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Original Research Article

Genotyping of vacA alleles of *Helicobacter pylori* strains recovered from some Iranian food items

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Abstract

Purpose: *To study the vacA genotype status of H. pylori isolated from some Iranian food items.*
Methods: *Three hundred assorted samples of fish, ham, chicken, vegetable and meat sandwiches, and minced meat were purchased and tested using culture method. Those that were H. pylori-positive were analyzed for presence of vacA genotypes using polymerase chain reaction (PCR).*
Results: *Sixty out of 300 (20 %) food samples were positive for H. pylori. Vegetable sandwich (45 %), minced meat (32 %) and meat sandwich (20 %) were the most commonly contaminated. The most commonly detected genotypes in the meat-based foods, viz, vegetable sandwich and ready to eat fish, were vacA s1a, vacA m1a and vacA m2, respectively. The most commonly detected combined genotypes were s1am2 (45 %), s1am1a (40 %) and m1am2 (35 %).*
Conclusion: *The presence of similar genotypes in H. pylori strains of foods and those of human clinical samples suggest that contaminated foods may be the source of bacteria for humans.*

Keywords: *Helicobacter pylori, VacA genotypes, Genotyping, Food items*

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INTRODUCTION

Helicobacter pylori is a microaerophilic gram-negative bacteria which is known as the causative agent of gastric adenocarcinoma, type B gastritis, peptic ulcer disease, and mucosa-associated lymphoid tissue lymphoma [1]. Recorded data show that 17 to 86 % of patients with peptic ulcers were positive for *H. pylori* [1-3]. Despite the high incidence of *H. pylori*, its exact routes of transmission and origin have not been well determined.

The role of foods in the transmission of *H. pylori* is still unknown but there are several studies on its isolation from various types of food like milk, vegetables and salad [4-7]. Favorable conditions for the growth of bacteria in various types of foods including ready to eat foods, meat, salads and vegetables would enhance *H. pylori* the survival [5,7].

To appraise the pathogenicity of *H. pylori*, evaluation of the status of genotypes is requisite. The most commonly known virulence markers among *H. pylori* strains of different clinical samples of animals and human beings are the vacuolating cytotoxin (vacA) [7-9]. The vacA gene is associated with injury to epithelial cells of gastric tract. This gene is polymorphic, comprising variable signal regions and mid-regions. The s-region is classified into s1 and s2

types and the m-region into m1 and m2 types. The s1 type is further subtyped into s1a, s1b and s1c subtypes, and the m1 into m1a and m1b subtypes [10,11]. Genotyping using vacA virulence marker is considered as one of the most effective approaches for the study of correlations between *H. pylori* isolates from different samples.

There were scarce published data on the genotyping of the vacA gene of the *H. pylori* isolated from food items. Also data about the epidemiology and routes of transmission of this bacterium are scarce too. With the high prevalence of *H. pylori* among Iranians [4,5,7,9] and based on the good features of foods in the survival of *H. pylori* [12], the present study was carried out to ascertain the status of vacA genotypes of *H. pylori* isolated from various types of food items.

EXPERIMENTAL

Sample collection

From March 2013 to July 2014, overall 300 samples of food items samples including ready to eat fish (n = 60), ham (n = 60), chicken sandwich (n = 40), vegetable sandwich (n = 40), meat sandwich (n = 50) and minced meat (n = 50) were purchased from the supermarkets of Shiraz province, Iran. Samples (100 mL, in sterile glass containers) were transported to the laboratory at ca. 4 ^{o}C within a maximum of 6 h after sampling. All samples were kept under refrigeration in plastic bags; information about dates of production and of assigned shelf lives was not presented.

Isolation of *Helicobacter pylori*

Twenty five milliliters of each homogenized sample were added to 225 mL of Wilkins Chalgren anaerobe broth (Oxoid, UK) supplemented with 5 % of horse serum (Sigma, St. Louis, MO, USA) and colistin methanesulfonate (30 mg/L), cycloheximide (100 mg/L), nalidixic acid (30 mg/L), trimethoprim (30 mg/L), and vancomycin (10 mg/L) (Sigma, St. Louis, MO, USA) and colistin methanesulfonate (30 mg/L), cycloheximide (100 mg/L), nalidixic acid (30 mg/L), trimethoprim (30 mg/L), and vancomycin (10 mg/L) (Sigma, St. Louis, MO, USA) and incubated for 7 days at 37 °C with shaking under microaerophilic conditions. Then, 0.1 mL of the enrichment selective broth was plated onto Wilkins Chalgren anaerobe agar (Oxoid, UK) supplemented with 5 % of defibrinated horse blood and 30 mg/L colistin methanesulfonate, 100 mg/L cycloheximide, 30 mg/L nalidixic acid, 30 mg/L trimethoprim, and 10 mg/L vancomycin (Sigma, St. Louis, MO, USA) and incubated for 7 days at 37 °C under microaerophilic conditions. For comparison, a reference strain of *H. pylori* (ATCC 43504) was employed.

DNA extraction and *Helicobacter pylori* 16S rRNA gene amplification

Suspected colonies were identified as *H. pylori* based on the PCR technique. Genomic DNA was extracted from the colonies with typical characters of *H. pylori* using a DNA extraction kit for cells and tissues (Roche Applied Science, Germany, 11814770001) according to the manufacturer's instructions and its density was assessed by optic densitometry. Extracted DNA was amplified for the 16S rRNA gene (primers: HP-F: 5'-CTGGAGAGACTAAGCCCTCC-3' and HP-R: 5'-ATTACTGACGCTGATTGTGC-3') [13]. PCR reactions were performed in a final volume of 50 µL containing 5 µL 10 × buffer + $MgCl_2$, 2 mM dNTP, 2 unit Taq DNA polymerase, 100 ng genomic DNA as a template, and 25 picomole of each primer. PCR was performed using a thermal cycler (Eppendorf Co., Germany) under the following conditions: an initial denaturation for 2 min at 94 °C; 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 8 min.

Genotyping of vacA gene of *Helicobacter pylori*

Presence of the genotypes of vacA alleles (s1a, s1b, s1c, m1a, m1b and m2) were determined by PCR. The primer sequences are shown in Table 1 [14].

The PCR were performed in a total volume of 50 µL containing 1 µM of each primers, 1 µL of genomic DNA (approximately 200 ng), 1 mM of dNTPs mix (invitrogen), 2 mM of $Mgcl_2$, and 0.05 U/µL Taq DNA polymerase (invitrogen). PCR amplifications were performed in an automated thermal cycler (Biometra Co., Germany). The following cycle conditions were used for PCR amplification: 32 cycles of 45 s at 95 °C, 50 s at 64 °C, and 70 s at 72 °C. All runs included one negative DNA control consisting of PCR grade water and two or more positive controls (26695, J99, SS1, Tx30, 88-23 and 84-183).

Gel electrophoresis

The PCR amplification products (10 µL) were subjected to electrophoresis in a 1 % agarose gel in 1X TBE buffer at 80 V for 30 min,

Table 1: Oligonucleotide primers used for genotyping of *Helicobacter pylori* isolated from ready to eat foods [14]

VacA alleles	Primer Sequence (5'-3')	Size of product (bp)
s_1a	F: CTCTCGCTTTAGTAGGAGC R: CTGCTTGAATGCGCCAAAC	213
s_1b	F: AGCGCCATACCGCAAGAG CTGCTTGAATGCGCCAAAC	187
s_1c	F: CTCTCGCTTTAGTGGGGYT R: CTGCTTGAATGCGCCAAAC	213
s_2	F: GCTAACACGCCAAATGATCC R: CTGCTTGAATGCGCCAAAC	199
m_1a	F: GGTCAAAATGCGGTCATGG R: CCATTGGTACCTGTAGAAAC	290
m_1b	F: GGCCCCAATGCAGTCATGGA R: GCTGTTAGTGCCTAAAGAAGCAT	291
m_2	F: GGAGCCCCAGGAAACATTG R: CATAACTAGCGCCTTGCA	352

stained with ethidium bromide, and images were obtained in a UVIdoc gel documentation systems (UK). The PCR products were identified by 100 bp DNA size marker (Fermentas, Germany).

Statistical analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), Chi-square test and Fisher's exact two-tailed test analysis was performed and differences were considered significant at $p < 0.05$. Distribution of *H. pylori* genotypes isolated from food items was statistically analyzed.

RESULTS

Prevalence of *Helicobacter pylori* in various types of food items

All of the food items samples were tested using the culture and PCR methods. Total distribution of *H. pylori* in various types of food items samples is shown in Table 2. Of 300 food items, 60 (20 %) were positive for *H. pylori*. All the food items were contaminated with *H. pylori*. The most commonly contaminated samples were vegetable sandwich (45 %), minced meat (32 %), and meat sandwich (20 %). There were statistically significant differences amongst the incidence of bacteria in vegetable sandwich and chicken sandwich ($p = 0.017$), minced meat and ham ($p = 0.025$) and vegetable sandwich and ham ($p = 0.023$).

VacA genotyping pattern

Figures 1, 2 and 3 show the results of gel electrophoresis for vacA genotypes of *H. pylori* strains of food samples. Total distribution of vacA genotypes in the *H. pylori* isolates of various types of food items is shown in table 3. Results indicated that the most commonly detected genotypes were vacA s1a (66.66 %), vacA m2 (56.66 %) and vacA m1a (51.66 %). The most commonly detected genotypes in the meat based food items (minced meat, meat sandwich and ham), vegetable sandwich and ready to eat fish were vacA s1a, vacA m1a and vacA m2, respectively. Significant differences were found between the incidence of s1a and s2 (p =0.017) and also between s1a and s1c ($p = 0.020$), s1a and m1b ($p = 0.024$), m2 and s1c (p =0.018), m1a and m1b ($p = 0.016$), m2 and s2 (p =0.022) and m2 and s1b (p =0.025) genotypes.

Table 2: Distribution *of Helicobacter pylori* in various types of food items

Type of sample	No. of samples collected	*H. pylori* (%)
Ready to eat fish	60	9 (15)
Ham	60	5 (8.33)
Chicken sandwich	40	2 (5)
Vegetable sandwich	40	18 (45)
Meat sandwich	50	10 (20)
Minced meat	50	16 (32)
Total	300	60 (20)

Combined genotype status

Twenty-one different genotypic combinations were found for the *H. pylori* strains of studied food samples (Table 4). The most commonly detected combined genotypes were s1am2 (45 %), s1am1a (40 %) and m1am2 (35 %). There were no positive results for m1as2, m1bs1c, m1bs2, s1as2, s1bs2 and s1cs2 genotype.

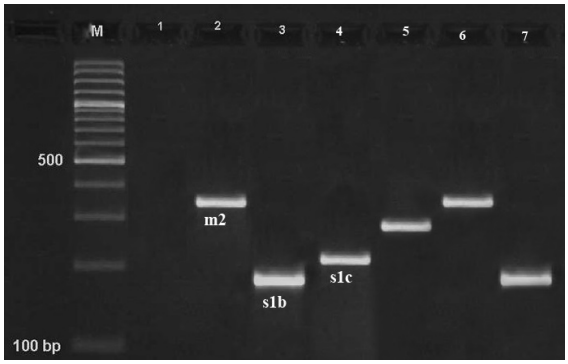

Figure 1: Results of the gel electrophoresis for the identification of *m2*, *s1b* and *s1c* genotypes of the *Helicobacter pylori* strains of food samples. Lanes 5 - 7: Positive controls, 1: Negative control, M: 100 bp DNA ladder (Fermentas, Germany), numbers 2 - 4: Positive samples

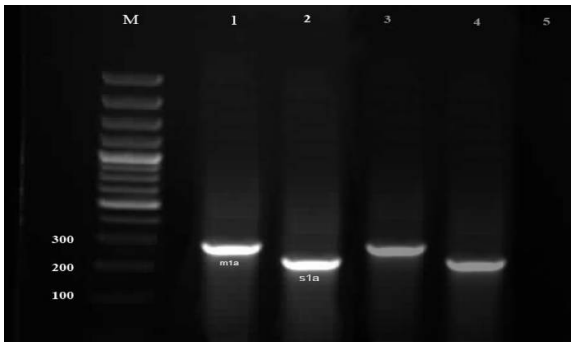

Figure 2: Identification of *m1a* and *s1a* genotypes of the *Helicobacter pylori* strains of food samples by gel electrophoresis. Lanes 3 and 4: positive controls, 5: negative control, M: 100 bp DNA ladder (Fermentas, Germany), numbers 1 - 2: positive samples

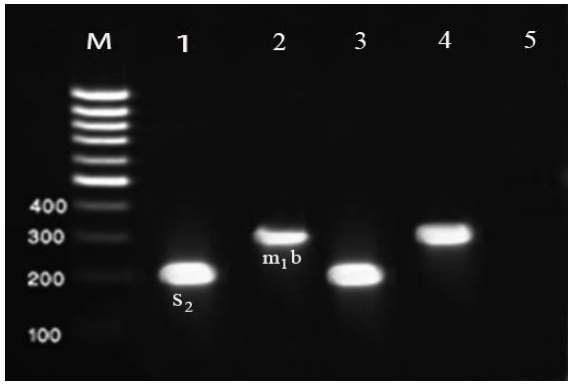

Figure 3: Lentification of *m1b* and *s2* genotypes of the *Helicobacter pylori* strains of food samples by gel electrophoresis. Lanes 3 and 4: positive controls, 5: negative control, M: 100 bp DNA ladder (Fermentas, Germany), numbers 1 - 2: positive samples

Table 4: Distribution of combined genotypes of *Helicobacter pylori* isolated from ready to eat foods

Genotype	Prevalence (%)*
M1as1a	24 (40)
M1as1b	6 (10)
M1as1c	2 (3.33)
M1as2	-
M1am1b	1 (1.66)
M1am2	21 (35)
M1bs1a	2 (3.33)
M1bs1b	1 (1.66)
M1bs1c	-
M1bs2	-
M1bm2	2 (3.33)
S1as1b	2 (3.33)
S1as1c	1 (1.66)
S1as2	-
S1bs1c	2 (3.33)
S1bs2	-
S1cs2	-
S1am2	27 (45)
S1bm2	5 (8.33)
S1cm2	2 (3.33)
S2m2	1 (1.66)

*From a total of 60 positive strains of *H. pylori*

Table 3: Distribution of *vacA* genotypes in *Helicobacter pylori* strains of ready to eat foods

Type of sample (No. positive)	*vacA* genotypes (%)						
	S1a	***S1b***	***S1c***	***S2***	***M1a***	***M1b***	***M2***
Ready to eat fish (9)	5 (5.55)	1 (1.11)	1 (1.11)	-	3 (3.33)	2 (2.22)	7 (7.77)
Ham (5)	3 (60)	1 (20)	-	-	3 (60)	-	2 (40)
Chicken sandwich (2)	1 (50)	-	-	-	-	1 (50)	-
Vegetable sandwich (18)	11 (61.11)	3 (16.66)	2 (11.11)	1 (5.55)	14 (77.77)	2 (11.11)	12 (66.66)
Meat sandwich (10)	8 (80)	4 (40)	3 (30)	2 (20)	5 (50)	1 (10)	7 (70)
Minced meat (16)	12 (75)	1 (6.25)	-	1 (6.25)	6 (37.5)	-	6 (37.5)
Total (60)	40 (66.66)	10 (16.66)	6 (10)	4 (6.66)	31 (51.66)	6 (10)	34 (56.66)

DISCUSSION

As far as we know, this is the first report of genotyping of *H. pylori* in the various types of food items in Iran and even other parts of the world. Our results showed high prevalence of *H. pylori* in various types of food items (20 %). The prevalence of *H. pylori* in our study (20 %) was higher than those of milk (12.5 %) [15], dairy products (19.2 %) [5], salad (10.86 %) [4], vegetable (13.68 %) [7]. In addition to vegetable sandwich (45 %), meat based food items including minced meat (32 %), meat sandwich (20 %) and ham (8.33 %) had high prevalence of *H. pylori*.

One possible explanation for the high prevalence of bacteria in vegetable sandwich is the fact that there was no sufficient time for well washing of vegetables used in the sandwich. In addition, vegetables are mainly growth in the context of animal manure and were irrigated using the contaminated water. Role of animal feces as a source for transmission of *H. pylori* has been reported previously [13]. High amount of activated water (AW), optimum levels of salt and pH and finally the role of infected staffs as sources of *H. pylori* infection [9,12] are other reasons for the high prevalence of *H. pylori* in vegetable sandwich samples.

Mhaskar *et al* [16] reported the high prevalence of peptic ulcer and *H. pylori* infection in patients associated with meat and meat products and restaurant foods which confirm the results of our study. High amount of Aw in meat base food samples, optimum pH and salt levels, presence of appropriate levels of amino acids including arginine, histidine, isoleucine, leucine, methionine, phenylalanine, alanine, valine, proline, serine, and tryptophan favors the for growth of *H. pylori* [12], this is in addition to unsanitary conditions in their preparation and cross contamination of these foods due to handling by factory and food units staffs. High prevalence of *H. pylori* in foods with animal origin has been reported previously [5,9,15,17,18,20].

Roles of contaminated water and even drinking water in transmission of *H. pylori* have been confirmed previously [17-20]. Probably, the water sources used for production, washing and processing of these foods samples were contaminated. Finally, unsanitary conditions, handling contamination, use of contaminated equipment and lack of public and individual hygiene could be the main reasons for the high prevalence of *H. pylori* in food samples of our study. Food safety regulations as well as quality standards – including hazard analysis and critical control points (HACCP), good agricultural practices (GAPs) and good manufacturing practices (GMPs) – should be introduced in Iranian food units to prevent proliferation of *H. pylori* in food samples.

Similar to the conducted studies on the clinical samples [8-10,21], the results of our investigation showed the high prevalence of vacA s1a (66.66 %), vacA m2 (56.66 %), vacA m1a (51.66 %), vacA s1am2 (45 %), vacA s1am1a (40 %) and vacA m1am2 (35 %) genotypes in food samples. High prevalence of these genotypes have been reported from the various types of foods [4,5,7] and even animals [8]. Yahaghi *et al* [7] reported that the vacA s1a (37.28 %) and vacA m1a (30.50 %) had the highest prevalence of genotypes in the *H. pylori* of vegetable and salad samples, while vacA s1c (10.16 %) had the lowest prevalence which was similar to our findings.

It seems that differences in the sources of infection in vegetable sandwich, ready to eat fish samples and meat based food items in our study, could have been the main factor for the differences in the prevalence of genotypes of *H. pylori*. The vacA s1a was the most commonly detected genotypes in the meat based food items which maybe showed that these samples were secondary infected (handling of samples by staffs of the factories).

In the case of vegetable sandwiches the vacA m1a was the most commonly detected which maybe showed that contact with contaminated water and animal manure are the main factors for primary infection of vegetables used in their preparation. It seems that in the ready to eat fish samples the most prevalent genotype was vacA m2 and it may be related to the type of *H. pylori* strains detected in this type of samples. Since *H. pylori* isolates in our study harbored vacA genotypes, consumption of food items contaminated with virulent strains may provoke duodenal ulceration, gastric mucosal atrophy and gastric cancer.

CONCLUSION

Some food items in Iran harbor *H. pylori* similar in the genotype of vacA alleles with isolates recovered from human clinical samples. High prevalence of *H. pylori* in food items suggests that contaminated foods may be the sources of the bacteria that entered the human population in Iran over a period of time. Diversity of *H. pylori* genotypes between various types of food samples may be indicative of the various sources of contamination of these food items.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Shrestha S, Paudel P, Pradhan GB, Shrestha L, Bhattachan CL. Prevalence study of H. pylori infection in dyspeptic patients coming to Nepal Medical College Teaching Hospital, Jorpati, Kathmandu. Nepal Med Coll J 2012; 14: 229-233.*
2. *Mastromarino P, Conti C, Donato K, Strappini PM, Cattaruzza MS, Orsi GB. Does hospital work constitute a risk factor for Helicobacter pylori infection J Hosp Infect 2005; 60: 261-268.*
3. *Vu C, Ng YY. Prevalence of Helicobacter pylori in peptic ulcer disease in a Singapore hospital. Singapore Med J 2000; 41: 478-481.*
4. *Atapoor S, Safarpoor Dehkordi F, Rahimi E. Detection of Helicobacter pylori in Various Types of Vegetables and Salads. Jundishapur J Microbiol 2014; 7: e10013.*
5. *Mousavi S, Safarpoor Dehkordi F, Rahimi E. Virulence factors and antibiotic resistance of Helicobacter pylori isolated from raw milk and unpasteurized dairy products in Iran. J Venom Anim Tox inclTrop Dis 2014; 20: 51.*
6. *Stevenson TH, Bauer N, Lucia LM, Acuff GR. Attempts to isolate Helicobacter from cattle and survival of Helicobacter pylori in beef products. J Food Prot 2000; 63: 174-178.*
7. *Yahaghi E, Khamesipour F, Mashayekhi F, Safarpoor Dehkordi F, Sakhaei MH, Masoudimanesh M, Khameneie MK. Helicobacter pylori in vegetables and salads: genotyping and antimicrobial resistance properties. Biomed Res Int 2014; 2014: 757941.*
8. *Momtaz H, Dabiri H, Souod N, Gholami M. Study of Helicobacter pylori genotype status in cows, sheep, goats and human beings. BMC Gastroenterol 2014; 14: 61.*
9. *Momtaz H, Souod N, Dabiri H, Sarshar M. Study of Helicobacter pylori genotype status in saliva, dental plaques, stool and gastric biopsy samples. World J Gastroenterol 2012; 18: 2105-2111.*
10. *Momtaz H, Souod N, Dabiri H. Comparison of the virulence factors of Helicobacter pylori isolated in stomach and saliva in Iran. Am J Med Sci 2010; 340: 345-349.*
11. *Torres LE, Melián K, Moreno A, Alonso J, Sabatier CA, Hernández M, Bermúdez L, Rodríguez BL. Prevalence of vacA, cagA and babA2 genes in Cuban Helicobacter pylori isolates. World J Gastroenterol 2009; 15: 204-210.*
12. *Van Duynhoven YT, de Jonge R. Transmission of Helicobacter pylori: a role for food. Bull World Health Org 2001; 79: 455-460.*
13. *Ho SA, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR. Direct polymerase chain reaction test for detection of Helicobacter pylori in humans and animals. J Clin Microbiol 1991; 29: 2543-2549.*
14. *Yamazaki S, Yamakawa A, Okuda T, Ohtani M, Suto H, Ito Y, Yamazaki Y, Keida Y, Higashi H, Hatakeyama M, Azuma T. Distinct diversity of vacA, cagA, and cagE genes of Helicobacter pylori associated with peptic ulcer in Japan. J Clin Microbiol 2005; 43: 3906-3916.*
15. *Rahimi E, Kheirabadi EK. Detection of Helicobacter pylori in Bovine, Buffalo, Camel, Ovine, and Caprine Milk in Iran. Foodborne Pathog Dis 2012; 9: 453-456.*
16. *Mhaskar RS, Ricardo I, Azliyati A, Laxminarayan R, Amol B, Santosh W, Boo K. Assessment of risk factors of helicobacter pylori infection and peptic ulcer disease. J Glob Infect Dis 2013; 5: 60-67.*
17. *Amirhooshang A, Ramin A, Ehsan A, Mansour R, Shahram B. High frequency of Helicobacter pylori DNA in drinking water in Kermanshah, Iran, during June-November 2012. J Water Health 2014; 12: 504-512.*
18. *Dube C, Tanih NF, Ndip RN. Helicobacter pylori in water sources: a global environmental health concern. Rev Environ Health 2009; 24: 1-14.*
19. *Queralt N, Bartolome` R, Araujo R. Detection of Helicobacter pylori DNA in human faeces and water with different levels of faecal pollution in the north–east of Spain. J Appl Microbiol 2005; 98: 889–895.*
20. *Samra ZQ, Javaid U, Ghafoor S, Batool A, Dar N, Athar MA. PCR assay targeting virulence genes of Helicobacter pylori isolated from drinking water and clinical samples in Lahore metropolitan, Pakistan. J Water Health 2011; 9: 208-216.*
21. *Havaei SA, Mohajeri P, Khashei R, Salehi R, Tavakoli H. Prevalence of Helicobacter pylori vacA different genotypes in Isfahan, Iran. Adv Biomed Res 2014; 3: 48-52.*