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# *Helicobacter pylori* in water, vegetables and foods of animal origin: A systematic review and meta-analysis on the prevalence, antibiotic resistance and genotype status in Iran

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#### ABSTRACT

This study was conducted to investigate the prevalence, antibiotic resistance and genotype status of *Helicobacter pylori* strains in water, vegetables and foods of animal origin in Iran. National and international databases were searched using MeSH-extracted keywords in English and Persian languages to find relevant publications by up to November 01, 2019. Among articles that were identified from national and international databases on antibiotic resistance as well as genotype and the prevalence of *Helicobacter pylori*, 20 articles were included in the meta-analysis according to the predefined inclusion and exclusion criteria. The prevalence of *Helicobacter pylori* strains isolated from various foods, vegetables and water in Iran was 11.4% (8.5–15.2). *VacA s1a* (69.3%) had the highest, while *VacA s1c* (11.1%) showed the lowest prevalence of *Helicobacter pylori* genes. Additionally, in the current study, *Helicobacter pylori* resistance rates were as follows: 66.3% to metronidazole, 42.4% to clari-thromycin, 72% to amoxicillin, 68% to teracycline, 33.4% to levofloxacin, 19.8% to rifampin, 17.2% to fur-azolidone, 22.6% to streptomycin, 61.1% to erythromycin, 84.8% to ampicillin, 49% to trimethoprim, 20.2% to cefsulodin and 13.4% to spiramycin. Our findings revealed that the prevalence and antibiotic resistance rates of *Helicobacter pylori* have reached alarming levels in water, vegetables and foods of animal origin in Iran. These issues can greatly affect the risk of bacterial transmission and efficacy of antibiotic treatment in human infections.

#### 1. Introduction

*Helicobacter pylori (H. pylori)* is the most common Gram-negative bacterium, which is colonized in the mucus of the stomach in more than 50% of the world's adult population. It has been estimated that around 70–90% of the population in the developing countries and less than 40% in the industrial countries are infected with *H. pylori* (Khademi et al., 2014; Khademi et al., 2013; Khademi et al., 2015; Murray et al., 2015). This microaerophilic organism is responsible for 70–100% of

gastritis, peptic and duodenal ulcers (Murray et al., 2015). Moreover, *H. pylori* has been classified as class I carcinogen because it expresses *cagA* (cytotoxin-associated gene A), *vacA* (vacuolating cytotoxin gene A) and *dupA* (duodenal ulcer promoting gene A) genes that are associated with the development of gastric adenocarcinomas and gastric mucosa-associated lymphoid tissue (MALT) B-cell lymphomas (Lee and Derakhshan, 2013; Mietzner et al., 2016; Murray et al., 2015). The sanitary status has an important role in *H. pylori* colonization, which mainly occurs during the childhood age (1–10% in the developed and > 50% in the

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Abbreviations: Helicobacter pylori, H. pylori; MTZ, metronidazole; CLR, clarithromycin; AMX, amoxicillin; TET, tetracycline; LVX, levofloxacin; RIF, rifampin; FRZ, furazolidone; STR, streptomycin; ERY, erythromycin; AMP, ampicillin; TMP, trimethoprim; SPI, spiramycin; CEF, cefsulodin; AST, antimicrobial susceptibility testing; ND, not determined; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay

developing countries), and persists throughout the entire life (Yousefi-Avarvand et al., 2018). H. pylori transmission routes and also its reservoirs other than the human stomach are poorly understood, but the microorganism can be isolated from endoscopes, dental plaque, saliva and feces of children and, rarely, adults. The transmission and ensuing colonization and development of gastritis and hypochlorhydria occur via three routes, i.e., iatrogenic, fecal-oral and oral-oral (Mietzner et al., 2016; Van Duynhoven and Jonge, 2001). One possible source of human acquisition of H. pylori is water and foods contaminated with feces (Van Duynhoven and Jonge, 2001). Several studies have focused on the role of water and food in *H. pylori* transmission in Iran. However, there is no comprehensive information about the prevalence and characteristics of H. pylori in water, vegetables and foods of animal origin in Iran. Another important point is the possible role of water and food sources as reservoirs of antibiotic-resistant H. pylori strains that can cause human infections. H. pylori can be omitted with antimicrobial chemotherapy; however, antibiotic-resistant H. pylori strains are the major cause of failure in the eradication of infection (Khademi et al., 2013). Therefore, the present systematic review and meta-analysis was conducted to estimate H. pylori prevalence, antibiotic resistance profile and genotype status in water, vegetables and foods of animal origin in Iran.

#### 2. Methods

#### 2.1. Search strategy and selection criteria

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Liberati et al., 2009). The ISI Web of Knowledge, PubMed, Scopus, Google Scholar and the Scientific Information Database (SID) were systematically searched to find eligible articles published by up to November 01, 2019. Database search was performed in English and Persian languages using the following MeSHextracted keywords and connectors (AND/OR): "Helicobacter pylori" OR "H. pylori" AND "drug resistance" OR "antibiotic resistance" OR "antimicrobial resistance" AND "prevalence" OR "incidence" AND "genotype" AND "food" OR "water" OR "milk" OR "meat" OR "vegetables" AND "Iran". The eligibility of retrieved articles was determined based on the predefined inclusion criteria including articles assessing the prevalence of H. pylori in raw and ready-to-use foods, drinking water and vegetables along with H. pylori genotype status and their antibiotic resistance. The exclusion criteria were articles assessing specimens from human and animal gastric biopsy, reporting other species of the genus of Helicobacter and duplicate publications. We also restricted the studies to Iran. At the end, to find further relevant publications, references of the included studies were also inspected.

#### 2.2. Quality assessment and data extraction

The second step of selecting eligible articles was quality assessment of the included studies using the Joanna Briggs Institute (JBI) critical appraisal checklist (Munn et al., 2015). Eligible studies were further analyzed based on the following criteria: target population, sample size, study subjects and statistical analysis. Only the high- and mediumquality studies (> 5 scores and 4–5 scores, respectively) were finally included in the meta-analysis and low-quality studies (< 4 scores) were excluded. At the end, the required information was extracted from the eligible studies, tabulated in Tables 1 and 2, and used in the metaanalysis. The first author surnames, sample origin, *H. pylori* identification method, sample size, number of tested isolates, methods for assessing bacterial antibiotic susceptibility and number of isolates resistant to different antibiotics (in Table 1) as well as the prevalence of *H. pylori* genotypes (in Table 2) were extracted from the included articles.

#### 2.3. Meta-analysis

Meta-analysis was performed using Comprehensive Meta-Analysis (CMA) software version 2.2 (Biostat, Englewood, NJ). Random- or fixed-effects models were applied depending on the existence of heterogeneity among included studies. The  $I^2$  statistic and the Chi-square test with the Cochrane Q statistic were used to determine inter-study heterogeneity. Due to a high level of heterogeneity, random-effects model was used for computing the prevalence of *H. pylori* in foods, water and vegetables as well as their antibiotic resistance rate and genotype status. The results were expressed as percentage and 95% confidence intervals (95% CIs). The presence of publication bias was evaluated using funnel plots.

#### 3. Results

As shown in Table 1, a total of 20 studies were identified as eligible in reporting data on the prevalence rate of H. pylori in foods, water and vegetables as well as its antibiotic resistance and genotype status in Iran. Briefly, 1249 articles were initially retrieved from the national and international databases by using relevant search terms related to H. pylori antibiotic resistance and also 2183 articles reporting the prevalence of *H. pylori* in foods, water and vegetables and their genotype status. The titles and abstracts were reviewed based on the predefined criteria and selected articles were further evaluated through reviewing full texts of the articles. Finally, 9 and 11 articles reporting antibiotic resistance and prevalence of H. pylori, respectively, were included in the meta-analysis (Fig. 1). All 20 studies evaluated the prevalence of H. pylori genotype status (Table 2). Studies were reported from various cities/provinces of Iran including Alborz, Chaharmahal va Bakhtiari, Isfahan, Kermanshah, Khuzestan, Shiraz and Yazd. Quality score of the included articles was between 4 and 8. Culture and polymerase chain reaction (PCR) were the two most commonly applied methods to identify H. pylori and its genotype status from water, vegetables and foods of animal origin. Additionally, disk diffusion was the only method used for evaluating H. pylori drug resistance. Overall, the prevalence of H. pylori strains isolated from various foods, vegetables and water in Iran was 11.4% (95% CI: 8.5–15.2;  $I^2 = 95.6\%$ ; Q = 437.3; p = 0.00) (Fig. 2A). As shown in Fig. 2B, asymmetrical distribution of the included articles showed the possibility of publication bias. The heterogeneity among included studies assessing drug resistance to the following antibiotics was high and thus we used a random-effects model to calculate percentage and 95% CIs for each antibiotic resistance. In the current study, 66.3% (95% CI: 54.5–76.4;  $I^2 = 76.6\%$ ; Q = 34.2; p = 0.00) of *H. pylori* strains were resistant to metronidazole, 42.4% (95% CI: 27.5–58.8;  $I^2 = 87.6\%$ ; Q = 64.8; p = 0.00) to clarithromycin, 72% (95% CI: 58.5–82.5;  $I^2 = 78.6\%$ ; Q = 37.5; p = 0.00) to amoxicillin, 68% (95% CI: 54.9–82.5;  $I^2 = 80.9\%$ ; Q = 31.5; p = 0.00) to tetracycline, 33.4% (95% CI: 20.2–49.8;  $I^2 = 86.7\%$ ; Q = 52.8; p = 0.00) to levofloxacin, 19.8% (95% CI: 10.5-34.4;  $I^2 = 70.3\%$ ; Q = 13.5; p = 0.00) to rifampin, 17.2% (95% CI: 12.8–22.9;  $I^2 = 34.9\%$ ; Q = 12.2; p = 0.13) to furazolidone, 22.6% (95% CI: 15.2–32.3;  $I^2 = 46.6\%$ ; Q = 5.6; p = 0.13) to streptomycin, 61.1% (95% CI: 46.9–73.6;  $I^2 = 83.5\%$ ; Q = 42.6; p = 0.00) to ervthromycin, 84.8% (95% CI: 74.8–91.3;  $I^2 = 74.1\%$ ; Q = 27; p = 0.00) to ampicillin, 49% (95% CI: 39.2–58.9;  $I^2 = 69.1\%$ ; Q = 22.6; p = 0.00) to trimethoprim and 20.2% (95% CI: 15.3–26.1;  $I^2 = 30\%$ ; Q = 8.5; p = 0.19) to cefsulodin. However, the prevalence of *H. pylori* drug resistance to spiramycin (13.4% (95% CI: 9.6–18.2;  $I^2 = 0.0\%$ ; Q = 2.3; p = 0.8)) was assessed by a fixed-effect model. Finally, the prevalence of H. pylori genotypes was determined among strains isolated from different types samples by random-effect model. The presence of genotypes in H. pylori strains was as follows: VacA s1a 69.3%  $(95\% CI: 54.9-80.8; I^2 = 89.1\%; Q = 110.9; p = 0.00), VacA s1b$ 28.4%; (95% CI: 18.3–41.3;  $I^2 = 88.9\%$ ; Q = 108.2; p = 0.00), VacA s1c 11.1% (95% CI: 5.6–20.7;  $I^2 = 87.5\%$ ; Q = 96.1; p = 0.00), VacA

#### Table 1

Extracted information from eligible studies included in the meta-analysis.

Author	Sample origin	H. pylori	Sample	Strain	AST	Antib	itibiotic resistance (n)											
(Ref)		method	(11)	(11)		MTZ	CLR	AMX	TET	LVX	RIF	FRZ	STR	ERY	AMP	TMP	SPI	CEF
Ranjbar et al., 2018	Raw milk	Culture	630	67	Disk	44	32	50	51	26	22	9	16	36	55	23	11	9
Ranjbar et al., 2019	Traditional dairy product	Culture	800	31	Disk diffusion	24	21	29	28	18	ND	9	ND	25	29	16	5	ND
Talimkhani and Mashak, 2017	Raw meat Raw milk Raw vegetables	Culture PCR	340	40	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gilani et al., 2017	Meat products	Culture	150	11	Disk diffusion	3	7	8	7	5	1	1	1	8	10	7	1	1
Ranjbar et al., 2016a, 2016b	Bottled mineral water	Culture	450	8	Disk diffusion	5	5	5	ND	4	ND	2	ND	5	6	5	ND	2
Ranjbar et al., 2016a, 2016b	Drinking water	Culture	400	12	Disk diffusion	6	9	6	ND	6	3	1	ND	9	12	7	2	3
Talaei et al., 2015	Raw milk	PCR	210	28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mousavi et al., 2017	Raw milk Cheese	PCR	250	37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mousavi and Dehkordi, 2014	Raw milk Traditional dairy product	Culture	920	180	Disk diffusion	126	32	117	138	23	ND	25	ND	127	152	61	ND	38
Hemmatinezhad et al., 2016	Ready to eat foods	Culture	550	74	Disk diffusion	66	34	70	54	27	21	19	23	43	69	40	9	22
Saeidi and Sheikhshahrokh, 2016	Raw milk Raw meat	Culture PCR	820	197	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ghorbani et al., 2016	Ready to eat foods	Culture PCR	300	60	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Momtaz et al., 2014	Raw meat	PCR	600	38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Amirhooshang et al., 2014	Drinking water	PCR	118	66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kazemi et al., 2012	Raw milk Drinking water Cheese Ice-cream	PCR	182	16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Rahimi and Kheirabadi, 2012	Raw milk	PCR	447	56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bahrami et al., 2011	Raw milk Drinking water	Culture	438	22	Disk diffusion	8	2	0	4	ND	ND	1	ND	ND	ND	ND	ND	ND
Safaei et al., 2011	Raw milk	ELISA PCR	92	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Atapoor et al., 2014	Vegetables Salads	Culture	460	44	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yahaghi et al., 2014	Vegetables Salads	Culture	430	59	Disk diffusion	46	13	40	35	5	2	9	9	14	36	35	5	8

Abbreviations: MTZ-metronidazole; CLR-clarithromycin; AMX-amoxicillin; TET-tetracycline; LVX-levofloxacin; RIF-rifampin; FRZ-furazolidone; STR-streptomycin; ERY-erythromycin; AMP-ampicillin; TMP-trimethoprim; SPI-spiramycin; CEF-cefsulodin; AST-antimicrobial susceptibility testing; ND-not determined; PCR-poly-merase chain reaction; ELISA-enzyme-linked immunosorbent assay.

s2 32.5% (95% CI: 18.7–50.2;  $I^2 = 91.7\%$ ; Q = 145.2; p = 0.00), VacA m1a 57.4% (95% CI: 41.9–71.5;  $I^2 = 90.4\%$ ; Q = 125.9; p = 0.00), VacA m1b 19.5% (95% CI: 10.8–32.8;  $I^2 = 90.1\%$ ; Q = 121.5; p = 0.00), VacA m2 48.9% (95% CI: 35.8–62.1;  $I^2 = 88.2\%$ ; Q = 101.7; p = 0.00), CagA 58.6% (95% CI: 46.5–69.7;  $I^2 = 83.8\%$ ; Q = 68.2; p = 0.00), IceA1 37.5% (95% CI: 24.2–53;  $I^2 = 77.9\%$ ; Q = 22.6; p = 0.00), IceA2 16.3% (95% CI: 7.5–32;  $I^2 = 80.4\%$ ; Q = 25.5; p = 0.00), OipA 38.1% (95% CI: 20.1–60.1;  $I^2 = 91.3\%$ ; Q = 57.5; p = 0.00) and BabA2 32.7% (95% CI: 16.5–54.4;  $I^2 = 46.9\%$ ; Q = 3.7; p = 0.15).

#### 4. Discussion

Determination of the reservoirs of *H. pylori* infection is faced with several challenges. However, there has been recent evidence suggesting that *H. pylori* may be a foodborne pathogen that can be isolated from water, vegetables and foods of animal origin (Quaglia and Dambrosio, 2018). It has been shown that the bacterium is able to survive for several days in various foodstuffs, vegetables and water, which were

polluted artificially (Quaglia and Dambrosio, 2018). For example, H. pylori can survive from 5 to 12 days in pasteurized and sterile milk stored at 4 °C, 7 days in ground beef stored at 4 °C and 3 to 5 days in vegetables stored at 8 °C (Quaglia and Dambrosio, 2018). Iran is known as a country with a high *H. pylori* prevalence in clinical specimens in the world (40% to 90%) (Khademi et al., 2017). Additionally, compared to the global average, the prevalence of H. pylori resistance to common drug therapeutic regimens is also high in Iran (Khademi et al., 2015). Considering the current situation, it seems that identification of other reservoirs of H. pylori infection and bacterial drug resistance in Iran is very important. According to the results of this systematic review and meta-analysis, the prevalence of H. pylori in water, vegetables and foods of animal origin was 11.4% (8.5-15.2) in Iran (Fig. 2A). This result can be attributed to poor hygiene management during production and preparation of foodstuff as well as contamination of drinking water sources. Approximately 91-100% of the Iranian population have access to safe drinking water service (WHO report, 2018). However, the situation is different in some geographical areas especially among people who live in rural areas and use river water for drinking with no efficient

#### Table 2

H. pylori genotype status among strains isolated from different types samples.

Author	Sample origin	Strain	H. pylori s	trains harbo	or each gen	h genotype (n)								
(Kei)		(11)	VacA s1a	VacA s1b	VacA s1c	VacA s2	VacA m1a	VacA m1b	VacA m2	CagA	IceA1	IceA2	OipA	BabA2
Ranjbar et al., 2018	Raw milk	67	56	22	7	52	54	19	46	49	31	13	25	30
Ranjbar et al., 2019	product	31	28	10	4	25	28	10	23	16	10	4	ND	ND
Talimkhani and Mashak,	Raw meat	40	35	12	6	33	35	12	25	32	ND	ND	ND	ND
2017	Raw vegetables													
Gilani et al., 2017	Meat products	11	3	1	1	1	2	1	0	2	ND	ND	ND	ND
Ranjbar et al., 2016a, 2016b	Bottled mineral water	8	8	2	0	4	7	2	4	5	5	1	2	2
Ranjbar et al., 2016a, 2016b	Drinking water	12	10	5	1	6	8	4	5	6	5	2	4	2
Talaei et al., 2015	Raw milk	28	15	8	2	4	5	3	17	20	ND	ND	ND	ND
Mousavi et al., 2017	Raw milk	37	10	3	1	5	9	2	4	8	ND	ND	ND	ND
	Cheese													
	Ice-cream													
Mousavi and Dehkordi, 2014	Raw milk	180	ND	ND	ND	ND	ND	ND	ND	138	ND	ND	45	ND
	Traditional dairy													
	product													
Hemmatinezhad et al., 2016	Ready to eat foods	74	58	20	13	6	38	8	56	31	10	3	14	ND
Saeidi and Sheikhshahrokh,	Raw milk	197	171	137	95	78	156	125	59	ND	ND	ND	ND	ND
2016	Raw meat													
Ghorbani et al., 2016	Ready to eat foods	60	40	10	6	4	31	6	34	ND	ND	ND	ND	ND
Momtaz et al., 2014	Raw meat	38	21	12	0	5	16	0	22	28	ND	ND	ND	ND
Amirhooshang et al., 2014	Drinking water	66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kazemi et al., 2012	Raw milk Drinking	16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	water													
	Cneese													
Dehimi and Khaimhadi 2012	Ice-cream	56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Rammi and Kneirabadi, 2012 Robromi et al. 2011	Raw IIIIK Row milk Drinking	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Baillailli et al., 2011	water	22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Safaei et al 2011	Raw milk	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Atapoor et al 2014	Vegetables	23 44	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
mapoor et al., 2017	Salads		110		110	110	110	110	110	ND.	.ND	ND .	1112	110
Yahaohi et al 2014	Vegetables	59	22	14	6	15	18	14	15	34	28	25	51	ND
1414611 Ct 41., 201 (	Salads	0,		- 1	~	10	10		10	51	20	20	51	

ND-not determined.

methods for purification. It seems that further epidemiological studies are needed to exactly determine *H. pylori* contamination in areas with limited access to safe drinking water in Iran. In the survey of Bianchini et al. (2015) and Osman et al. (2015), 0% and 22% of raw cow milk samples were positive for *H. pylori*, respectively, according to culture and nested PCR results. In another study, Angelidis et al. (2011)

recovered *H. pylori* in 20% of raw cow milk samples using fluorescence *in situ* hybridization (FISH) assay. Furthermore, several reports have assessed the prevalence of *H. pylori* in water. For example, Boehnke et al. in 2018 (49 positives out of 241 drinking water samples using PCR), Holman et al. in 2013 (4 positives out of 31 seawater samples using culture and PCR), Twing et al. in 2011 (21% using PCR) and Al-



Fig. 1. Flow chart of study selection.

Study name		Statist	ics for e	Event rate and 95% CI					
	Event rate	Lower limit	Upper limit	Z-Value	p-Value	Total			
Ranjbar-1	0.106	0.085	0.133	-16.471	0.000	67 / 630			
Ranjbar-2	0.039	0.027	0.055	-17.529	0.000	31/800			
Talimkhani	0.118	0.087	0.156	-11.970	0.000	40/340			
Gilani	0.073	0.041	0.128	-8.099	0.000	11/150			
Ranjbar-3	0.018	0.009	0.035	-11.246	0.000	8/450			
Ranjbar-4	0.030	0.017	0.052	-11.860	0.000	12/400			
Talaei	0.133	0.094	0.186	-9.221	0.000	28/210			
Mousavi-1	0.148	0.109	0.198	-9.828	0.000	37/250			
Mousavi-2	0.196	0.171	0.223	-17.010	0.000	180/920			
Hemmatinezhad	0.135	0.108	0.166	-14.896	0.000	74 / 550			
Saeidi	0.240	0.212	0.271	-14.086	0.000	197 / 820			
Ghorbani	0.200	0.159	0.249	-9.605	0.000	60/300			
Momtaz	0.063	0.046	0.086	-16.072	0.000	38/600			
Alvandi	0.559	0.469	0.646	1.286	0.199	66 / 118			
Kazemi	0.088	0.055	0.139	-8.937	0.000	16/182			
Rahimi	0.125	0.098	0.159	-13.601	0.000	56/447			
Bahrami	0.050	0.033	0.075	-13.437	0.000	22/438			
Ghasemian Safaei	0.272	0.191	0.371	-4.206	0.000	25/92			
Atapoor	0.096	0.072	0.126	-14.171	0.000	44 / 460			
Yahaghi	0.137	0.108	0.173	-13.118	0.000	59/430			
	0.114	0.085	0.152	-12.223	0.000				
							-1.00 -0.50 0.00 0.50 1.00		

## Meta-analysis

(A)



**(B)** 

Fig. 2. Forest plot (A) and funnel plot (B) showing the prevalence of H. pylori strains isolated from water, vegetables and foods of animal origin in Iran.

Sulami et al. in 2010 (10 out of 469 isolates using culture) identified H. pylori in water (Quaglia and Dambrosio, 2018). Divergent results reported from various countries can be due to differences in bacterial detection methods and hygiene management. H. pylori ability to survive in reservoirs other than human stomach may depend on the capacity to form biofilms in contaminated water and vegetables as well as ureadependent acid resistance in milk (Quaglia and Dambrosio, 2018). An important concern pertains to the introduction of antibiotic-resistant H. pylori strains into the human population through water, vegetables and foods of animal origin. Our results on the prevalence of H. pylori resistance in Iran is worrisome because compared to our previous study on clinical specimens in 2013 (Khademi et al., 2015), the resistance rate to commonly used antibiotics in human infections (66.3% to metronidazole, 42.4% to clarithromycin and 72% to amoxicillin) was high. This data is important for public health in Iran and is consistent with the World Health Organization (WHO) concerns on the prevalence of resistant strains, especially clarithromycin-resistant strains, in the world (WHO report, 2017). One possible explanation for the high prevalence of antibiotic resistance in H. pylori strains in Iran is extreme prescription of antibiotics in veterinary.

#### 5. Conclusion

Our findings revealed that isolation of H. pylori strains and identification of their virulence genes through culture and molecular methods in water, vegetables and foods of animal origin in Iran was considerable. The findings suggest that H. pylori can be transmitted similar to a waterborne and foodborne pathogen and various foodstuff and water may serve as natural reservoirs of the bacteria in Iran. However, further documents on genetic homology of H. pylori isolated from theses samples as well as clinical specimens are needed. On the other hand, our findings suggest that water, vegetables and foods of animal origin may also act as reservoirs of antibiotic-resistant H. pylori strains and greatly affect the efficacy of treatment in human infection. Therefore, to control the spread of virulent and resistant strains of H. pylori in water and foodstuff in Iran, the following approaches are recommended: continuous surveillance on the incidence of H. pylori and bacterial drug resistance through further epidemiological studies in wider geographical regions, supplying safe water and foodstuff via implementing decontamination and sterilization strategies, and monitoring staffs of food-producing companies.

#### Declaration of competing interest

The authors declare that there is no conflict of interest.

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