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Helicobacter pylori outer membrane protein and virulence marker differences in expatriate patients

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SUMMARY

We studied the prevalence of *Helicobacter pylori* virulence markers, e.g. cytotoxin associated gene (*cag*A), *cag*A promoter, vacuolating associated cytotoxin A (*vac*A) alleles induced by contact with epithelium (*ice*A type), and outer membrane protein Q (*hop*Q) in expatriates and compared them with those in local residents. Gastric biopsies were obtained at endoscopy for culture, histology and PCR for virulence marker and *hop*Q. Of 309 patients, 236 (76%) were males with a mean age of 45 years. A total of 102 patients were expatriates. *hop*Q type 1 was present in 98 (47%) local residents compared to 88 (86%) expatriates ($P < 0{\cdot}001$), while *hop*Q type 2 was present in 176 (85%) local residents, compared to 60 (59%) expatriates ($P < 0{\cdot}001$). *H. pylori* virulence marker *cag*A was positive in 97 (47%) local residents compared to 86 (84%) expatriates ($P < 0{\cdot}001$) while *cag*A-P was positive in 72 (35%) local residents compared to 87 (85%) expatriates ($P < 0{\cdot}001$). *ice*A type 1 was positive in 157 (76%) local residents compared to 45 (44%) expatriates ($P < 0{\cdot}001$), while *ice*A type 2 was positive in 81 (39%) local residents compared to 86 (84%) expatriates ($P < 0{\cdot}001$). Distribution of *H. pylori cag*A, *cag*A promoter, *ice*A and *hop*Q type in local residents and expatriates was different. *H. pylori* virulence markers were associated with severe pathology in expatriates.

Key words: *cag*A, *cag*A promoter, gastric carcinoma, gastric ulcer, gastritis, *Helicobacter pylori*, *ice*A, outer membrane protein Q.

INTRODUCTION

Helicobacter pylori is a major cause of gastroduodenal diseases like gastritis, peptic ulcer, gastric carcinoma (GC) and mucosal associated lymphoid tissue lymphoma [1, 2]. The prevalence of *H. pylori* is very heterogeneous in Asia with some populations having extremely low prevalence, e.g. Malays [3]. In developing countries, like Pakistan and Bangladesh, infection with *H. pylori* is more frequent in the general population and is acquired at an early age. It has been shown previously that *H. pylori* is acquired by most individuals in early childhood [4]. In Pakistan, the prevalence of *H. pylori* seropositivity in children aged 11–15 years was 53·5% [4]. As *H. pylori* is transmitted via the gastro-oral and faeco-oral route, overcrowding, poor sanitation, lower socioeconomic status and poor water supply are the major factors that result in its acquisition at a higher frequency and lower age in less developed Asian countries [5, 6]. By contrast, in industrialized and developed countries like the United States, the prevalence of *H. pylori* has decreased [7]. In Asian countries like

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Japan, Singapore and China, the frequency of *H. pylori* infection has been reported to be somewhat lower [8]. By contrast, people living in less developed countries of Asia with high frequency of *H. pylori* infection [4–6] acquired at an earlier age have the lowest risk of developing GC [8]. It is interesting to note that in Japan despite a lower frequency of *H. pylori* infection, this country has the highest frequency of GC.

Genetic diversity among *H. pylori* strains and host characteristics play a role in the varying clinical outcomes in persons colonized with *H. pylori*. Candidate markers for distinguishing disease-associated *H. pylori* strains from less virulent strains include the presence of the *cag* pathogenicity island, *vac*A alleles *s1/m1*, induced on contact with epithelium (*ice*A types 1/2), intact outer immunoprotein A (*oip*A) alleles, outer membrane protein (OMP) Q (*hop*Q), etc. [9–13]. However, presence of these OMPs and virulence markers does not always correlate well with gastroduodenal diseases [14].

The inflammatory immune response during acute *H. pylori* infection of the gastric epithelium triggers a mutation burst and an increased frequency of mutation and recombination events occur in *H. pylori* OMP genes [15, 16]. It is known that changes in immunogenic OMPs facilitates rapid adaptation of *H. pylori* to an individual host, evasion of the host's immune system resulting in chronic infection [16]. Horizontal transmission of *H. pylori* is common in populations living in low socioeconomic conditions found in developing countries and is known to be associated with infection by multiple *H. pylori* strains [17, 18]. In view of the high frequency of DNA transformation and lateral gene transfer reported in *H. pylori* strains we wanted to see whether there was any differences in the *H. pylori* strains isolated from overseas Pakistani residents (expatriates) compared to local residents. Existence of differences may suggest that expatriates' *H. pylori* genome acquires characteristics of the country of temporary residence. It is also possible that these newly acquired *H. pylori* genomic characteristics may be transfered to the *H. pylori* gene pool of their country of origin once individuals return. There is a high prevalence of virulent *H. pylori* strains in countries like China, South Korea and Japan that are known to be associated with severe gastroduodenal diseases.We studied the prevalence of *H. pylori*, its virulence marker, e.g. *cag*A, *cag*A promoter, *vac*A alleles *s1a/1b, m1, m2* and *s2, iceA* types 1 and 2 and OMP Q (*hop*Q) in expatriates and compared them with those in local residents.

MATERIALS AND METHODS

Patients

Three hundred and nine patients were enrolled from an endoscopy suite providing upper gastrointestinal endoscopy for upper gastrointestinal symptoms and who were positive by rapid urease test for *H. pylori* infection at endoscopy. There were 236 (76%) males and 73 (24%) females with a mean age of 45 ± 13 years (range 18–79 years). These patients attended the gastroenterology outpatient and endoscopy suite from January 2013 to December 2014. Of these, 207 (67%) were local residents with a mean age of 46 ± 14 years (male:female ratio 148:59) while 102 (33%) were expatriates with a mean age of 44 ± 9 years (male:female ratio 88:14). There was no significant difference in the mean age of the two groups. There were significantly ($P = 0{\cdot}004$) fewer female patients in the expatriate group compared to local residents due to males being the family earner and travelling abroad in the Pakistani culture. These expatriate patients had lived abroad for more than 10 years for various reasons and had moved in their mid-twenties to a foreign country. These expatriates included 48 (15%) from China; 43 (14%) from South Korea and 11 (4%) from Japan. These expatriates had lived abroad for more than 10 years and had paid infrequent visits to their country of origin for social reasons or in seeking medical healthcare. The study was approved by the Ethics Review Committee of Aga Khan University. All patients gave informed consent for endoscopy and participation in the study. None of the patients had received antibiotics, acid reducing drugs such as H2 receptor antagonists, proton pump inhibitors, non-steroidal anti-inflammatory drugs or bismuth compounds in the last 8 weeks. The clinical symptoms at the time of presentation and endoscopic findings were noted. Gastric biopsy specimens were taken from an area of inflammation in the antrum and corpus. Two biopsy specimens were taken for each of: rapid urease test (Pronto Dry, Medical Instruments Corporation, Switzerland), histology and polymerase chain reaction (PCR). Two gastric biopsy specimens were used for a rapid urease test (Pronto Dry). Specimens for histology were dispatched in formalin and for PCR in 0·9% normal saline. PCR for *cag*A 5′-terminal, *cag*A promoter region, *vac*A alleles for the signal (s), i.e. *s1a, s1b, s2* and middle (m), i.e. *m1, m2, ice*A (types 1 and 2) and *hop*Q alleles (types 1 and 2) were analysed.

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Bacterial culture

The specimens were transported immediately in sterile normal saline to isolate *H. pylori*. Each specimen was homogenized in a sterile Eppendorf tube with electric homogenizer and inoculated onto Columbia blood agar (Oxoid, UK) medium supplemented with 10% defibrinated sheep blood and Dent's supplement (containing vancomycin, trimethoprim, cefsulodin and amphotericin B) and incubated at 37 °C under microaerobic conditions using anaerobic jars and strips (Campygen strips, Oxoid) for isolation and growth for 5–7 days. Plates were then examined for bacterial growth and typical colonies were selected for identification. The identity of *H. pylori* was confirmed by Gram stain, and production of urease and catalase. *H. pylori* isolates were defined as Gram-negative spiral-shaped bacilli that were catalase positive and rapidly (<30 min) urease positive.

Histology

Gastric biopsy specimens for histopathology were stained with haematoxylin and eosin (H&E) stain for the detection of *H. pylori* and degree of gastritis. The degree of gastritis as determined on H&E stain was scored in accordance with the Sydney system [19].

Extraction of genomic DNA

The bacterial cells on chocolate agar plate were washed twice with phosphate-buffered saline (PBS, pH 8·0) then centrifuged at 1008 ***g*** for 20 min. *H. pylori* DNA was extracted by the phenol/chloroform method similar to a method described previously [20].

PCR

*cag*A *and vac*A *genotyping*

Amplification of *cag*A, *cag*A promoter, and *vac*A alleles by PCR was performed in a volume of 25 μl containing 10 mmol/l Tris–HCl (pH 8·3), 50 mmol KCl, 1·5–2·5 mmol/l $MgCl_2$, 200 μmol/l deoxynucleoside triphosphates, 2 U *Taq* DNA polymerase (Promega, USA) and 25 pmol of both forward and reverse primers (MWG automatic synthesizer, Germany) (Table 1) as used previously [21]. PCR was performed in a PerkinElmer 9700 thermal cycler (PerkinElmer, USA). The amplification cycles for *cag*A, *cag*A promoter, and *vac*A alleles are given in Table 1. Positive and negative reagent control reactions were performed with each batch of amplifications. DNA from *H. pylori* strains ATCC 43504 (*vac*A *s1am1, cag*A positive), ATCC 51932 (*vac*A *s2m2, cag*A negative) and ATCC 43526 (*vacA s1bm1, cag*A positive) was used to define the accuracy of the *cag*A and *vac*A alleles [21]. After PCR, the amplified PCR products were electrophoresed in 2% agarose gels containing Tris/acetate/EDTA acid, stained with ethidium bromide, and visualized under a short wavelength ultraviolet light source.

*ice*A *genotyping*

For analysis of the *ice*A genotype, primers previously described [22] were used. Primers iceA1 F and iceA1R yielded a fragment of 247 bp for the *ice*A1 allele, and primers iceA2 F and iceA2R yielded a fragment of 229 or 334 bp, respectively, according to the existence of repeated sequences of 105 nt.

*hop*Q *genotyping*

The *hop*Q genotype (types 1 and 2) were determined by PCR methods [23]. Primers and conditions used for PCR amplification of *hop*Q sequences of types 1 and 2 are shown in Table 1. Primers used for PCR amplification of *hop*Q alleles are given in (Table 1) [23].

Sample size

Two different sample sizes were calculated in keeping with the aim of the study. The first sample size was calculated to estimate the prevalence of *H. pylori* in overseas Pakistani residents (expatriates), taking the prevalence of 58% (as reported in the Pakistani population) that gives the maximum sample size, with 95% level of confidence and 6% bound on the error of estimation [4]. After accounting for a non-response rate of about 10%, the minimum sample size required is about 285 participants using the formula [24]:

$$N = \frac{4(z_{\text{crit}})^2 p(1-p)}{D^2},$$

The second sample size of 160 was derived using the formula

$$N = 2 \cdot \left[Z_{\text{crit}} \sqrt{2\bar{p}(1-\bar{p})} + z_{\text{pwr}} \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right]^2 / D^{2s},$$

assuming that 73 patients in each group will help achieve a 5% significance level using a two-sided equivalence test of proportions [24]. This number of patients would provide the study with the ability to

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Table 1. *Oligonucleotide primers used in typing of* Helicobacter pylori

Region amplified	Primer designation	Primer sequence (5′–3′)	PCR product (bp)	PCR cycles
*cag*A	D008 R008	GGTCAAAATGCGGTCATGG TTAGAATAATCAACAAACATCACGCCAT	297	1 cycle of 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 90 s, 1 cycle of 72 °C for 5 min
*cag*A-P	cagAP-F1 cagAP-R1	GTGGGTAAAAATGTGAATCG CTGCAAAAGATTGTTTGGCAGA	730	1 cycle of 94 °C for 5 min followed by 35 cycles of 1 min at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. The final cycle 72 °C for 7 min
*vac*A *s1a*	SS1-F VA1-R	GTCAGCATCACACCGCAAC CTGCTTGAATGCGCCAAAC	190	1 cycle of 95 °C for 5 min; 35 cycles of 95 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min, 1 cycle of 72 °C for 5 min
*vac*A *s1b*	SS3-F VA1-R	AGCGCCATACCGCAAGAG CTGCTTGAATGCGCCAAAC	187	
*vac*A *m1*	VA3-F VA3-R	GGTCAAAATGCGGTCATGG CCATTGGTACCTGTAGAAAC3′	190	
*vac*A *m2*	VA4-F VA4-R	GGAGCCCCAGGAAACATTG CATAACTAGCGCCTTGCAC	352	
*ice*A1	iceA1 F iceA1R	GTGTTTTTAACCAAAGTATC CTATAGCCASTYTCTTTGCA	247	1 cycle consisting of 1 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 52 °C, and 1 min at 72 °C. The final cycle 72 °C for 7 min
*ice*A2	iceA2 F iceA2R	GTTGGGTATATCACAATTTAT TTRCCCTATTTTCTAGTAGGT	229/334	
*hop*Q type 1 region A	OP5136 F OP4829R	CAACGATAATGGCACAAACT GTCGTATCAATAACAGAAGTTG	524	95 °C for 1 min, 50 °C or 55 °C for 1 min and 72 °C for 2 min, for a total of 30 cycles
*hop*Q type 1 region B	OP4070 F BA8705R	CAATTCCCCTGCCTATCAAGCC TGATGTGGTTACATGCGCTTC	372	
*hop*Q type 2	BA8363 F BA8364R	TCCAATCCAGAAGCGATTAA GTTTTAATGGTTACTTCCACC	430	

Source: Covacci & Rappuoli [21]; Van Doorn *et al.* [22]; Cao & Cover [23].

detect a 20% difference in the *H. pylori* virulence marker and HopQ protein in local residents and expatriates, with a power of 80%. Taking into account a dropout rate of 10%, the final sample size of 300 participants would suffice for both objectives.

Statistical analysis

The descriptive analysis was performed for demographic and clinical features. Results were presented as mean ± standard deviation for quantitative variables and number (percentage) for qualitative variables. Differences in proportions were assessed using Pearson's χ^2, Fisher's exact or the likelihood ratio tests, as appropriate. To assess the univariate association between the outcomes and potential factors, odds ratios (ORs) and their 95% confidence intervals (CIs) were computed by logistic regression analysis. All significant factors in the univariate analysis were considered for inclusion in the multivariable logistic model. A P value of <0·05 was considered statistically significant. All P values were two sided. Statistical interpretation of data was performed using the computerized software program SPSS v. 19.0 (IBM Corp., USA).

RESULTS

Abdominal pain was significantly more common in local residents (183, 88%) compared to expatriates (72, 71%) ($P < 0·001$), while GC was diagnosed in 30 (29%) expatriates compared to 24 (12%) local residents ($P < 0·001$) (Table 2).

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Table 2. *Comparison of residents symptoms, diagnosis and histological changes in local citizens and expatriates*

	Local resident	Expatriates	*P* value
Symptoms			
Abdominal pain			
Positive	183 (88)	72 (71)	<0·001
Negative	24 (12)	30 (29)	
Nausea			
Positive	4 (2)	0 (0)	0·306
Negative	203 (98)	102 (100)	
Haematemesis			
Positive	13 (6)	2 (2)	0·157
Negative	194 (94)	100 (98)	
Melaena			
Positive	4 (2)	6 (6)	0·087
Negative	203 (98)	96 (94)	
Weight loss			
Positive	3 (2)	22 (22)	<0·001
Negative	204 (98)	80 (78)	
Diagnosis			
Non-ulcer dyspepsia (gastric erythema)	143 (69)	42 (41)	<0·001
Gastric ulcer	23 (11)	20 (20)	0·042
Duodenal ulcer	17 (8)	10 (10)	0·641
Gastric carcinoma	24 (12)	30 (29)	<0·001
Histology			
Grade of gastritis			
Chronic active gastritis	151 (73)	89 (87)	0·005
Chronic inflammation	56 (27)	13 (13)	
Lymphoid aggregates			
Positive	20 (10)	50 (49)	<0·001
Negative	187 (90)	52 (51)	
Intestinal metaplasia			
Positive	32 (15)	6 (6)	0·016
Negative	175 (85)	96 (94)	
Severity of inflammation			
Mild	160 (77)	43 (42)	<0·001
Moderate	47 (23)	59 (58)	
*hop*Q type 1			
Positive	98 (47)	88 (86)	<0·001
Negative	109 (53)	14 (14)	
*hop*Q type 2			
Positive	176 (85)	60 (59)	<0·001
Negative	31 (15)	42 (41)	

Values given are *n* (%).

H. pylori OMP (*hop*Q) types

In local residents, *hop*Q type 1 was associated with abdominal pain in 84 (86%, $P = 0·001$), gastric erythema on endoscopic examination (GST) in 63 (64%, $P < 0·001$), chronic active gastritis in 65 (66%, $P = 0·002$), and *cag*A was positive in 56 (57%, $P = 0·005$) individuals. *hop*Q type 2 was also associated with abdominal pain in 158 (90%, $P = 0·02$), GST in 122 (69%, $P = 0·014$) and *vac*A *s1a* in 119 (68%, $P = 0·016$) local residents.

In expatriates, *hop*Q was associated with abdominal pain in 62 (70%, $P = 0·001$) and weight loss in 22 (25%, $P = 0·001$) individuals, GST in 32 (36%, $P = 0·019$) and GC in 30 (34%, $P = 0·009$) individuals, respectively. *hop*Q type 1 was associated with histological mild gastritis in 43 (49%, $P < 0·001$), *cag*A in 82 (93%, $P < 0·001$) and *cag*A promoter in 81 (92%, $P < 0·001$) individuals, respectively. *hop*Q type 2 was associated with abdominal pain in 46 (77%, $P < 0·001$), and GST in 28 (47%, $P < 0·001$) individuals. On histology, *hop*Q type 2 was associated with histological mild gastritis in 35 (58%, $P < 0·001$) local residents, and in expatriates, it was associated with *vac*A *s1a* in 34 (57%, $P < 0·001$) individuals.

A single *hop*Q type was present in 105 (58%) local residents compared to 56 (55%) expatriates ($P = 0·563$), while multiple types of *hop*Q in were present in 76 (42%) local residents compared to 46 (45%) expatriates ($P = 0·563$). Both types were absent in one (0·5%) local resident ($P = 0·563$).

*cag*A and *cag*A promoter

H. pylori virulence marker *cag*A was positive in 97 (47%) local residents compared to 86 (84%) expatriates ($P < 0·001$), while *cag*A-P was positive in 72 (35%) local residents compared to 87 (85%) expatriates ($P < 0·001$) (Table 3).

*vac*A alleles

There was no difference noted in the distribution of *vac*A allele *s1a, s1b* and *m1* in local residents and expatriates (Table 3).

iceA types

iceA was positive in 157 (76%) and *iceA* type 2 in 81 (39%) local residents, respectively (Table 4). *iceA* type 1 was associated with gastritis (non-ulcer dyspepsia; NUD) in 95 (61%) and GC in 24 (15%) ($P < 0·001$) individuals. It was also associated with intestinal metaplasia in 32 (80%, $P < 0·001$) individuals. *ice*A type 1 was associated with *cag*A, c*ag*A promoter and *vac*A *s1a* in 86 (55%, $P < 0·001$), 68 (43%, $P < 0·001$), and 95 (60%, $P = 0·047$), individuals, respectively.

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Table 3. *Comparison of* Helicobacter pylori *virulence markers in the study groups*

	Local resident	Expatriates	*P* value
*cag*A			
Positive	97 (47)	86 (84)*	<0·001
Negative	110 (53)	16 (16)	
*cag*A promoter			
Positive	72 (35)	87 (85)*	<0·001
Negative	135 (65)	15 (15)	
*vac*A allele			
S1a			
Positive	133 (64)	73 (72)	0·199
Negative	74 (36)	29 (28)	
S1b			
Positive	42 (20)	13 (13)	0·103
Negative	165 (80)	89 (87)	
m1			
Positive	126 (61)	66 (65)	0·513
Negative	81 (39)	36 (35)	
m2			
Positive	96 (46)*	15 (15)	<0·001
Negative	111 (54)	87 (85)	
s2			
Positive	59 (28)	49 (48)*	0·001
Negative	148 (72)	53 (52)	
*ice*A type 1			
Positive	157 (76)*	45 (44)	<0·001
Negative	50 (24)	57 (56)	
*ice*A type 2			
Positive	81 (39)	86 (84)*	<0·001
Negative	126 (61)	16 (16)	

Values given are *n* (%).
* $P < 0·05$ significant.

In expatriates distribution of *iceA* type 1 was 45 (44%) and *ice*A type 2 was 86 (84%), respectively. *ice*A type 1 was associated with abdominal pain, lymphoid aggregates, intestinal metaplasia and mild gastric mucosal inflammation while *ice*A type 2 was associated with GST and GC. On histology *ice*A type 2 was also associated with chronic active gastritis, lymphoid aggregates and mild to moderate degree of inflammation. *ice*A type 1 was associated with c*ag*A [44 (98%), $P = 0·001$] and *cag*A promoter [45 (100%), $P < 0·001$], respectively.

Multiple *ice*A type was positive in 29 (28%) expatriates compared to 33 (18%) local residents ($P = 0·044$). *ice*A types were absent in 11 (6%) local residents compared to none in expatriates ($P = 0·009$). The *H. pylori* genotype *cag*A *ice*A2 in local residents was 40 (19%) compared to 67 (66%) in expatriates ($P < 0·001$).

Multivariate analysis showed that expatriates' *H. pylori* strains were associated with moderately severe mucosal inflammation (OR 4·75, 95% CI 1·49–15·08, $P = 0·008$), lymphoid aggregates (OR 7·25, 95% CI 2·10–25·0, $P = 0·002$), *hop*Q type 1 (OR 3·19, 95% CI 1·06–9·63, $P < 0·03$), *cag*A promoter (OR 22·54, 95% CI 7·53–67·40, $P < 0·001$), *vac*A *m2* (OR 0·075, 95% CI 0·02–0·23, $P < 0·001$), *vacA s2* (OR 8·77, 95% CI 2·87–26·74, $P < 0·001$) and *ice*A type 1 (OR 0·03, 95% CI 0·01–0·10, $P < 0·001$) (Table 4).

DISCUSSION

This study shows that patients in both groups presented with abdominal pain associated with NUD. Weight loss was common in expatriates and was associated with GC. Gastric mucosal changes of *H. pylori* infection were evident in expatriates as chronic active inflammation while mild chronic inflammation was more common in local residents. The virulence marker *cag*A and *cag*A promoter region positive *H. pylori* infection were frequent in expatriates and was associated with severe *H. pylori* related pathology (Table 3). No difference was noted in the distribution of *vac*A alleles *s1a, s1b* and *m1* in the two groups (Table 3). *H. pylori* infection in expatriates was predominantly with *ice*A type 2 and it was significantly associated with *vac*A *s1a* [58 (67%), $P = 0·036$]. However, in this group, *ice*A type 1 was also seen to be associated with *cag*A [44 (98%), $P = 0·001$] and *cag*A promoter region [45 (100%), $P < 0·001$] (Table 3). In local residents, *hop*Q type 2 was predominant compared to *hop*Q type 1 in expatriates (Table 2). In both groups there were *H. pylori* strains that demonstrated multiple types of *hop*Q. Their number was greater in expatriates compared to local residents suggesting co-infection was marked in expatriate patients.

The implications of this study are that NUD with *H. pylori* infection was also common in expatriates residing abroad for a lengthy period. Gastric ulcer was marginally significant in expatriates compared to local residents, whereas duodenal ulcer was not. GC in expatriates was associated with chronic active inflammatory changes and intestinal metaplasia.

The distribution of *H. pylori* virulence markers in expatriates was different from local residents in that *H. pylori* were 84–85% *cag*A and *cag*A promoter positive compared to the distribution of 45–51% previously described in local residents [25, 26]. The expatriates' *H. pylori* strain increase in *cag*A and *cag*A promoter region positivity is in keeping with *H. pylori* strains described in East Asian countries. In a previous local

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Table 4. *Multivariable model for factor predicting expatriate* Helicobacter pylori *virulence*

Characteristics	OR (95% CI)	*P* value
Lymphoid aggregate		
Negative	1·0	0·002
Positive	7·25 (2·10–25·0)	
Severity of inflammation		
Mild	1·0	0·008
Moderate	4·75 (1·49–15·08)	
*cag*A promoter		
Negative	1·0	<0·001
Positive	22·54 (7·53–67·40)	
m2		
Negative	1·0	<0·001
Positive	0·075 (0·02–0·23)	
s2		
Negative	1·0	<0·001
Positive	8·77 (2·87–26·74)	
*ice*A type 1		
Negative	1·0	<0·001
Positive	0·03 (0·01–0·10)	
*hop*Q type 1		
Negative	1·0	0·03
Positive	3·19 (1·06–9·63)	

OR, Odds ratio; CI, confidence interval.
Values given are *n* (%).
* $P < 0{\cdot}05$ significant.

study that looked at the intactness of the *cag* pathogenecity activity island (*cag*-PAI) in 115 clinical strains of *H. pylori* only 31 (28%) were positive for the five *cag*-PAI loci [27]. It has been reported that the *ice*A allelic type distribution was independent of *cag*A and *vac*A status. Previously, a significant association between the presence of the *ice*A type 1 allele and peptic ulcer disease has been described [28]. The *ice*A type 1 allele is reported to be predominant in Japan and Korea, and the *ice*A type 2 allele in the United States and Colombia [29]. In expatriates, infection with *H. pylori* strains demonstrating multiple *ice*A types was frequent at 28% compared to 18% in local residents.

The wide CI of factors in the multivariable analysis suggests that the study may be underpowered (Table 4). However, the findings from the multivariable analysis support our conclusion that expatriates have more virulence markers and also more gastroduodenal diseases. Further, significance of *vac*A *m2* and *ice*A type 1 with ORs of 0·075 and 0·03, respectively, in local resident suggests that they are associated with nonulcer gastritis and less florid *H. pylori* associated disease in keeping with our previous results [25, 30].

There was clearly a difference in the distribution of virulence marker and *hop*Q types in local residents and expatriates. This is in keeping with the exogenous DNA taken up by *H. pylori* and its integration into the chromosome by homologous recombination or replication as a plasmid [31]. For survival and persistent growth in the presence of a constant immune response permanent adaptation of the bacteria is required [31]. Such adaptive processes include mechanisms of reversible or irreversible genome changes. It is known that clonal transmission is followed by a rapid adaptation to the new host, so that *H. pylori* isolates from different subjects are almost always unique [31]. Each *H. pylori* isolate contains a distinct set of strain-specific genes often located in plasticity regions. They generally contain complete sets of genes required to produce type IV secretion machineries, as well as genes encoding different DNA-processing proteins [32–34] that are mobile genetic elements capable of horizontal gene transfer between bacterial cells. In an earlier study which was conducted in the Malaya population [35], the virulence genotype of *H. pylori* strain was different in the immigrant Chinese and Indian population but the native Malays showed a mixture of both [35].

The limitation of this study includes an absence of information regarding smoking and salt intake that are independent risk factors for GC. Dietary intake of red meat, high fat, and heavy alcohol use positively influences carcinogenesis while fresh fruit, vegetables and vitamin C reduce the risk. Certain dietary constituents of local cuisine reduce *H. pylori* viability, colonization and infection may also reduce the GC risk [36–38]. In this study, the chance of possible selection bias could not be ignored. All the patients attending the endoscopy suite of the tertiary-care private hospital in the study period were included. All expatriates and residents were sufficiently well off financially to access healthcare services at this hospital. There is a possibility of information bias in regards to the length of time expatriates had spent in the foreign country. The stated length of time all expatriates were away was >10 years and all left their country of origin as adults. An earlier study from China foundthat children aged 5–6 years and adults had comparable rates of *H. pylori* infection at ~70% [39]. A review of *H. pylori* prevalence in USA found that individuals who immigrated as adults (aged >20 years) had a rate of infection, consistent with their country of origin [40]. Moreover, spontaneous elimination of *H. pylori* infection was found rarely in adults [41]. Socioeconomic

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factors play a distinct role in transmission due to differences in waste disposal, hygiene, and practices such as sharing of eating utensils, e.g. chopsticks and premastication by parents [42]. There is a possibility that nonviable oral *H. pylori* participate in horizontal gene transfer that is increased in unsanitary, crowded environments, making multiple infection and recombination among strains more common in populations living in crowded conditions [40, 43]. Hence, these expatriates were likely to have maintained their *H. pylori* infection and experienced change in their infecting *H. pylori* strains' dynamic genome.

In conclusion, the local residents and expatriates demonstrated differences in the distribution of *H. pylori* virulence marker *cag*A, *cag*A promoter, *ice*A and *hop*Q types. In expatriates severe gastroduodenal disease could be explained by the distribution of virulence markers, although the causes of GC and gastroduodenal diseases are multifactorial in nature.

DECLARATION OF INTEREST

None.

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