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ORIGINAL ARTICLE

Detection of Helicobacter Pylori in Saliva and Oral Swab Versus Biopsy Samples Using Urease Test, Culture, PCR Technique

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ABSTRACT

The aim of this study was to compare between different methods in identifying Helicobacter pylori in saliva, oral swabs and biopsy specimens obtained from patients' chronic gastritis and peptic ulcer disease.

Methods: A total of 100 tissue biopsy, saliva and oral swab samples were collected and tested for identification of H. pylori using urease test, culture media and PCR for detection of 16sRNA and virulence CagA and VagA genes.

Results: It was found that the biopsy results in all methods have high percentage 85% with the exception of CagA gene that gave a high percentage 71% in saliva samples, there were 82.05% urease positive using saliva sample and 43.59% using swab sample, 59.65% positive saliva culture and 38,60% positive swab culture, regarding 16sRNA, there were 70.59% saliva sample positive and 43,53% positive swab sample. H pylori detection by cag A gene of saliva in comparison with biopsy was 87.50% and of oral swab 41.67%, however by VagA gene the result of saliva was 31.25% and of oral swab was 16.67%.

Conclusion: All detection method that done by using biopsy sample more accurate and give reliable result. Saliva sample using in detection H.pylori is attractive for use in population based prevalence surveys for H. pylori infection.

Keywords: H. pylori, saliva, oral swab ,16SrRNA, VacA,GagA

INTRODUCTION

H. pylori is a Gram-negative, microaerophilic bacterium found in the stomach and associated with chronic gastritis, peptic ulcers, atrophic gastritis, intestinal metaplasia, gastric adenomas. (Hooi et al., 2017)

H. pylori infection can be diagnosed by invasive techniques requiring endoscopy and biopsy (histological examination, culture, polymerase chain reaction) and by noninvasive techniques (serology, urea breath test, detection of H. pylori antigen in stool specimen) (Machado, 2002).

The first documentation of the presence of H. pylori in the oral cavity was reported in 1989, when the bacterium was cultured from the dental plaque of one of 29 patients with H. pylori associated gastric disease (Krajden et al., 1989). Since then, some reports indicated that Helicobacter may be present in oral cavity (particularly gingival pockets) which can serve as a reservoir for bacteria and a source of gastric reinfection (Dowsett, Kowolik, & Medicine, 2003).

Many virulence-associated genes of H. pylori, including vacuolating cyto-toxin gene a (vacA), cytotoxin-associated gene a (cagA). (Talebi Bezmin Abadi et al., 2013)

The only known reservoir is the human stomach (Schwarz et al., 2008), the bacterium appears to travel to its host through direct contact with another human or by way of a contaminated environment (Selgrad, Kandulski, & Malfertheiner, 2009) .

The saliva is another possible source of H. pylori, since the gastric microbiome can reach and colonize the mouth after regurgitation or vomiting. (Gebara et al., 2006).

MATERIALS AND METHODS

In this cross-sectional study, one hundred patients suffering from severe epigastric pain from Baquba teaching hospital was involved. A total of 100 tissue, saliva and oral swab samples were collected during a period from October 2020 to March 2021. Patients admitted to gastrointestinal center, Aged (18-80) years and from both sexes

Sample Collection: Tissue biopsy samples: Subjects were advised to fast for overnight before endoscopy. Biopsy taken by specialist physician, three tissue biopsies were obtained from antrum. A rapid urease test and culture skirrows media was performed on one of the antral biopsies, the second one was sent to histological lab for cytological study and the third one was placed in 1 ml of normal saline and preserved at -20°C for molecular analysis. Three ml of saliva taken from patients and put in small capped jar for another test. Oral swab

was taken from patient and added to 1ml of normal saline

Molecular Methods

DNA Extraction: Genomic DNA extraction from tissue, saliva and swab was isolated according to the protocol of ABIOpure Extraction

Gene amplification by conventional polymerase chain reaction.

Primers: Three sets of published primers (table1) were used in this study to amplify the corresponding fragments of vacA cagA by nested-PCR and16s rRNA gene by conventional PCR. The PCR program conditions(Al Thwai & Ali, 2013; Huang Y, 2009; Tirapattanun A, 2016) were set in thermal cycler

Nested PCR was done for amplification of H.pylori vag A and cagA genes

Agrose Gel Electrophoresis: Gel was prepared and PCR product was Examined in U. V. trans illuminator and then photographed with a camera (Flowgen, UK)

Statistical Methods: The Statistical Analysis was done by using package for social sciences (SPSS) 21.0software and Microsoft Excel 2013. Categorical data were described as count and percentage. Chi-square test or Fisher exact test was used to describe the association between variables.

Table 1: Comparison between different H. pylori diagnostic methods using different clinical samples.

		Saliva		Biopsy		Swab	
		Count	%	Count	%	Count	%
Urease	Positive	64	64	78	78	34	34
	Negative	36	36	22	22	66	66
Culture	Positive	38	38	57	57	23	23
	Negative	62	62	43	43	77	77
16sRNA	Positive	62	62	85	85	38	38
	Negative	38	38	15	15	62	62
CagA	Positive	71	71	48	48	34	34
	Negative	29	29	52	52	66	66
VacA	Positive	25	25	48	48	13	13
	Negative	75	75	52	52	87	87

RESULTS

Table 4 illustrate the sensitivity, specificity, positive predictive value and negative predictive value of culture from saliva and swab samples in comparison with biopsy.

Table 2: Sensitivity and specificity of urease test for identification of H. pylori using different samples.

		Urease test (biopsy)		Sensitivity	Specificity	PPV	NPV
		Positive N=78	Negative N=22				
Urease test (Saliva) N=64	Positive	64	0	82.05%	100.00%	100.00%	61.11%
	%	82.05%	0.00%				
	Negative	14	22	72.10 - 89.00	85.13 - 100	94.34 - 100	44.86 - 75.22
	%	17.95%	100.00%				
Urease test (Swab) N=34	Positive	34	0	43.59%	100.00%	100.00%	33.33%
	%	43.59%	0.00%				
	Negative	44	22	33.14 - 54.64	85.13 - 100	89.85 - 100	23.16 - 45.34
	%	56.41%	100.00%				
Total		78	22				

Table 3: The association between urease test with age group, gender using different clinical samples.

		Urease test (Saliva)		Urease test (Biopsy)		Urease test (Swab)	
		Positive	Negative	Positive	Negative	Positive	Negative
Age groups	<21 years	8	2	10	0	2	8
		80.0%	20.0%	100.0%	0.0%	20.0%	80.0%
	21-30 years	5	6	5	6	5	6
		45.5%	54.5%	45.5%	54.5%	45.5%	54.5%
	31-40 years	17	6	21	2	9	14
		73.9%	26.1%	91.3%	8.7%	39.1%	60.9%
	41-50 years	14	10	16	8	10	14
		58.3%	41.7%	66.7%	33.3%	41.7%	58.3%
	51-60 years	12	6	16	2	6	12
		66.7%	33.3%	88.9%	11.1%	33.3%	66.7%
	>60 years	8	6	10	4	2	12
		57.1%	42.9%	71.4%	28.6%	14.3%	85.7%
p value		.492		.009		.435	
Sex	Female	39	24	49	14	25	38
		61.9%	38.1%	77.8%	22.2%	39.7%	60.3%
	Male	25	12	29	8	9	28
		67.6%	32.4%	78.4%	21.6%	24.3%	75.7%
		.569		.944		.118	

Result of Culture in Skirrows Media: Table 4: illustrate the sensitivity, specificity, positive predictive value and negative predictive value of culture from saliva and swab samples in comparison with biopsy.

Table 4: Sensitivity and specificity of culture for identification of H. pylori using different clinical samples.

		Culture (biopsy)		Sensitivity	Specificity	PPV	NPV
		Positive N=57	Negative N=43				
Culture (Saliva) N=38	Positive	34	4	59.65%	90.70%	89.47%	62.90%
	%	59.65%	9.30%				
	Negative	23	39	46.70 - 71.38	78.40 - 96.32	75.87 - 95.83	50.46 - 73.84
	%	40.35%	90.70%				
Culture (Swab) N=23	Positive	22	1	38.60%	97.67%	95.65%	54.55%
	%	38.60%	2.33%				
	Negative	35	42	27.06 - 51.57	87.94 - 99.88	79.01 - 99.78	43.47 - 65.19
	%	61.40%	97.67%				
Total		57	43				

Pcr Screening For H Pylori by Detection of 16srrna Gene: Regarding 16sRNA gene which was used for detection of H. pylori in different clinical samples, the current result showed that 85% were biopsy positive for this gene, figure (1), as presented in table (5)

Table 5: Comparison between different clinical samples for detection of H. pylori by using 16sRNA

		16 sRNA (biopsy)		Sensitivity	Specificity	PPV	NPV
		Positive N=85	Negative N=15				
16 sRNA (Saliva) N=62	Positive	60	2	70.59%	86.67%	96.77%	34.21%
	%	70.59%	13.33%				
	Negative	25	13	60.18 - 79.21	62.12 - 97.63	88.98 - 99.43	21.21 - 50.11
	%	29.41%	86.67%				
16 sRNA (Swab) N=38	Positive	37	1	43.53%	93.33%	97.37%	22.58%
	%	43.53%	6.67%				
	Negative	48	14	33.50 - 54.12	70.18 - 99.66	86.51 - 99.87	13.96 - 34.41
	%	56.47%	93.33%				
Total		85	15				

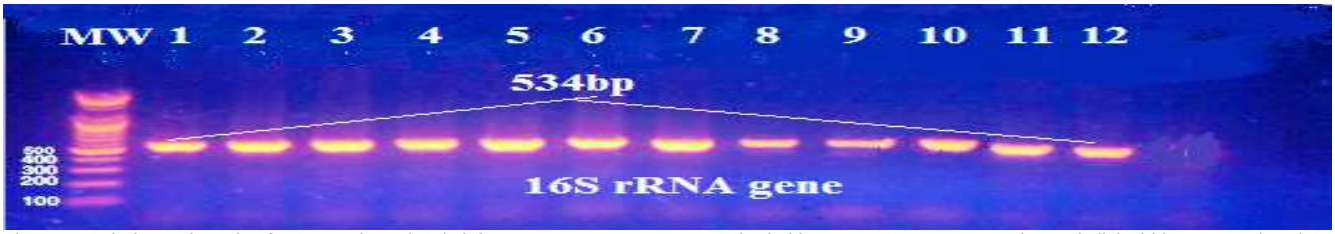

Figure 1: Gel electrophoresis of PCR products (534bp) for16 sRNA gene. Lane 1: 100bp ladder. Lanes 2-12: PCR products of clinical biopsy samples. (2% agarose, 7 v/cm^2, 45 min).

Nested PCR Screening For Cag a and VAG a Genes in H Pylori: Nested PCR were used for detection cag A and vag A genes in H. pylori(figure2). Regarding sensitivity, specificity, positive predictive value and negative predictive value of detection cagA in saliva and swabs in comparison with biopsy samples, as illustrated in (table6)

Table 6: Comparison in result of cagA gene between different clinical samples

		Cag A (Tissue)		Sensitivity	Specificity	PPV	NPV
		Positive N=48	Negative N=52				
Cag A (Saliva) N=71	Positive	42	29	87.50%	44.23%	59.15%	79.31%
	%	87.50%	55.77%				
	Negative	6	23	75.30 - 94.14	31.60 - 57.66	47.54 - 69.83	61.61 - 90.15
	%	12.50%	44.23%				
Cag A (Swab) N=34	Positive	20	14	41.67%	73.08%	58.82%	57.58%
	%	41.67%	26.92%				
	Negative	28	38	28.85 - 55.72	59.75 - 83.23	42.22 - 73.63	45.56 - 68.76
	%	58.33%	73.08%				
Total		48	52				

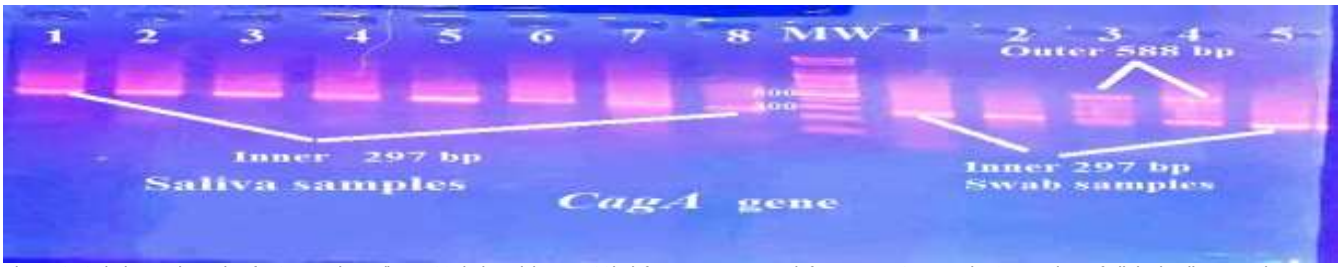

Figure 2: Gel electrophoresis of PCR products (inner 297bp) and (outer 588bp) for cag A gene. At left, Lanes 1-8: nested PCR product of clinical saliva sample. Lane MW: 100bp ladder. At right, Lanes 1-5: PCR products of clinical oral swab samples. (2% agarose, 7 v/cm2, 45 min).

Regarding sensitivity, specificity, positive predictive value and negative predictive value of Vag A for biopsy samples in comparison with saliva and swab, as illustrated in table (7)

Table 7: compression in result of vagA gene between different clinical samples

		VacA (Tissue)		Sensitivity	Specificity	PPV	NPV
		Positive N=48	Negative N=52				
VacA (Saliva) N=25	Positive	15	10	31.25%	80.77%	60.00%	56.00%
	%	31.25%	19.23%				
	Negative	33	42	19.95 - 45.33	68.10 - 89.20	40.74 - 76.60	44.75 - 66.67
	%	68.75%	80.77%				
VacA (Swab) N=13	Positive	8	5	16.67%	90.38%	61.54%	54.02%
	%	16.67%	9.62%				
	Negative	40	47	8.7 - 29.58	79.39 - 95.82	35.52 - 82.29	43.60 - 64.10
	%	83.33%	90.38%				
Total		48	52				

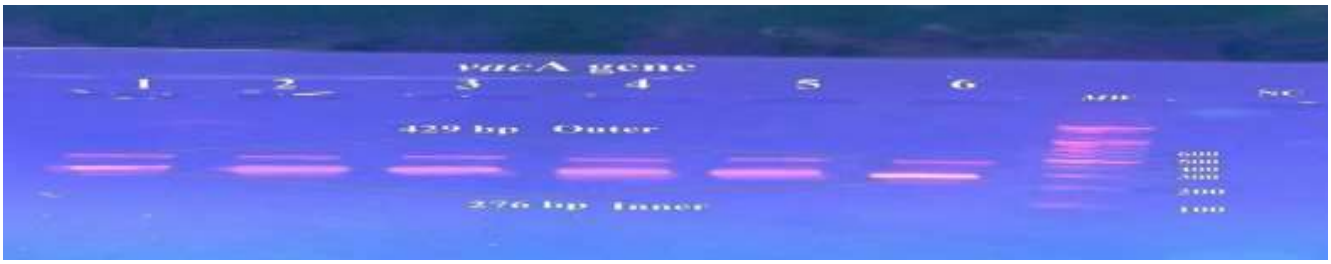

Figure 3: Gel electrophoresis of PCR products (inner 276 bp) and (outer 429bp) for Vag A gene. Lane 1: PCR products of clinical biopsy samples. Lane 2: 100 bp ladder. (2% agarose, 7 v/cm2, 45 min).

DISCUSSION

The current study was the first, that investigated different techniques for detection of H. pylori in saliva and oral swab and compare the result with biopsy sample in Iraqi patients with gastrointestinal diseases symptoms.

Rapid urease test (RUT) is the popular invasive and cost-effective test for the detection of H. pylori infection (Dandin A S, 2012). The result showed that 78 out of 100 (78%) biopsy specimen taken from patients and assisted by RUT were positive, while 22out of 100 (22%) patients gave negative results. Dandin et al. (2012) (Hu et al., 2019) they found that 43 of 48(89.6%) patient infected with H.pylori gave positive results by RUT.

In the present study urease test was positive in 82,05% saliva sample and 43,05% oral swab sample while other study reported that the urease test in 89.8% in saliva and in dental plaque was positive in 99.3% of the patients. (De Sousa, Vásquez, Velasco, & Parlapiano, 2006)

Culture of biopsy, saliva and oral swab samples is used for diagnosis of H. pylori infection by Skirrow's media. This study aimed to detect the frequency of H.pylori in clinical samples, the percentage were in biopsy 57(57%), in saliva 38(38%), and in swab 23(23%), regarding comparison between samples the present result reported that out of 57 positive biopsy culture, there were 34(59.65%) positive saliva culture and 22(38.60%) positive swab culture, the sensitivity of culture from saliva sample in comparison with culture from biopsy sample was 97.67% and specificity was 90.70%

Aftab et al., (2018) (Aftab et al., 2018) reported H. pylori positive biopsy culture in 56(42.1%) with high sensitivity and specificity. Rui et al., (2014) (Rui et al., 2014) revealed that the rate of H. pylori-positive saliva culture in Lanzhou was 42.72%. The rate of H. pylori-positive saliva among females was 47.89%, which was greater compared with the rate among males 38.45%. This result was agreed with the present study that the rate of H.pylori which was 38(38%) in saliva, and the rate of h pylori among females 41.3% which was greater than male 32.4%. The present study also exhibited that the percentage of isolates were 23(23%) in oral swab sample. While Okuda et al., (2000) (Okuda et al., 2000) exhibited that detection rates of H. pylori culture were 12.1% in oral swab samples.

This study revealed that the molecular detection of H. pylori using 16SRNA showed high percentage of these bacteria in tissue biopsy 85(85%) However, the sensitivity and specificity of 16S rRNA PCR in biopsy were found 70.59% and 86.67% respectively, this study agreed with a study by Vagarali et al., (2021) (Vagarali, Metgud, Bannur, & Dodamani, 2021).

Also, this study was amid to investigate the rate of H. pylori in saliva by 16SrRNA gene detection using PCR. The positivity was 62(62%), likewise the sensitivity and specificity of 16S rRNA PCR in saliva were 70.59% and 86.67% respectively. In addition, the study also determined the sensitivity and specificity of PCR as 80% and 70%, respectively This study revealed that the rate of h. pylori in oral swab was 38(38%) cases with low sensitivity 43.53% and high specificity 93.33%.

This study revealed that the prevalence of cagA gene was 48% in biopsy samples However. These results agreed with the study conducted in Saudi by Akeel et al., (2019) (Sallas et al., 2017). The VacA gene was detected in 48% of biopsy samples in the current investigation. while in recent study done by Kishk et al., (2021) (Kishk et al., 2021) found that the vacA was identified in 61.6% of H. pylori strains.

Comparison of five techniques of H. pylori detection showed that microbiome diagnostics by 16SRNA offered gold standard method for H. pylori identification.

Detection of 16sRNA from biopsy samples in comparison with urease test from biopsy were with high sensitivity (90, 59%) and specificity (93.33%). Also, this study agreed with a study done in Iran by Khalifehgholi et al., (2013) (Khalifehgholi et al., 2013). In comparison with culture, there was low sensitivity 65.88% and high specificity 93.33%. However, Atkinson et al., (Atkinson, Braden, & sciences, 2016) demonstrated the sensitivity and specificity of 60% and 100% for this method, respectively. Other results by Hussein et al., (2021) (Hussein, Al-Ouqaili, & Majeed, 2021) who found that low sensitivity and specificity (67.9%) (79.4%).

The present study also showed that, there was highest specificity appear in CagA gene (100.00%) and low sensitivity (56.47%). This study was not identical to a study done by Vagarali et al., (2021) (Vagarali et al., 2021). This study revealed that the association between 16sRNA and vacA gene from biopsy samples had high specificity 93.33% and low sensitivity 55.29%. The current study differs from another study found that the Vac A assay giving a specificity of 99.0% and sensitivity (89.5%) (Chisholm, Owen, Teare, & Saverymuttu, 2001).

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REFERENCES

1. Aftab, H., Yamaoka, Y., Ahmed, F., Khan, A. A., Subsomwong, P., Miftahussurur, M., . . . Malaty, H. M. J. T. J. o. I. i. D. C. (2018). Validation of diagnostic tests and epidemiology of Helicobacter pylori infection in Bangladesh. 12(05), 305-312.
2. Al Thwai, A. N., & Ali, S. F. J. I. J. o. A. B. R. (2013). Detection of Helicobacter pylori in saliva and biopsy specimens of some Iraqui patients using PCR technique. 3(4), 593-598.
3. Atkinson, N. S., Braden, B. J. D. d., & sciences. (2016). Helicobacter pylori infection: diagnostic strategies in primary diagnosis and after therapy. 61(1), 19-24.
4. Chisholm, S. A., Owen, R. J., Teare, E. L., & Saverymuttu, S. J. J. o. c. m. (2001). PCR-based diagnosis of Helicobacter pylori infection and real-time determination of clarithromycin resistance directly from human gastric biopsy samples. 39(4), 1217-1220.
5. Dandin A S, P. J., and AthanikarVS. . (2012). Helicobacter Pylori AssociatedGastritis. J. of Clin. and Diag. Res, 211-214.
6. De Sousa, L., Vásquez, L., Velasco, J., & Parlapiano, D. J. I. C. (2006). Aislamiento de Helicobacter pylori en mucosa gástrica, placa dental y saliva en una población de los Andes venezolanos. 47(2), 109-116.
7. Dowsett, S., Kowolik, M. J. C. R. i. O. B., & Medicine. (2003). Oral Helicobacter pylori: can we stomach it? , 14(3), 226-233.
8. Gebara, E., Faria, C., Pannuti, C., Chehter, L., Mayer, M., & Lima, L. J. J. o. c. p. (2006). Persistence of Helicobacter pylori in the oral cavity after systemic eradication therapy. 33(5), 329-333.
9. Hooi, J. K., Lai, W. Y., Ng, W. K., Suen, M. M., Underwood, F. E., Tanyingoh, D., . . . Wu, J. C. J. G. (2017). Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. 153(2), 420-429.
10. Hu, S., Gao, Y., Wu, Y., Guo, X., Ying, Y., Wen, Y., . . . Bioelectronics. (2019). Raman tracking the activity of urease in saliva for healthcare. 129, 24-28.
11. Huang Y, T. X., Fan XG, Fu CY, Zhu C. . (2009). The pathological effect of Helicobacter pylori infection on liver tissues in mice. Clin Microbiol Infect, 15, 843-849.
12. Hussein, R. A., Al-Ouqaili, M. T., & Majeed, Y. H. J. P. o. (2021). Detection of Helicobacter Pylori infection by invasive and non-invasive techniques in patients with gastrointestinal diseases from Iraq: A validation study. 16(8), e0256393.
13. Khalifehgholi, M., Shamsipour, F., Ajhdarkosh, H., Daryani, N. E., Pourmand, M. R., Hosseini, M., . . . Shirazi, M. H. J. I. j. o. m. (2013). Comparison of five diagnostic methods for Helicobacter pylori. 5(4), 396.
14. Kishk, R. M., Soliman, N. M., Anani, M. M., Nemr, N., Salem, A., Attia, F., . . . Fouad, M. J. I. J. o. M. (2021). Genotyping of Helicobacter pylori virulence genes cagA and vacA: Regional and National Study. 2021.
15. Krajden, S., Fuksa, M., Anderson, J., Kempston, J., Boccia, A., Petrea, C., . . . Penner, J. J. J. o. c. m. (1989). Examination of human stomach biopsies, saliva, and dental plaque for Campylobacter pylori. 27(6), 1397-1398.
16. Machado, R. S. (2002). Validação do teste respiratório com uréia-13C realizado com espectroscopia infravermelho em pacientes menores de 12 anos de idade.
17. Okuda, K., Ishihara, K., Miura, T., Katakura, A., Noma, H., Ebihara, Y. J. M., & immunology. (2000). Helicobacter pylori may have only a

transient presence in the oral cavity and on the surface of oral cancer. 44(5), 385-388.
18. Rui, G., Tuanjie, C., Jun, J., Sen, Y., Xiangyi, H., & Ying, Z. J. W. C. J. o. S. (2014). Analysis of the infection status of saliva Helicobacter pylori in Lanzhou. 32(4).
19. Sallas, M. L., Melchiades, J. L., Zabaglia, L. M., do Prado Moreno, J. R., Orcini, W. A., Chen, E. S., . . . Rasmussen, L. T. J. A. i. M. (2017). Prevalence of Helicobacter pylori vacA, cagA, dupA and oipA genotypes in patients with Gastric Disease. 7(1), 1-9.
20. Schwarz, S., Morelli, G., Kusecek, B., Manica, A., Balloux, F., Owen, R. J., . . . Suerbaum, S. J. P. p. (2008). Horizontal versus familial transmission of Helicobacter pylori. 4(10), e1000180.
21. Selgrad, M., Kandulski, A., & Malfertheiner, P. J. C. o. i. g. (2009). Helicobacter pylori: diagnosis and treatment. 25(6), 549-556.
22. Talebi Bezmin Abadi, A., Taghvaei, T., Mohabbati Mobarez, A., Vaira, G., Vaira, D. J. I., & medicine, e. (2013). High correlation of babA 2-positive strains of Helicobacter pylori with the presence of gastric cancer. 8(6), 497-501.
23. Tirapattanun A, N. W., Kanthawong S, Wongboot W, Wongwajana S, Wongphutorn P, et al. . (2016). Detection of Heicobacter pylori and virulence-associated genes in saliva samples of asymptomatic person in northeast of Thailand. The Southeast Asian journal of tropical medicine and public health., 47(6), 1246-1256.
24. Vagarali, M. A., Metgud, S. C., Bannur, H., & Dodamani, S. S. J. J. o. G. I. D. (2021). A cross-sectional study on molecular detection of Helicobacter pylori cytotoxin-associated gene A and 16SrRNA gene from gastric biopsy specimens. 13(3), 120.