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• BRIEF REPORTS •

Comparison of three PCR methods for detection of *Helicobacter pylori* DNA and detection of *cag*A gene in gastric biopsy specimens

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Abstract

AIM: To comparatively evaluate PCR and other diagnostic methods (the rapid urease test and / or culture) in order to determine which of the three PCR methods (*ureA*, *glmM* and 26-kDa, SSA gene) was most appropriate in the diagnosis of *Helicobacter pylori* (*H pylori*) infection and also to evaluate the detection of a putative virulence marker of *H pylori*, the *cag*A gene, by PCR in biopsy specimens.

METHODS: One hundred and eighty-nine biopsy specimens were collected from 63 patients (three biopsies each) undergoing upper gastroduodenal endoscopy for various dyspeptic symptoms. The PCR methods used to detect *H pylori* DNA directly from biopsies were the *glmM*, 26-kDa, *ureA* and then *cag*A was used to compare the culture technique and CLO for urease with the culture technique being used as the gold standard.

RESULTS: Thirty-five percent of the biopsies were positive for *H pylori* DNA using the 3 PCR methods, while 68% of these were positive for the *cagA* gene. Twenty-four percent of the biopsies were negative for *H pylori* DNA in all PCR methods screened. The remaining 41% were either positive for *ureA* gene only, *glmM* only, 26-kDa only, or *ureA* + *glmM*, *ureA* + 26-kDa, *glmM* + 26-kDa. Out of the 35% positive biopsies, 41% and 82% were positive by culture and CLO respectively, while all negative biopsies were also negative by culture and *cagA*. Cag A+ infection was also predominantly found in *H pylori* DNA of the biopsies irrespective of the clinical diagnosis.

CONCLUSION: This method is useful for correctly identifying infections caused by *H pylori* and can be easily applied in our laboratory for diagnostic purposes.

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INTRODUCTION

Culture has been for long the method of choice to detect infectious agents. However, for some organisms that are growing slowly like *Helicobacter pylori* (*H pylori*), it may take several days to obtain a result. Furthermore, culture is very much dependent on infrastructure conditions and in developing countries may be jeopardized by shortage in electrical power supply.

Recently, assays based on PCR technology have been developed to detect the presence of microbial DNA, including *H pylori* DNA, by using several gene targets directly from the biopsies[1-3]. The targets of these PCR methods include the urease A (*ureA*) gene[4], the 26-kDa species-specific antigen (SSA) gene[5] and the phosphosamine mutase (*glmM*) gene[3] to mention a few. It can be standardized to diagnose different agents. With such methods, all the experiments are not lost in case of shortage of power supply as they can be easily repeated. A similar approach has been applied in difficult environments such as in Russia.

The present study was therefore aimed (i) to comparatively evaluate PCR and other diagnostic methods (the rapid urease test and / or culture) in order to determine which of the three PCR methods was most appropriate in the diagnosis of *H pylori* infection, and to evaluate the detection of a putative virulence marker of *H pylori*, the *cag*A gene, by PCR in biopsy specimens.

MATERIALS AND METHODS

Patients

A total of 189 specimens from 63 patients (three biopsies each) undergoing upper gastroduodenal endoscopy for various dyspeptic symptoms were included in this study and 3 biopsies each were taken from the antrum of the patients for CLO test, culture and DNA, respectively.

The specimens were obtained from four centres in Nigeria: Lagos University Teaching Hospital (LUTH), Lagos; Mount Pleasant Medical and Endoscopy clinic, Ojuelegba, Lagos; University College Hospital, Ibadan; and Obafemi Awolowo Teaching Hospital Complex (OAUTHC), Ile-Ife.

Bacterial strains

Twelve *H pylori* isolates were used in this study: one *cag*A-positive reference strain (26695), and 11 clinical isolates.

Other strains tested included local isolates of *Campylobacter jejuni* (4), *C. coli* (4) and *C. fetus* (3). The strains were tested by PCR to assess the specificity of the primers.

DNA extraction from biopsies was by the method of Marais *et al*.[6]. Briefly, the biopsy samples were ground and centrifuged for 5 min at 10 000×g. The pellet was resuspended in 300 μL extraction buffer (20 mmol/L Tris-HCl, pH 8.0; 0.5% Tween 20) and proteinase K (0.5 mg/mL final concentration). The mixture was incubated at 56 ℃ for one hour after which

Table 1 Conditions for four different PCR methods

Target (reference), nucleotide (nt) positions amplified, and size of PCR products	Primer names and sequences	PCR conditions
26-kDa SSA gene (5),	Primer 3, 5'-TGGCGTGTCTATTGACAGCGAGC-3'	98 ℃, 10 min (1cycle);
nt 474–776, 303 bp	Primer 4, 5'-CCTGCTGGGCATACTTCACCAG-3'	92 ℃, 30 s; 68 ℃, 1 min (37cycles); 92 ℃, 30 s 68 ℃, 1 min; 72 ℃, 2 min (6 cycles)
Urease A gene (4),	HPU1, 5'-GCCAATGGTAAATTAGTT-3'	94 ℃, 1 min; 45 ℃, 1 min
nt 304 – 714, 411 bp	HPU2, 5'-CTCCTTAATTGTTTTTAC-3'	72 ℃, 1 min (35 cycles)
*glm*M gene (3)	Forward primer, 5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3'	93 ℃, 1 min; 55 ℃, 1 min;
nt 784–1 077, 294 bp	Reverse primer, 5'-AAGCTTACTTTCTAACACTAACGC-3'	72 ℃, 1 min (35 cycles)
CagA gene	Primer 1, 5'-CCATGAATTTTTGATCCGTTCGG-3'	94 ℃, 1 min, 58 ℃, 1 min; 72 ℃, 1 min (40 cycles)
nt 394 bp	Primer 2, 5'-GATAACAGGCAAGCTTTTGAGGGA-3'	

the enzyme was inactivated by boiling for 10 min.

Five μL of DNA was used as the template for each PCR. Each sample was examined by four different PCRs. Primers used in this study were from, 26-kDa SSA gene (303 bp), urease A gene (411 bp), *glm*M gene (294 bp) and the *cag*A1 gene sequence (394 bp).

PCR reaction was carried out in a 50 μL volume in GeneAmp 9700 (Perkin Elmer). The primer sequences, conditions and sizes of these PCR methods are listed in Table 1.

Detection of amplified DNA products

A volume of seven μL of each PCR mixture was subjected to gel electrophoresis (1%) and ethidium -bromide staining for the detection of amplified DNA products.

RESULTS

Specificity of PCR assays with bacterial isolates

The specificity of PCR primers targeting *ure*A, *glm*M, 26-kDa and *cag*A gene was determined by testing 12 bacterial strains from related genus.

The 26-kDa PCR amplified the expected 303-bp fragment from the reference *H pylori* strain, while none from the other *Campylobacter* strains was amplified. Likewise, the *glm*M gene amplified the expected 294-bp fragment, *ure*A, 411-bp fragment and *cag*A, 394-bp fragment in *H pylori* reference and not in *Campylobacter* spp.

Detection of the three genes by PCR

Out of the 63 biopsies screened, 22(35%) were positive in all three PCR methods (26-kDa, *glm*M, *ure*A), 11 were positive for *ureA*, five for *glmM*, two for 26-kDa, two each for *ureA* and *glmM* and one each for *glmM*/ 26-kDa and *ureA* /26-kDa. Fifteen (24%) of the biopsies screened were negative for all three genes screened. Of these, 22 were positive for *H pylori* DNA, only nine (41%) were culture positive (Table 2). All negative biopsies from the 3 genes were negative by culture and CLO test. Two culture negative biopsies were positive in all three genes screened.

Detection of the cagA gene

Of the 22 biopsies that showed positive amplification in all three genes, 15(68%) were positive for the *cag*A gene. These comprised three biopsies from patients with cancer positive for the cagA gene. The patients with normal findings also had two out of three biopsies positive for the *cag*A gene. Out of the ten biopsies screened from patients with duodenitis or duodenal ulcer, only three (30%) were negative for the *cag*A gene (Table 3). All the biopsies that were positive for *cagA* were also positive for their corresponding isolates. All negative biopsies were also negative for *cag*A gene.

Table 2 Results of three PCR methods and cagA gene for the detection of *H pylori* from gastric biopsy

Biopsy (*n*=63)	*ureA*	*glmM*	26-kDa	Cag A +	Culture	CLO
22	+	+	+	15	9+, 13-	18+, 4-
11	+	-	-	-	11-	3+, 8-
5	-	+	-	-	5 -	3+, 2-
2	-	-	+	-	2 -	2-
4	+	+	-	-	4 -	2-, 2+
2	+	-	+	-	1+, 1-	2 +
2	-	+	+	-	1+, 1-	2 +
15	-	-	-	-	15 -	15 -

–: negative, +: positive.

Table 3 Positive and negative predictive values of three different PCR methods

Value	Results [(%, No. of samples with value / total No.)] for PCR method		
	ureA gene	*glmM* gene	26-kDa gene
Positive predictive[1]	91 (10 / 11)	91 (10 / 11)	100 (11 / 11)
Negative predictive[2]	44 (23 / 52)	56 (29 / 52)	67 (35 / 52)

[1]Compared with 11 culture- positive samples, [2]Compared with 52 culture- negative samples.

DISCUSSION

The *ureA* gene PCR had a very poor specificity in our study, as it amplified 29 of the 52 (npv=44%) culture negative biopsy specimens. This was contrary to the report by Lu *et al*.[1], but they concluded that the sensitivity was unsatisfactory and could be due to sequence polymorphism in the loci.

However, the positive predictive value was 91%. The 26-kDa gene primer amplified all 100%(11/11) (ppv=100%) of *H pylori* culture positive biopsy samples and produced 17 false positive results on 52 culture negative specimens (npv=67%) (Table 2).

The *glmM* gene PCR amplified 10 out of the 11 culture positive biopsy specimens (ppv, 91%), with a low sensitivity, as 23 of 52 culture negative biopsy specimens (npv, 56%) were amplified.

Lage *et al*.[2] however, reported in their study that there

were no false positive or negative biopsies amplified by the *glmM*. The reason for this is quite obvious as there are no problems of power outages in their environment and so it is easy to culture *H pylori* as a result of the steady power supply as opposed to our environment where constant power outages threaten the isolation of *H pylori*.

A comparison of the urease test using the CLO test kit (Delta West, pty, Australia) showed that a total of 25 biopsies positive for urease test were negative by culture. This was possible as a result of the fact that the CLO test kit could detect the presence of *H pylori* even when they were very small, while when there was power outage the possibility of detecting the organism was small. In addition, occassionally the biopsy forcep could be contaminated during the passage of the endoscope in the stomach, resulting in growth of some other urease positive organisms from the biopsies[7]. Another general explanation for the poor specificity of all tests compared to culture was as a result of incessant power outages in our country, thus decreasing the possibility of isolating *H pylori* (a fastidious organism) by culture.

Sixty-eight percent of the biopsies that were positve for all three PCR methods were positive for *cagA*. The presence of *cagA*, a virulence factor, was found to be common irrespective of the clinical diagnosis, similar to a previous study by Smith *et al*.[8].

In conclusion, the 26-kDa gene, was found to be the most appropriate of the three different PCR methods for the detection of *H pylori* from biopsies. The study also showed that PCR had a potential value for studying *cagA* and possibly other virulent factors directly from biopsies, although it might not be important in rapidly detecting a patient that is at high risk of peptic ulcer since the frequency is similar to those of non-ulcer dyspepsia and more importantly the method could be adapted for our environment, where there is constant power outage.

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