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Análise comparativa entre alvos moleculares para detecção do *Helicobacter pylori*

Comparative analysis of molecular targets for Helicobacter pylori detection

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Chronic active gastritis and other gastric disorders can be associated with *Helicobacter pylori* infection. Detection of the *H. pylori* in clinical samples by molecular methods can provide more accurate results than conventional methods, such as, histology, rapid urease test and urea breath test.[1] It have been proposed the *ureA*, *glmM* and *hsp60* as main targets to Polymerase Chain Reaction (PCR) for *H. pylori* detection.[2] This study aimed to evaluate the accuracy ofthese three genes as target for the detection of *H. pylori*, using as gold standard the combination between histology and in-house urease test.[3] It was analyzed gastric biopsy specimens obtained from 95 patients submitted to endoscopyin the Integrated Center for Gastroenterology of the Hospital Dr. Miguel Riet Corrêa Jr., Rio Grande (RS), Brazil. This study was approved by the Research Ethics Committee (FURG - process number: 23116.001044/2011-16). Informed consent was obtained from all patients.

Gastric biopsy specimens were processed for histological examination and evaluated according to the updated Sydney system of classification and grading of gastritis.[4,5] The in-house urease test was carried out as previously described.[3] The gastric biopsies used for PCR were maintained in 1.0 mL of Brain Heart Infusion broth (Acumedia®, USA) with 20% glycerol after collection and stored at -70 °C for further DNA extraction, as described previously.[6] *ureA*, *glmM* and *hsp60* were used as target for the detection of *H. pylori*. The primers used to PCR, conditions and the size of the amplified fragments are listed in Table 1.[7-9] Amplification of the *hsp60* was done by nested protocoland of the *ureA* and *glmM* was carried out by single step PCR. Amplicons were visualized by electrophoresis in agarose gel 1.5%, stained with ethidium bromide and examined under UV illumination.

Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracyand degree of agreement by Kappa test were calculated for each of the testing methods, using as the gold standard a positive result in both histology and in-house urease test, as previously stated.

Based on the gold standard used in this study 34/95 (36%) patients were infected with *H. pylori*. The sensitivity, specificity, and accuracy of the *ureA*, *glmM*, and *hsp60* comparing to the gold standard are showed in table 2. The *glmM* and *hsp60* were the most sensitive methods (97% and 100%, respectively). While *urea* was the most specific test (95%), the *hsp60* showed the lowest specificity (7%). PCR with *ureA* and *glmM*as target showed accuracy of 93% and 95%, respectively. Although *ureA* and *glmM* are conserved gene the difference of sensitivity found in this study could be related to sequence polymorphism in the *ureA* loci or to heterogeneity of clones in the same patient.[8]

Our study showed a sensitivity of 100%, specificity of 7% and accuracy of 40% when *hsp60* was used as target. In contrast, a previous study tested the same primers

Table 1. Primers and PCR conditions used for *H. pylori* detection.

Genes	Primers (5'-3')	PCR Conditions	PCR product size	Reference
Urea	UREA1 (GCCAATGGTAAATTAGTT) UREA2 (CTCCTTAATTGTTTTTAC)	94°C-5 min; 94°C-60s, 45°C-60s, 72°C-60s (35 cycles); 72°C-7 min	394 bp	7
GlmM	GLM MF (GGATAAGCTTTTAGGGGTGTTAGGGG) GLM MR (GCATTCACAAACTTATCCCCAATC)	94°C-10 min; 94°C-60s, 58°C-60s, 72°C-60s (35 cycles); 72°C-10 min	140 bp	8
*hsp*60	HSP1 (AAGGCATGCAATTTGATAGAGGCT) HSP2 (CTTTTTTCTCTTTCATTTCCACTT)	94°C-30s, 56°C-30s, 72°C-30s (30 cycles)	609 bp	9
*hsp*60	HSPN1 (TTGATAGAGGCTACCTCTCC) HSPN2 (TGTCATAATCGCTTGTCGTGC)	94°C-30s, 56°C-30s, 72°C-30s (30 cycles)	501 bp	9

and showed that nested amplification targeting *hsp60* gene was the most sensitive and specific, with 100% of PPV and NPV.[9] The very low specificity observed in our study for *hsp60* can be due that the of the primers used are presents in the human genome, beside *H. pylori*. In order to confirm this hypothesis, we performed an *in silico* analysis in the Basic Local Alignment Search Tool (BLAST) and we verified that theof the primers used beside to annealing in the *H. pylori* genome (Accession no. NC-0091; gene ID 899089), also annealing in the human genome, specifically in chromosomes 1 (Sequence ID: ref|NC_0189122), 2 (Sequence ID: ref|NC_0189132) and 12 (Sequence ID: ref|NC_0189232). For check this information by wet lab experiments, we selected randomly four DNA samples from cervicovaginal brush collected to perform *hsp60* amplification with same primers used to detect *hsp60* in *H. pylori*. We found in all of them anamplified fragment of 501bp, which is equivalent to the expected length of amplicon. This result suggests that these primers could be used with DNA samples extracted from culture, but not from clinical samples.

On the other hand, the PPV and NPV (Table 2) of *ureA* and *glmM* indicated good acuracy, with reduced risk of false-positive or false-negative results. Regarding the results of Kappa test (Table 3), the *ureA* and *glmM* showed an excellent agreement with the gold standard (0.839 and 0.888, respectively), while, *hsp60* (0.048) indicated a very low agreement.

Table 3. Kappa value and p-value of each test according with the gold standard (association between histology and in-house urease test).

Agreement	Kappa value	*p* - value
gold standard with *ureA*-PCR	0.839	<0.001
gold standard with *glmM*-PCR	0.888	<0.001
gold standard with *hsp60*-PCR	0.048	0.127

In conclusion, for detection of *H. pylori* in gastric biopsy the *glmM* was the most accurate target studied, and this is inagreement with findings by Luet al(1999).[10] Therefore we can consider the *glmM* gene PCR to be the most appropriate PCR method for detection of *H. pylori* in gastric clinical specimens.

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Table 2. Comparative evaluation of *ureA*-PCR, *glmM*-PCRand *hsp60*-PCR considering the combination of histology and in-house urease test as gold standard.

Gold Standard	***ureA*-PCR**		***glmM*-PCR**		***hsp60*-PCR**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
	30	4	33	1	34	0
	3	58	4	57	57	4
	%		**%**		**%**	
Sensitivity	88		97		100	
Specificity	95		93		7	
PPV	91		89		37	
NPV	93		98		100	
Accuracy	93		95		40	

PPV: positive predictive value; NPV: negative predictive value

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