urease gene, it was observed that the gene gave a clearer amplification curve compared to 16S rRNA gene. In this study, the mean temperature separation between *ureA* (81.57 °C) and 16S rRNA (82.90 °C) was 1.33 °C. Thus it could be asserted that amplification products of *ureA* gene could easily be
distinguished from 16S rRNA even in a multiplex qPCR reaction. Contreras et al. [13] reported a melting temperature range between 57.0 and 57.4 °C that enabled them detect 16S rRNA single mutation associated with antibiotic resistance in H. pylori strains isolated from biopsies in Venezuela compared to a higher Tm that was observed in wild type strains. The detection of H. pylori in biopsies collected from the antrum and corpus is widely reported. Pichon et al. [14], reported the detection of H. pylori in both biopsies of antrum and corpus obtained from patients with H. pylori infection. However, in this present study there was zero detection of H. pylori in the corpus of two patients in which H. pylori was detected in their antrum biopsy samples. Similarly, in one patient, H. pylori was detected in corpus biopsy but absent in the antrum. Palamides et al. [15] reported similar findings in which H. pylori was detected in biopsies from either antrum or corpus of some patients using conventional PCR. This suggest that there are variations in the distribution of H. pylori in the gut of H. pylori infected patients. Lan et al. [16] reported in their study that corpus biopsy enhances the detection of H. pylori infection. Similarly, Latif et al. [17] opined that in addition to antral biopsy, corpus biopsy increases the sensitivity in the detection of H. pylori infection.

**Conclusion**

Real-time PCR in the diagnosis of H. pylori can be considered an alternative or in addition to the gold standard and including histology since it relies on the detection of DNA isolated from biopsies and not necessarily viable bacteria coupled with its competitive cost [11]. Furthermore, direct detection of H. pylori from biopsies can circumvent the difficulty and extended time lapse encountered with culture.

**Limitation of study**

It would be difficult to make far reaching conclusion as the number of samples in this study are few and other diagnostic methods such as histology and culture were not evaluated alongside real-time PCR. Hence, future study should increase the number of samples and evaluate other diagnostic methods alongside.

**Abbreviations**

ATCC: American Type Culture Collection; BHQ: Black Hole Quencher; Ct: Cycle threshold; MALT: Mucosa-associated Lymphoid Tissue; Tm: Melting Temperature.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s13104-021-05505-y.

**Additional file 1:** Table S1. Primers used in the detection of H. pylori by TaqMan and EvaGreen qPCR assay.

**Additional file 2:** Figure S1. Melting curve of EvaGreen real-time PCR targeting a 16S rRNA b ureA. Melting peaks were derived by the plot of derivative reporter (∆R) against temperature (°C).
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Authors' contributions
Conception and design: SSI; Laboratory experiment: AA; JT; Result Analysis and manuscript writing/review: AA; JT; SSI. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
Written consent was obtained from participants before they will recruited for the study and ethical approval was obtained from Institutional Review Board of the Nigerian Institute of Medical Research with number IORG0002656.

Consent for publication
Not applicable.

Competing interests
There is no conflict of interest.

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