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Lijuan Fan[a], Ran Li[a], Hongyun Li, Jian Zhang and Lingyun Wang*

Detection of *CagA*, *VacA*, *IceA1* and *IceA2* virulent genes in *Helicobacter pylori* isolated from gastric ulcer patients

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Abstract

Background: Virulence factors of *Helicobacter pylori* including *cagA*, *vacA*, *iceA* and their association with clinical manifestation varied widely with different subpopulations. The objective of the study was to determine the prevalence of *cagA*, *iceA1*, *iceA2*, *vacA*, *vacA s1/s2*, *vacA m1/m2*, Western type *cagA* and East Asian type *cagA* virulence genes in *H. pylori* isolated from gastric ulcer patients and evaluate the association of these genes with gender, age, smoking and alcohol consumption.
Methods: Gastric biopsy samples from 172 patients were collected. *H. pylori* virulence genes, *cagA*, *vacA*, *iceA1*, *iceA2*, *vacA s1/s2*, *vacA m1/m2*, Western type *cagA* and East Asian type *cagA* were detected using polymerase chain reaction (PCR).
Results: Of the gastric biopsy samples collected, 48.3% of samples grew *H. pylori*. The *vacA* (68.7%) was the predominant virulence gene detected and associated with male patients and patients within the age group of 31–40 years. The *cagA* was the second most common gene detected and significantly associated with alcoholic patients.
Conclusions: *H. pylori* infection rate was 48.3% and was associated with patients who were smokers or had a history of smoking. The majority of our isolates were positive for any one of the virulence genes tested indicating that these isolates were highly virulent in nature.

Keywords: gastric ulcer; *H. pylori*; virulence genes.

Introduction

Helicobacter pylori is associated with gastrointestinal disorders including peptic ulcers, gastric cancer, chronic gastritis and lymphoma [1, 2]. Various factors such as age, ethnicity, geography and socio-economic status contribute to the varied prevalence of *H. pylori* [1]. *H. pylori* incidence tends to be low in many developed countries due to improved social-economic status, while it remains high in developing countries [3, 4]. *H. pylori* is highly prevalent in some countries; however, the disease severity varies from one region to another mainly due to the differences in the virulence factors among the strains [5]. Several virulence factors play an important role in the pathogenesis of the disease, which includs the cytotoxin-associated gene A (*cagA*), vacuolating cytotoxin A (*vacA*) and *iceA* genes [5–7]. The *cagA* gene encoded by the CagA protein is found in more than 50% of the *H. pylori* isolates. The *cagA* gene is associated with increased production of interleukin-8 (IL-8), mucosal inflammation, nuclear factor-kB activation and the development of peptic ulcer disease (PUD) and gastric cancer (GC). The *cagA* gene, a marker of the cag pathogenicity island has been associated with PUD, atrophic gastritis and adenocarcinoma [7–9]. About 60%–70% of *H. pylori* strains tend to possess the *cagA* gene [8, 9]. The vacuolating toxin encodes the *vacA* gene, consists of three variable regions, the signal peptide encoding *s* region and two alleles (*s1* and *s2*). The *s1* region is further subdivided into *s1a*, *s1b* and *s1c* subtypes. The middle region (m region) of the *vac* gene has two alleles, *m1* and *m2* [10]. The activity of the *vac* gene was determined by the combination of *s*- and *m*-regions, the *s1m1* combination was considered as the most virulent and produces a high amount of toxin, while the combination of *s2m2* produces an inactive toxin [10, 11]. The *cagA* and *vacA* genes play an important role in determining the clinical outcomes of the infected patients [12]. The *iceA* gene induced by the contact

[a]Lijuan Fan and Ran Li contributed equally to this work.
***Corresponding author: Lingyun Wang,** Department of Gastroenterology, Jining First People's Hospital, Shandong Province, 272011, P.R. China, Tel./Fax: +86 537 225 3431, E-Mail: AlvinkThomasod@yahoo.com
Lijuan Fan, Hongyun Li and Jian Zhang: Department of Gastroenterology, Jining First People's Hospital, Shandong Province, P.R. China
Ran Li: Department of Gastroenterology, Third Affiliated Hospital of Shandong Medical College, Shandong Province, P.R. China

with human gastric cells produces a high level of IL8 and is highly associated with PUD [13]. The *iceA* has two alleles, *iceA1* and *iceA2* [14].

The prevalence of *H. pylori* virulence factors including *cagA*, *iceA* and *vacA* and their association with clinical manifestation varied widely with different subpopulations [15–17]. However, studies regarding the prevalence of *cagA* and *iceA*, *vacA* genes are scarce. Hence, the present study determined the prevalence of *cagA*, *iceA1*, *iceA2*, *vacA*, *vacA s1/s2*, *vacA m1/m2*, Western type *cagA* and East Asian type *cagA* virulence genes in *H. pylori* isolated from gastric ulcer patients. The study also evaluated the association of these virulence genes with gender, age, smoking and alcohol consumption.

Materials and methods

One hundred and seventy-two consecutive gastric biopsy samples from 172 patients with a gastric ulcer, admitted to the endoscopic section at the Department of Gastroenterology, Jining First People's Hospital, Shandong Province and the Department of Gastroenterology, Third Affiliated Hospital of Shandong Medical College, Shandong Province, China between September 2015 and December 2017 were collected. Patients who received prior antibiotic therapy within the previous 4 weeks were excluded from the study. An informed consent was obtained from all patients or their legal heir. The hospital Ethics Committee approved the study. Using a sterile container sample was collected aseptically and immediately sent for *H. pylori* culture.

Isolation and identification of *H. pylori*

The collected samples were aseptically dissected into two pieces for rapid urease test and *H. pylori* culture. Using the modified rapid urease agar medium, the rapid urease test was performed [18]. Briefly, after collection one part of the biopsy samples were immediately inoculated into urea agar and incubated at room temperature for 2 h. The change in color of the medium from yellow to red indicates a positive reaction. *Helicobacter pylori* culture was performed by inoculating the biopsy samples on the modified Columbia agar plates (Merck, Germany) supplemented with horse lysed blood (10%, v/v). The plates were then incubated in an anaerobic condition at 37 °C for 2–3 days under microaerobic conditions [19]. After incubation, based on the colony morphology, gram staining, positive oxidase, catalase, and urease tests the organisms were identified as *H. pylori*. The isolates were stored in Columbia broth supplemented with sterile glycerol (20%, v/v) at –70 °C until used.

DNA extraction

The *H. pylori* isolates were subjected to DNA extraction by the boiling lysis method, briefly, 200 µL of *H. pylori* culture was incubated at 95 °C for 10 min, spun at 14,000 *g* for 10 min to collect the supernatant containing DNA. Both the DNA samples were stored at –20 °C until further analysis. DNA quantification and purity was determined using NanoDrop ND 1000 (NanoDrop Technologies, Wilmington, DE, USA).

Detection of virulence genes

Detection of *cagA*, *vacA*, *iceA1* and *iceA2* virulence genes were performed using polymerase chain reaction (PCR). The primer sequences, cycling conditions and expected amplicon sizes are described in Table 1. Two duplex PCR was performed to detect the *iceA1/iceA2* and *cagA/vacA* genes separately. PCR was done using a 50 µL master mix containing 5 µL of template DNA, 0.4 µM of each primer, 200 µM of dNTPs, one unit of Taq polymerase enzyme and 5 µL of 10× reaction buffer. After PCR, the amplicons were resolved in 1.5% agarose gel and visualized under a UV transilluminator.

Detection of *cagA* and *VacA* gene variance

The *cagA* and *vacA* variant genes, Western type *cagA*, East Asian *cagA* and *vacA s1/s2* and *vacA m1/m2* genes were detected by PCR. The primer sequences, cycling conditions and expected amplicon sizes were described in Table 1. Four different PCRs were performed to detect the Western type *cagA*, East Asian *cagA*, *vacA s1/s2* and *vacA m1/m2* genes, respectively. The Western type *cagA* gene PCR was expected to yield two amplicons of sizes ~218–227 and ~174–177 bp. Similarly, the East Asian *cagA* was expected to yield two amplicons of size ~293–299 bp and ~455–461 bp. The PCR master mix was prepared as described above. After PCR, the amplicons were resolved in 1.8% agarose gel and visualized under a UV transilluminator.

Statistics

Continuous variables were presented as mean and ranges; categorical variables as numbers and percentages.

Table 1: Primer sequence of *H. pylori* virulence genes.

Genes	Primers	Size, bp	Cycling conditions	References
iceA1	F: 5′-GTGTTTTTAACCAAAGTATC-3′ R: 5′-CTATAGCCATTATCTTTGCA-3′	246	95 °C, 1 min; 50 °C, 1 min; 72 °C, 1 min (30 cycles)	[20]
iceA2	F: 5′-GTTGGGTATATCACAATTTAT-3′ R: 5′-TTTCCCTATTTTCTAGTAGGT-3′	229/334[a]		
cagA	F-5′-GATAACAGCCAAGCTTTTGAGG-3′ R-5′-CTGCAAAAGATTGTTTGG*CAGA*-3′	349	94 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min (35 cycles)	[21]
vacA	F-5′-ATGGAAATACAACAAACACAC-3′ R-5′-CTGCTTGAATGCGCCAAAC-3′	286		
vacA s1/s2	F-5′-ATGGAAATACAACAAACACAC-3′ R-5′-CTGCTTGAATGCGCCAAAC-3′	259/286	95 °C, 50 s; 58 °C, 50 s; 72 °C,50 s (35 cycles)	[1]
vacA m1/m2	F-5′-CAATCTGTCCAATCAAGCGAG-3′ R-5′-GCGTCAAAATAATTCCAAGG-3′	567/642	95 °C, 50 s; 54 °C, 50 s; 72 °C,50 s (35 cycles)	
Western type *cagA* (*cagT/cag W*)	F-5′-ACCCTAGTCGGTAATGGG-3′ R-5′-TGCCCTACAMCACCSAAACCAC-3′ F-5′-AAAAATTGACCRACTCAATC-3′ R-5′-GCTTTAGCTTCTGAYACYGC-3′	~218–227 and ~174–177	95 °C, 1 min; 52 °C, 1 min; 72 °C, 1 min (35 cycles)	[22]
East Asian type *cagA* (*cagT/cag j*)	F-5′-ACCCTAGTCGGTAATGGG-3′ R-5′-GCAATTTTGTTAATCCGGTC-3′ F-5′-GCATCAGCAGGTAAAGGAGT-3′ R-5′-GCTTTAGCTTCTGAYACYGC-3′	~293–299 ~455–461	95 °C, 1 min; 52 °C, 1 min; 72 °C, 1 min (35 cycles)	[5]

[a]The primers yield a fragment of 229 or 334 bp depending on the presence of a repetitive sequence of 105 nucleotides codifying for 35 amino acids in some *iceA2* alleles.

Chi-square tests were performed to determine the statistical significance using MINITAB statistical software (MINITAB ver. 13.1; MINITAB Inc., PA, USA). The data was considered statistically significant if the p-value was <0.05.

Results

Of the 172 patients, 97 (56.4%) were male and 75 (43.6%) were female. The mean age was 46 ± 7.2 (range 19–63) years. Of the included patients, 113 (65.7%) were smokers or had a history of smoking and 108 (62.8%) were alcoholic patients. Demographic details of the patients are given in Table 2. Of the 172 gastric biopsy samples, 118 (68.6%) samples were positive for rapid urease test and 83 (48.3%) samples (48 from male and 35 from female) grew *H. pylori*. All the samples which isolated *H. pylori* were found to be positive for the rapid urease test. No significant association was found between *H. pylori* infection in male and female (27.9% vs. 20.3%; p = 0.714) patients and between alcoholic and non-alcoholic (30.2% vs. 18.0%; p = 0.971) patients. However, we observed a significant association between *H. pylori* infection and patients who were smokers or had a history of smoking (26.7%; p = 0.006). We found that patients within the age group of 41–50 years were highly likely to be *H. pylori* positive (18.0%) followed by patients within the age group of 31–40 years (14.5%). However, no significant association was found between the *H. pylori* infection and different age groups (p = 0.979).

Table 2: Baseline characteristics of patients.

Variables	No. of patients
Male	97 (56.4%)
Female	75 (43.6%)
Mean age (SD) years	46 ± 7.2
Age, years	
19–30	32 (18.6%)
31–40	53 (30.8%)
41–50	64 (37.2%)
51–63	23 (13.4%)
Smoker	113 (65.7%)
Non-smoker	59 (34.3%)
Alcoholic	108 (62.8%)
Non-alcoholic	64 (37.2%)

SD, standard deviation.

Of the 83 isolates subjected to PCR, 57 (68.7%) isolates were positive for the *vacA* gene, 53 (63.9%) isolates were positive for the *cagA* gene, 46 (55.4%) isolates were positive for the *iceA2* gene and 37 (44.6%) isolates were positive for the *iceA1* gene. We found that the presence of *vacA* (49.4% vs. 19.3%; p = 0.000) and *iceA2* (26.5% vs. 29%; p = 0.040) genes were significantly associated with male and female patients, respectively. When analyzed

Table 3: Detection and distribution of *H. pylori* virulence genes.

Variables	Culture positive (n=172)	Distribution of virulence genes among the isolates (n=83)			
		cagA	*vacA*	*iceA1*	*iceA2*
Male	48 (27.9%)	34 (41.0%)	41 (49.4%)	20 (24.1%)	22 (26.5%)
Female	35 (20.3%)	19 (22.9%)	16 (19.3%)	17 (20.5%)	24 (29.0%)
χ^2-Test	p=0.714	p=0.121	**p=0.000**	p=0.532	**p=0.040**
19–30 years	15 (8.7%)	8 (9.6%)	12 (14.5%)	7 (8.4%)	10 (12.0%)
31–40 years	25 (14.5%)	21 (25.3%)	28 (33.7%)	18 (21.7%)	14 (16.9%)
41–50 years	31 (18.0%)	17 (20.5%)	14 (16.9%)	6 (7.2%)	16 (19.3%)
51–63 years	12 (7.0%)	7 (8.4%)	3 (3.6%)	7 (7.2%)	6 (7.2%)
χ^2-Test	p=0.979	p=0.095	**p=0.000**	**p=0.010**	p=0.815
Smoker	46 (26.7%)	28 (33.7%)	33 (39.8%)	19 (22.9%)	24 (29.0%)
Non-smoker	37 (21.5%)	25 (30.1%)	24 (29.0%)	18 (21.7%)	22 (26.5%)
χ^2-Test	**p=0.006**	p=0.528	p=0.502	p=0.503	p=0.507
Alcoholic	52 (30.2%)	38 (45.8%)	37 (44.6%)	27 (32.5%)	31 (37.3%)
Non-alcoholic	31 (18.0%)	15 (18.1%)	20 (24.1%)	10 (12.0%)	15 (18.1%)
χ^2-Test	p=0.971	**p=0.024**	p=0.528	p=0.081	p=0.076

Bold values indicates statistical significant values.

between different age groups *vacA* (33.7%; p=0.000) and *iceA1* (21.7%; p=0.010) genes were significantly associated with the age group of 31–40 years. The presence of *cagA* gene was significantly associated with alcoholic patients (45.8% vs. 18.1%; p=0.024). There was no significant association between the tested virulence genes and smoking history of the patients (p>0.05). We also found that there was no statistically significant association of the *iceA2* gene in relation to gender, age, smoking and alcoholic history of the patients (p>0.05). Details on the distribution of virulence genes among the isolates are given in Table 3.

Among the isolates tested for virulence genes, 20 isolates, each were positive for *cagA* and *iceA2* genes alone, respectively. In the combinational analysis, we found that 18 (21.7%) isolates were positive for both *vacA* and *iceA2* genes, which was the most predominant combination detected. The *cagA* and *vacA*, *cagA* and *iceA1* were the next most common combinations detected, respectively, in each of the 12 (14.5%) isolates. Three isolates were positive for the *cagA*, *vacA* and *iceA1* genes, three isolates were positive for the *cagA*, *iceA1* and *iceA2* genes. Another three isolates were positive for all the tested (*cagA*, *vacA*, *iceA1*, *iceA2*) virulence genes (Table 4). In the present study, 89.2% (74/83) of *H. pylori* isolates were positive for at least one of the tested virulence genes (*cagA*, *vacA*, *iceA1*, *iceA2*) which implies that a majority of the *H. pylori* isolates had virulence potential as evidenced by the detection of virulence genes.

Of the 83 isolates tested for *vacA* and *cagA* gene variance, 52 (62.7%) isolates were positive for the *vacA s1/s2* gene, 61 (73.5%) isolates were positive for the *vacA m1/m2* gene, 12 (14.5%) isolates were positive for the Western type *cagA* gene and 56 (67.5%) isolates were positive for the East Asian type *cagA* gene. We found that the presence of *vacA s1/s2* (42.2% vs. 20.5%; p=0.024) and East Asian type *cagA* (28.9% vs. 38.6%; p=0.004) genes were significantly associated with male and female patients, respectively. When analyzed between different age groups the *vacA s1/s2* (28.9%; p=0.000) gene was significantly associated with the age group of 31–40 years. The presence of the *vacA m1/m2* gene was significantly associated with non-smoking patients (42.2% vs. 31.3%; p=0.000). The presence of the *vacA s1/s2* gene was significantly associated with alcoholic patients (49.4% vs. 13.3%; p=0.000). We also found that there was no statistically significant association of the Western type *cagA* gene in relation to gender, age, smoking and alcoholic history of the patients (p>0.05). Details on the distribution of *vacA* and *cagA*

Table 4: Detection and combination of *H. pylori* virulence genes.

Gene combinations	No. of isolates (n=83)
cagA	20 (24.1%)
vacA	13 (15.7%)
iceA1	8 (9.6%)
iceA2	20 (24.1%)
cagA+*vacA*	12 (14.5%)
cagA+*iceA1*	12 (14.5%)
vacA+*iceA1*	6 (7.2%)
vacA+*iceA2*	18 (21.7%)
cagA+*vacA*+*iceA1*	3 (3.6%)
cagA+*iceA1*+*iceA2*	3 (3.6%)
vacA+*iceA1*+*iceA2*	2 (2.4%)
cagA+*vacA*+*iceA1*+*iceA2*	3 (3.6%)

Table 5: Distribution of *vacA* and *cagA* gene variance among the isolates (n = 83).

Variables	Distribution of *vacA* and *cagA* gene variance among the isolates (n = 83)			
	vacA s1/s2	*vacA m1/m2*	Western type *cagA*	East Asian type *cagA*
Male	35 (42.2%)	32 (38.6%)	5 (6%)	24 (28.9%)
Female	17 (20.5%)	29 (34.9%)	7 (8.4%)	32 (38.6%)
χ^2-Test	**p = 0.024**	p = 0.099	p = 0.220	**p = 0.004**
19–30 years	8 (9.6%)	12 (14.5%)	1 (1.2%)	13 (15.7%)
31–40 years	24 (28.9%)	21 (25.3%)	1 (1.2%)	15 (18.1%)
41–50 years	11 (13.3%)	18 (21.7%)	8 (9.6%)	20 (24.1%)
51–63 years	9 (10.8%)	10 (12%)	2 (2.4%)	8 (9.6%)
χ^2-Test	**p = 0.000**	p = 0.106	p = 0.101	p = 0.350
Smoker	28 (33.7%)	26 (31.3%)	5 (3.6%)	29 (34.9%)
Non-smoker	24 (28.9%)	35 (42.2%)	7 (10.8%)	27 (32.5%)
χ^2-Test	p = 0.706	**p = 0.000**	p = 0.322	p = 0.337
Alcoholic	41 (49.4%)	39 (47%)	6 (7.2%)	36 (43.4%)
Non-alcoholic	11 (13.3%)	22 (26.5%)	6 (7.2%)	20 (24.1%)
χ^2-Test	**p = 0.000**	p = 0.687	p = 0.327	p = 0.657

Bold values indicates statistical significant values.

variance genes among the isolates are given in Table 5. Analysis of the *vacA* variance genes showed that five (6%) isolates amplified only the *vacA s1* gene, seven (8.4%) isolates amplified only the *vacA s2* gene, four (4.8%) isolates amplified only *vacA m1* gene and six (7.2%) isolates amplified the only *vacA m2* gene, the presence of these genes did not differ significantly ($p > 0.05$).

When compared between *vacA* and *vacA s1/s2*, *vacA m1/m2* gene-positive isolates; a total of 32 (38.6%) isolates amplified the *vacA s1/s2*, *vacA m1/m2* genes. Three isolates which were positive for the *vacA* gene did not amplify either *vacA s1/s2* or *vacA m1/m2* genes. Five and seven isolates which were positive for the *vacA s1/s2* and *vacA m1/m2* genes, respectively, did not amplify the *vacA* gene. When compared between *cagA* and *cagA* Western and East Asian type gene-positive isolates; four isolates which were positive for the *cagA* gene did not amplify either the *cagA* Western type or *cagA* East Asian type genes. Two and eight isolates which were positive for the *cagA* Western type and *cagA* East-Asian type genes, respectively, did not amplify the *cagA* gene.

Discussion

H. pylori, a causative agent of gastric ulcers, can lead to the development of GC [23]. Although the *H. pylori* prevalence is declining in many developed countries [24, 25], it remains high in developing countries and ranges from 70% to 90% [3, 4, 26, 27]. The association of *H. pylori* with various gastrointestinal diseases makes it a major health problem, worldwide [1, 2, 28]. In our study, the *H. pylori* prevalence rate among patients with gastric ulcer was 48.3%. A study from Pakistan, reported the prevalence of *H. pylori* at a rate of 53% and 80%, respectively, in patients with duodenal ulcer and individuals presenting symptoms of various upper gastrointestinal diseases [29, 30]. This was higher than that reported (48.3%) in our study from gastric ulcer patients. Another study from Bangladesh conducted in patients with a gastric ulcer reported *H. pylori* prevalence at a rate of 75% which was higher than that reported in our study [26]. A study from Singapore which assessed *H. pylori* infection in patients with gastric ulcer using a *Campylobacter*-like organism (CLO) test and the histology reported a prevalence rate of 67.5%, which is higher than that reported in our study [31]. A study from Mongolia which included dyspeptic patients from all parts of Mongolia reported an overall *H. pylori* infection at a rate of 80%, which was higher than that reported in our study [32]. The prevalence of *H. pylori* in our study is lower than that reported from dyspeptic patients in Bhutan (86%) [33]. Among the dyspeptic patients, the prevalence of *H. pylori* in African countries including Morocco (75.5%), Ethiopia (65.7%) and Nigeria (93.6%) was higher than that reported in our study [34–36]. Due to rapid urbanization in China, the prevalence of *H. pylori* infection has decreased and could be a reason for the low prevalence of *H. pylori* in our study [24]. In our study, there was no significant association between the presence of *H. pylori* infection and gender ($p = 0.714$) and alcohol consumption ($p = 0.971$). Smoking is considered as one of the independent risk factors for *H. pylori* infection; in our study, we found a significant ($p = 0.006$) association between the presence of *H. pylori* and patients who were smokers or had a history of smoking [37].

H. pylori possess several virulence factors which are associated with various gastrointestinal diseases [5–7]. *H. pylori* adheres to the mucosa of the stomach, colonizes it, persists for a long time and leads to an inflammatory response. The colonization of *H. pylori* as such does not cause infection; however, it can increase the risk of developing various gastrointestinal diseases including gastric ulcer, GC and lymphoma [38]. Specific analysis of *H. pylori* virulence factors can predict post *H. pylori* infectious disorders [38]. In our study, *vacA* (68.7%) was the predominant gene detected among our isolates followed by *cagA* (63.9%), *iceA2* (55.4%) and *iceA1* (44.6%) genes. A meta-analysis which investigated

the association of *H. pylori* virulence factors with GC reported that the presence of *cagA* and *vacA* genes were significantly associated with GC [38]. In our study, 63.9% isolates were positive for the *cagA* gene which is higher (50%) than that reported in the biopsy samples collected from gastric patients from Northeast China [23]. The presence of *cagA* in our study is lower than that reported in India (90.9%), detected in the biopsy samples from gastric ulcer patients, which also reported a significant association between peptic ulcer and the presence of the *cagA* gene [39]. Another study from India reported that 77.27% of biopsy samples from acid-peptic disease were positive for the *cagA* gene, which was higher than that reported in our study. The study also reported that the presence of the *cagA* gene was significant in female patients; however, in our study there was no significant ($p = 0.121$) difference found between the two genders [40]. Another study from Thailand reported that 98.2% of their gastric biopsy samples from dyspeptic patients were positive for the *cagA* gene [41]. In contrast, a lower rate of the *cagA* gene was reported in biopsy samples from gastric ulcer patients from Saudi Arabia (30%) and stool samples from asymptomatic individuals from Japan [1, 42]. The presence of the *cagA* in our study is much lower than that reported in *H. pylori* isolated from gastric biopsy samples from Korea (99%) [22].

We reported that 14.5% and 67.5% of our isolates were positive for Western and East Asian type *cagA* genes, respectively. As previously reported, East Asia type *cagA* was more prevalent in Asian countries, the majority of our isolates were positive for the East Asian type *cagA* gene compared to the Western type *cagA* gene [43]. In contrast, a study from Northeast China reported that 31% of their biopsy samples from gastric ulcer patients were positive for the Western type *cagA* gene which is higher and 20% of their samples were positive for the East Asian type *cagA* gene which is lower than that reported in our study [23]. In contrast, a study which determined the prevalence of *H. pylori* infection and genotypes of virulence factors in a Nepalese population reported, a higher rate of the Western type *cagA* gene (94.1%) compared to the East Asian type *cagA* gene (5.9%) [44]. The East Asian *cagA* is more virulent than Western type *cagA* [45, 46]. Additionally, the East Asian *cagA* gene plays a potential role in the development of gastric diseases caused by *H. pylori* infections [47]. Thus, the high presence of the East Asian *cagA* gene in our isolates indicates that our isolates are highly virulent in nature. Also, the significant association of the East Asian *cagA* gene with female patients ($p = 0.004$) in our study indicates that they may be at high risk of *H. pylori* infection with significant clinical manifestation.

In our study, 68.7% isolates were positive for the *vacA* gene which was higher than that reported from India (4.5%) [40]. The *vacA m1/m2* (73.5%) was the predominant *vacA* variant gene reported in our study. It has been reported that the presence of *vacA s1* and *m1* genotypes may indicate an increased risk of peptic ulcer and GC in Latin America and the Middle East [38]. In our study, 38.6% of isolates were positive for *vacA s1/s2* and *vacA m1/m2* genes indicating that the patients from whom these isolates were isolated are at a risk of GC in addition to the gastric ulcer they harbor. A study from Saudi Arabia reported the combined *vacA s1/s2* gene in 30% of isolates and *vacA m1/m2* in 0% of *H. pylori* isolates from gastric ulcer patients which were lower than that reported from our study [1]. The study reported that *vacA s1/s2* was the predominant genotype in gastric ulcer patients; in contrast, *vacA m1/m2* was the predominant genotype in our study [1]. We found that the *vacA* and *vacA s1/s2* genes were significantly associated with male patients and patients within the age group of 23–40 years. Additionally, *vacA s1/s2* was significantly associated with alcoholic patients. In our study, the co-occurrence of *vacA* variance and *cagA* variance gene combinations did not differ significantly ($p > 0.05$), hence were not discussed in detail.

In our study, 46 (55.4%) isolates were positive for the *iceA2* gene and 37 (44.6%) isolates were positive for the *iceA1* gene. Chomvarin et al. [41] who determined the association between the various genotypes of *H. pylori* and its clinical manifestations in Thai dyspeptic patients, reported that an overall 45.5% and 33.1% of the samples were positive for the *iceA1* and *iceA2* genes, respectively. Of this 35% and 40% of the gastric ulcer samples were positive for *iceA1* and *iceA2*, respectively, which is lower than that reported in our study [41]. A study from Saudi Arabia which determined the prevalence of *cagA* and *iceA* genes from biopsy samples of patients with both gastric and peptic ulcers reported that 81.7% of the samples were positive for the *iceA* gene, of which 92.3% were from peptic ulcer and 75% were from gastritis cases [48]. We found that a majority of our isolates were positive for any one of the tested virulence genes indicating that these isolates are highly virulent in nature. As reported earlier, the presence of *vacA* and *cagA* genes is associated with GC, the high prevalence of these genes among our isolates may warrant a monitoring for GC in patients from whom these strains are isolated.

Conclusions

The *H. pylori* infection rate in our study was 48.3% and was associated with patients who were smokers or had a

history of smoking. The *vacA* gene (68.7%) was the predominant virulence gene detected and was associated with male patients and patients within the age group of 31–40 years. The *cagA* gene was the second most common gene detected and was significantly associated with alcoholic patients. As reported earlier, the predominance of this gene warrants a close monitoring for GC in patients from whom these strains were isolated. The majority of our isolates were positive for any one of the virulence genes tested indicating that these isolates were highly virulent in nature.

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