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BRIEF ARTICLE

Ethnicity association of *Helicobacter pylori* virulence genotype and metronidazole susceptibility

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Abstract

AIM: To characterise the *cag* pathogenicity island in *Helicobacter pylori* (*H. pylori*) isolates by analysing the strains' *vacA* alleles and metronidazole susceptibilities in light of patient ethnicity and clinical outcome.

METHODS: Ninety-five *H. pylori* clinical isolates obtained from patients with dyspepsia living in Malaysia were analysed in this study. Six genes in the *cag*PAI region (*cagE*, *cagM*, *cagT*, *cag13*, *cag10* and *cag67*) and *vacA* alleles of the *H. pylori* isolates were identified by polymerase chain reaction. The isolates' metronidazole susceptibility was also determined using the *E*-test method, and the resistant gene was characterised by sequencing.

RESULTS: More than 90% of the tested isolates had at least one gene in the *cag*PAI region, and *cag67* was predominantly detected in the strains isolated from the Chinese patients, compared with the Malay and Indian patients ($P < 0.0001$). The majority of the isolates (88%) exhibited partial deletion (rearrangement) in the *cag*PAI region, with nineteen different patterns observed. Strains with intact or deleted *cag*PAI regions were detected in 3.2% and 8.4% of isolates, respectively. The prevalence of *vacA* s1m1 was significantly higher in the Malay and Indian isolates, whereas the isolates from the Chinese patients were predominantly genotyped as *vacA* s1m2 ($P = 0.018$). Additionally, the isolates from the Chinese patients were more sensitive to metronidazole than the isolates from the Malay and Indian patients ($P = 0.047$). Although we attempted to relate the *cag*PAI genotypes, *vacA* alleles and metronidazole susceptibilities to disease outcome, no association was observed. The *vacA* alleles were distributed evenly among the strains with intact, partially deleted or deleted *cag*PAI regions. Interestingly, the strains exhibiting an intact *cag*PAI region were sensitive to metronidazole, whereas the strains with a deleted *cag*PAI were more resistant.

CONCLUSION: Successful colonisation by different *H. pylori* genotypes is dependent on the host's genetic makeup and may play an important role in the clinical outcome.

Key words: *Helicobacter pylori*; *cag* pathogenicity island; *vacA* alleles; Metronidazole susceptibility

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INTRODUCTION

Helicobacter pylori (*H. pylori*) demonstrates great genetic diversity, as evidenced by the apparently unlimited number of unique strains differing in genome size, gene order, gene content and allelic profile[1]. The high levels of genetic diversity in clinical isolates and the presence of certain virulence genotypes may confer a selective advantage to certain strains in different hosts. Therefore, the virulence genotype of *H. pylori* is a potentially useful predictor of the clinical outcome of gastric mucosal colonisation.

H. pylori possess a number of virulence determinants that modulate the organism's interaction with a host[2,3]. These virulence factors include the secreted, vacuolating cytotoxin VacA and the gene products of the pathogenicity island (*cag*PAI). The presence of these genetic loci correlates with the more severe *H. pylori*-associated pathologies, peptic ulcers and gastric cancer. Screening for the *cag*PAI genes is frequently performed due to the genes' involvement in virulence characteristics. An infection with *cag*PAI-positive *H. pylori* strains significantly increases the risk of developing severe gastric mucosal inflammation, duodenal ulceration and gastric cancer[4-6]. It has been observed that only one-half to two-thirds of isolates from Western patients express the *cag*PAI region, whereas almost all of the East Asian strains express this region. As there is limited information on the structure of the *cag*PAI in Malaysian patients, the role of *cag*PAI rearrangement in *H. pylori* isolates from various ethnic populations within this country should be investigated.

The *vacA* gene is another *H. pylori* virulence factor that exhibits great polymorphism and diverse allelic combination. The variable structure, resulting in different allelic arrangements in the gene, is related to differences in cytotoxin production and to the distinct clinical outcomes of *H. pylori* infection[7-9]. For example, a recent study performed at our institute has demonstrated a correlation between the presence of genetic polymorphisms in the *vacA* gene and the severity of gastritis[10].

H. pylori infection can be effectively cured by antibiotics. More specifically, metronidazole is commonly used in conjunction with either amoxicillin or clarithromycin and a proton-pump inhibitor to eradicate *H. pylori* infection[11,12]. Metronidazole is also used in the treatment of other diseases, such as gynaecological infections, and has contributed to the increased antibiotic resistance of *H. pylori*[13]. In Malaysia, the prevalence of metronidazole-resistant strains is 37%, which may be one of the factors contributing to the failed eradication of *H. pylori* infection in the country[14].

The population of Malaysia consists of three major ethnic groups (Malay, Chinese and Indian) that are historically associated with differences in *H. pylori* infection[15-17]. *H. pylori*-associated gastroduodenal diseases typically do not reflect the prevalence of *H. pylori* infection[15]. Furthermore, geographical and population differences in *H. pylori* virulence factors and disease severity underscore the importance of investigating the relationship between the genotypes of the causative strains and the clinical outcome. Therefore, to further understand the dynamics of the genetic traits of the *H. pylori cag*PAI regions, *vacA* alleles and metronidazole susceptibilities in Malaysia's multi-ethnic population, we investigated the possible association between these *H. pylori* virulence factors and infection in patients of different ethnicities and clinical outcomes.

MATERIALS AND METHODS

Patients

Patients with dyspepsia who had undergone an upper gastrointestinal scope (OGDS) at Universiti Kebangsaan Malaysia Medical Centre (Kuala Lumpur, Malaysia) between May 2004 and December 2007 were recruited. Any patients who had received antibiotics 4 wk prior to OGDS were excluded. Written informed consent was obtained from each patient, and the protocol was approved by the Research Ethics Committee (protocol number FF-075-2003) of the University.

H. pylori culture

Biopsies were subcultured for *H. pylori* on Columbia agar base (Oxoid, Basingstoke, England) containing Dent's supplement (Oxoid, Basingstoke, England) and 7% defibrinated sheep blood. The plates were incubated at 37 ℃ for 5 d under microaerophilic conditions.

DNA extraction

H. pylori genomic DNA was prepared using a High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Detection of metronidazole susceptibility and resistance genotype

Metronidazole susceptibility was determined using the E-test method (AB Biodisk, Solna, Sweden). First, an *H. pylori* culture suspension with a McFarland turbidity of 3.0 was used to inoculate Columbia agar supplemented with 10% defibrinated sheep blood. The plates were then incubated at 35 ± 2 ℃ for 3-5 d under microaerophilic conditions. The isolates were classified as resistant to metronidazole when the MIC value was > 8 μg/mL[18]. The *rdxA* genes were amplified by PCR using specific primers, as previously described[19], and then subjected to sequencing.

Detection of cagPAI genes

Two loci were selected in the *cag* I region (*cagE* and

cagM), and four loci were chosen from the *cag* Ⅱ region (*cagT*, *cag13*, *cag10* and *cag67*). Additionally, the insertion sequence IS605 was selected. The presence of these selected genes was determined by PCR using specific primers, as previously described[20]. PCR was conducted in 25 μL volumes containing 1 × PCR buffer, 0.2 mmol/L dNTP mix, 10 pmol of each primer, 1 U Taq polymerase (BioTherm, GeneCraft, Germany) and 1 μL of the DNA sample. The PCR amplification conditions consisted of 35 cycles at 94 ℃ for 1 min, 50-55 ℃ for 30 s, 72 ℃ for 1 min, and one cycle for the final extension at 72 ℃ for 10 min. The amplified product was electrophoresed on a 1% agarose gel.

Detection of vacA alleles

The *vacA* genotyping was performed by multiplex PCR using specific primers, as previously described[10,21]. PCR was conducted in 25-μL volumes containing 1 × PCR buffer, 0.2 mmol/L dNTP mix, 6 pmol of each primer, 0.75 U Taq polymerase (BioTherm, GeneCraft, Germany) and 1 μL of the DNA sample. The PCR amplification conditions consisted of an initial denaturation step at 94 ℃ for 4 min, followed by 30 cycles of denaturation at 94 ℃ for 30 s, primer annealing at 55 ℃ for 30 s, extension at 72 ℃ for 30 s, and one cycle for the final extension at 72 ℃ for 7 min. The amplified products were electrophoresed on a 1.5% agarose gel.

Statistical analysis

A statistical analysis was performed using the χ^2 test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

H. pylori clinical isolates

A total of 95 clinical *H. pylori* isolates were obtained from 40 male and 55 female patients with a mean age of 55.47 ± 16.87 years (age range of 17-89 years) and from three different ethnic groups (12 Malays, 59 Chinese and 24 Indians). The endoscopic findings were as follows: nonulcer dyspepsia (n = 76), gastric ulcer (n = 9), duodenal ulcer (n = 6) and both gastric and duodenal ulcers (n = 2). The OGDS findings were not available for two of the patients. Nonulcer dyspepsia was further classified as endoscopically normal stomach (n = 12), antrum-predominant gastritis (n = 43), corpus-predominant gastritis (n = 2) and pangastritis (n = 19). No significant association was found between patient ethnicity (Chinese versus non-Chinese) and the endoscopic findings (nonulcer dyspepsia versus peptic ulcer).

cagPAI genotype

Of the 95 *H. pylori* isolates, 87 (91.6%) were *cag*PAI-positive, and the remaining 8 (8.4%) lacked all selected *cag*PAI genes. For the *cag*PAI-positive strains, intact *cag*PAI genes were detected only in 3 isolates, whereas other isolates exhibited partially deleted (rearranged) *cag*PAI genes. Additionally, the *IS605* gene was detected in 4 (4.2%) isolates. Further analyses indicated that the positivity of the *H. pylori* isolates was 61.1% (58/95), 53.7% (51/95), 4.2% (4/95), 82.1% (78/95), 76.8% (73/95) and 65.3% (62/95) for *cag67*, *cag10*, *cag13*, *cagT*, *cagM* and *cagE*, respectively.

Table 1 Distribution of *Helicobacter pylori cag*PAI genes isolated from patients of different ethnicities *n* (%)

*cag*PAI genes	Ethnic groups			*P* value
	Malay	Chinese	Indian	
cag Ⅰ region				
cagE	7 (58.3)	41 (69.5)	14 (58.3)	0.541
cagM	9 (75.0)	45 (76.3)	19 (79.2)	0.948
cag Ⅱ region				
cagT	11 (91.7)	49 (83.1)	18 (75.0)	
cag13	0	4 (6.8)	0	
cag10	6 (50.0)	29 (49.2)	16 (66.7)	0.336
cag67	6 (50.0)	47 (79.7)	5 (20.8)	< 0.0001

As shown in Table 1, the presence of *cagE* and *cagM* in the *cag*I region was distributed evenly among the isolates from patients of different ethnicities, with no significant differences between the ethnicities. The *cagT* gene was frequently detected in isolates from the Malay patients, whereas *cag13* was only detected in the isolates from the Chinese patients. The presence of *cag67* was significantly higher in the isolates from the Chinese patients than in the isolates from the Malay patients and was lowest in the isolates from the Indian patients. The *cag*PAI genes were detected in greater than 50% of the isolates from the nonulcer and ulcer patients, except for *cag13*, which was only detected in the patients with a normal stomach or gastritis. Thus, different *cag*PAI genes were present in the majority of the isolates examined, irrespective of disease state.

Of the 95 clinical isolates, only 3 (3.2%) contained an intact *cag*PAI, whereas 84 (88.4%) exhibited partial deletions within the *cag*PAI region, and 8 (8.4%) lacked the entire selected gene in the *cag*PAI. As shown in Table 2, thirteen isolates appeared to have a diverged *cag*PAI, in which the two selected genes for the *cag* Ⅰ region had been deleted, although the genes in the *cag* Ⅱ region were conserved, with at least one gene present. In contrast, ten strains had only one gene in either the *cag* Ⅰ or the *cag* Ⅱ region. The single gene detected was *cagM*, *cagT* and *cag67* in 3, 6 and 1 isolate, respectively. The most commonly partially deleted *cag*PAI genotypes were detected in 26 (27.4%), 16 (16.8%) and 13 (13.7%) isolates, with *cag*PAI regions of types C, I and N, respectively. These isolates exhibited at least four genes in the *cag*PAI region, and the genes in the *cag*I region were conserved in these isolates. Additionally, the *IS605* gene was detected in the isolates from the Chinese (n = 2) and Indian (n = 2) patients. Three of these isolates contained all of the selected genes for *cag*PAI, except *cag13*, and one isolate lacked the *cag13* and *cag10* genes.

As shown in Table 3, three isolates with an intact *cag*-

Table 2 *cag*PAI genotypes of *Helicobacter pylori* clinical isolates *n* (%)

*cag*PAI type	*cag* Ⅱ region				*cag* Ⅰ region		Total
	cag67	*cag10*	*cag13*	*cagT*	*cagM*	*cagE*	
A	+	+	+	+	+	+	3 (3.2)
B	+	-	+	-	-	-	1 (1.1)
C	+	+	-	+	+	+	26 (27.4)
D	+	+	-	+	+	-	2 (2.1)
E	+	+	-	-	+	+	1 (1.1)
F	+	+	-	-	+	-	1 (1.1)
G	+	+	-	-	-	-	1 (1.1)
H	+	-	-	-	-	-	1 (1.1)
I	+	-	-	+	+	+	16 (16.8)
J	+	-	-	+	+	-	2 (2.1)
K	+	-	-	+	-	+	1 (1.1)
L	+	-	-	-	+	+	1 (1.1)
M	+	-	-	+	-	-	2 (2.1)
N	-	+	-	+	+	+	13 (13.7)
O	-	+	-	+	+	-	2 (2.1)
P	-	+	-	+	-	-	2 (2.1)
Q	-	-	-	+	+	+	1 (1.1)
R	-	-	-	+	+	-	2 (2.1)
S	-	-	-	+	-	-	6 (6.3)
T	-	-	-	-	+	-	3 (3.2)
U	-	-	-	-	-	-	8 (8.4)

PAI were obtained from the Chinese patients. Nineteen of the 59 (32.2%) isolates from the Chinese patients possessed partially deleted *cag*PAI regions of type C, compared with 25% and 16.7% of the isolates from the Malay and Indian patients, respectively. Interestingly, the isolates from the Indian patients showed a high proportion of partially deleted *cag*PAI regions of type N (37.5%) and deleted *cag*PAI regions (16.7%). An analysis of the disease state, with reference to strain genotype, revealed a relationship between the *cag*PAI genotype and disease severity. The isolates from those patients with peptic-ulcer disease (PUD) primarily exhibited *cag*PAI regions of type C, which contained more genes in the rearranged *cag*PAI genotypes. In contrast, a larger number of isolates from the patients with pangastritis or antrum-predominant gastritis possessed *cag*PAI regions of type I or N, respectively, compared with the isolates from the patients with peptic ulcers.

vacA genotype

The *vacA* s1m1, s1m2 and s2m2 genotypes were identified in 51.6% (49/95), 42.1% (40/95) and 4.2% (4/94) of the isolates, respectively. Additionally, two (2.1%) isolates possessed the *vacA* s1m1m2 genotype. The *vacA* s1m1 genotype was further classified as *vacA* s1am1 (63.3%, 31/49), s1cm1 (22.4%, 11/49) or s1as1cm1 (14.3%, 7/49), and the *vacA* s1m2 genotype was further classified as *vacA* s1am2 (65%, 26/40), s1cm2 (22.5%, 9/40) or s1as1cm2 (12.5%, 5/40). An analysis of the distribution of the *vacA* alleles with respect to patient ethnicity and disease state is shown in Table 4. We found a significant distribution of different *vacA* alleles according to patient ethnicity. The *vacA* s1m1 genotype was significantly more prevalent in the isolates from the Malay and Indian patients, whereas the *vacA* s1m2 genotype was predominantly detected in the isolates from the Chinese patients ($P = 0.018$). The *vacA* s2m2 genotype was only isolated from the Malay and Indian patients, and a mixed genotype of the m region was identified in the isolates from the Chinese patients. Further analyses of the *vacA* subgenotypes with respect to patient ethnicity revealed that the highest proportion of isolates harbouring the *vacA* s1am1 subgenotype were from Indian patients (93.8%, 15/16). The prevalence of the *vacA* s1cm1 genotype was similar in proportion in the isolates from the Malay (37.5%, 3/8) and Chinese (32%, 8/25) patients, although this subgenotype was not detected in the isolates from the Indian patients. In contrast, the *vacA* s1am2 subgenotype was detected in all of the ethnic groups, whereas the *vacA* s1cm2 subgenotype was only identified in the isolates from the Chinese patients. No significant difference was observed in the distribution of the *vacA* s1m1 and s1m2 strains with respect to the disease state ($P = 0.686$).

Metronidazole susceptibility

In this study, metronidazole resistance was observed in 45.3% (43/95) of the isolates. The resistant strains exhibited MIC values ranging from 8 to > 256 mg/L, with an MIC value > 256 mg/L for the majority of the strains (62.8%, 27/43). As shown in Table 4, a strong association was observed between metronidazole susceptibility and patient ethnicity. The Chinese patients were more prone to infection with metronidazole-sensitive *H. pylori* strains, whereas the Malay and Indian patients were more likely to be infected with the antibiotic-resistant strains. The strains isolated from the patients with PUD were more sensitive to metronidazole than the strains from the patients with pangastritis, although this difference was not statistically significant. DNA sequence analyses of the *rdxA* gene revealed the presence of missense, frameshift and nonsense mutations in 30, 3 and 4 isolates, respectively. All of the tested metronidazole-sensitive strains harboured only missense mutations, whereas all three types of mutations were identified in the antibiotic-resistant strains.

Distribution of *H. pylori* cagPAI genotypes, vacA alleles and metronidazole susceptibilities

As shown in Table 5, the isolates with intact *cag*PAI regions carrying the *vacA* s1m2 allele were sensitive to metronidazole. Of the isolates with a rearranged *cag*PAI region, the type C strains predominantly harboured the *vacA* s1m2 allele, whereas a high proportion of the types I and N *cag*PAI strains harboured the *vacA* s1m1 allele. However, these strains were found to be more sensitive to metronidazole. In contrast, the isolates exhibiting a deleted *cag*PAI region predominantly carried the *vacA* s1m2 allele and were more resistant to metronidazole.

Table 3 Distribution of strains with intact, partially deleted (rearranged) or deleted *cag*PAI regions among patients of different ethnicities and disease states *n* (%)

*cag*PAI status	Ethnic group			Disease state			
	Malay (*n* = 12)	Chinese (*n* = 59)	Indian (*n* = 24)	Normal (*n* = 12)	Gastritis (antral) (*n* = 43)	Pangastritis (*n* = 19)	PUD (*n* = 17)
Intact							
Type A	0	3 (5.1)	0	1 (8.3)	2 (4.7)	0	0
Partially deleted							
Type C	3 (25.0)	19 (32.2)	4 (16.7)	3 (25.0)	9 (20.9)	7 (36.8)	7 (41.2)
Type I	2 (16.7)	14 (23.7)	0	2 (16.7)	5 (11.6)	5 (26.3)	3 (17.6)
Type N	2 (16.7)	2 (3.4)	9 (37.5)	1 (8.3)	7 (16.3)	2 (10.5)	2 (11.8)
Deleted							
Type U	1 (8.3)	3 (5.1)	4 (16.7)	1 (8.3)	3 (7.0)	1 (5.3)	2 (11.8)

PUD: Peptic-ulcer disease.

Table 4 Distribution of *vacA* alleles and metronidazole susceptibilities among *Helicobacter pylori* clinical isolates from patients of different ethnicities and disease states *n* (%)

*cag*PAI status	Ethnic group			Disease state			
	Malay (*n* = 12)	Chinese (*n* = 59)	Indian (*n* = 24)	Normal (*n* = 12)	Gastritis (antral) (*n* = 43)	Pangastritis (*n* = 19)	PUD (*n* = 17)
Intact							
Type A	0	3 (5.1)	0	1 (8.3)	2 (4.7)	0	0
Partially deleted							
Type C	3 (25.0)	19 (32.2)	4 (16.7)	3 (25.0)	9 (20.9)	7 (36.8)	7 (41.2)
Type I	2 (16.7)	14 (23.7)	0	2 (16.7)	5 (11.6)	5 (26.3)	3 (17.6)
Type N	2 (16.7)	2 (3.4)	9 (37.5)	1 (8.3)	7 (16.3)	2 (10.5)	2 (11.8)
Deleted							
Type U	1 (8.3)	3 (5.1)	4 (16.7)	1 (8.3)	3 (7.0)	1 (5.3)	2 (11.8)

PUD: Peptic-ulcer disease.

DISCUSSION

The *cag*PAI and *vacA* genotypes are widely used to characterise the virulence of *H. pylori* and the relationship of such virulence to disease severity, although direct associations with peptic ulcers and gastric cancer have not been established[22,23]. Rather, the development of severe histological changes in the gastric mucosa may depend on the synergistic effect of bacterial and host factors[24].

The genotypic characteristics of the *H. pylori cag*PAI genes show great variability worldwide. The rearrangement of the *cag*PAI varies between different populations and geographical regions, with variations of 50%-65% in areas of the Indian subcontinent[25,26], 32% in France[27], 9% in Sweden[28] and the United States[29] and 1% in Japan[30]. In the present study, more than 90% of the clinical isolates were positive for at least one of the selected *cag*PAI genes. The *cag*PAI genes tested in this study did not include the *cagA* gene, as we previously demonstrated[31] that the majority of the *H. pylori* strains circulating in our study population were *cagA*-positive. In the six genetic loci studied, the most frequently deleted gene was *cag13*, and the least frequently deleted gene was *cagT*. The IS605 insertion sequence was present in less than 5% of the *H. pylori* isolates examined, and the majority of the isolates harboured a rearranged *cag*PAI gene. Analyses of the individual *cag*PAI genes showed that *cag67* was more conserved in the isolates from the Chinese patients, whereas the rearrangement of *cagE* occurred at a higher frequency in the isolates from the Malay and Indian patients, although this difference was not significant.

We also observed that the majority of the *H. pylori* clinical isolates expressed partially deleted *cag*PAI regions and that only few isolates exhibited either intact or deleted *cag*PAI regions. Previous studies have reported the occurrence of different proportions of intact *cag*PAI in their isolates. Ali *et al*[32] found that 37.4% of isolates exhibited intact *cag*PAI regions, whereas other researchers[30,33] reported that more than 96% of isolates contained intact *cag*PAI. In the present study, the isolates expressing intermediate genotypes (deletions within the *cag*PAI region) were more commonly encountered than those isolates with intact or deleted *cag*PAI regions. Moreover, a large proportion of the isolates exhibiting *cag*PAI rearrangement lacked an IS605 sequence, suggesting that this insertion sequence did not play a role in *cag*PAI disruption in the clinical isolates.

Additionally, we found that no single gene in the *cag*PAI region can be used as a genetic marker for an intact *cag*PAI because a large proportion of the clinical isolates exhibited gene rearrangement in the *cag*PAI region. Due

Table 5 Distribution of *Helicobacter pylori cag*PAI genotypes, *vacA* alleles and metronidazole susceptibilities *n* (%)

*cag*PAI status	*vacA*		Metronidazole susceptibility		*vacA*/metronidazole susceptibility			
	s1m1	s1m2	Sensitive	Resistant	s1m1/sensitive	s1m1/resistant	s1m2/sensitive	s1m2/resistant
Intact (n = 2)[1]	0	2 (100)	2 (100)	0	0	0	2 (100)	0
Partially deleted								
Type C (n = 26)	10 (38.5)	16 (61.5)	14 (53.8)	12 (46.2)	5 (19.2)	5 (19.2)	9 (34.6)	7 (26.9)
Type I (n = 16)	12 (75.0)	4 (25.0)	10 (62.5)	6 (37.5)	7 (43.8)	5 (31.2)	3 (18.8)	1 (6.2)
Type N (n = 13)	11 (84.6)	2 (15.4)	8 (61.5)	5 (38.5)	7 (53.8)	4 (50.0)	1 (12.5)	1 (12.5)
Deleted (n = 6)[2]	1 (16.7)	5 (83.3)	3 (37.5)	5 (62.5)	0	1 (16.7)	2 (33.3)	3 (50.0)

[1]One strain with the *vacA* s1m1m2 genotype was not included; [2]Two strains with the *vacA* s2m2 genotype were not included.

to the region's large size (approximately 40 kbp), the absence of these genes in certain clinical isolates did not necessarily indicate the complete absence of the *cag*PAI, as indicated in 9 isolates positive for a single gene. Regarding *cag*PAI rearrangement, 19 different genotypes (types B to T) have been identified. The three most common *cag*PAI genotypes identified in the clinical strains revealed that the genes at the left end of the *cag*PAI region were more unstable and prone to rearrangement than the genes in the middle and at the right end of the *cag*PAI region. This finding was supported by analyses of the individual genes, which demonstrated more gene rearrangement at the left end of the *cag*PAI region (*cag13*, *cag10* and *cag67*). Therefore, the genes in the middle and at the right end of the *cag*PAI region may play an important role in the pathogenesis of *H. pylori* infection in the study population.

In the current study, specific *cag*PAI genotypes were isolated from patients of different ethnicities. All of the isolates with intact *cag*PAI regions and less *cag*PAI rearrangement were from the Chinese patients, whereas the Indian patients were infected with strains exhibiting more rearranged or deleted *cag*PAI regions, and the Malay patients tended to be infected with various *cag*PAI genotypes. Thus, the presence of different *cag*PAI genotypes in different ethnicities may be related to the genetic characteristics of both the colonising *H. pylori* strain and the host. Although the relationship between the *cag*PAI genotype and disease state was unclear, we observed that certain types of infecting strains exhibited different *cag*PAI rearrangements in nonulcer and ulcer patients.

A significant difference was observed in the prevalence of *vacA* genotypes among patients of distinct ethnicities. As the Chinese patients were more likely to be infected with the *vacA* s1m2 strains, these strains may be regarded as more pathogenic, consistent with previous reports showing a higher frequency of peptic ulcers and gastric cancer in the Chinese patients in a similar study population[15,34]. In our study population, the smaller proportion of patients with PUD than NUD may have contributed to the lack of a significant association between the endoscopic findings and patient ethnicity, as analysis was conducted on the samples with positive cultures. Additionally, we demonstrated that the *vacA* s1m2 genotype was significantly associated with enhanced gastric inflammation[10]. In contrast, the *vacA* s2m2 strains were isolated only from the Malay and Indian patients, suggesting that these strains are less virulent. Consistent with this finding, Chinese ethnicity has been associated with infection by strains lacking the *vacA* s2m2 allele[35,36]. As Miernyk *et al*[37] recently reported a high proportion of *vacA* s2m2 strains isolated from Alaskan natives, it would be interesting to examine whether the disease outcome in Alaskan natives is similar to the outcome in the Malay and Indian patients in our study population. The current study also revealed the proportions of different *vacA* subgenotypes in each ethnic population. More specifically, the *vacA* s1cm1 genotype was not detected in the isolates from the Indian patients, whereas the *vacA* s1cm2 genotype was only detected in the isolates from the Chinese patients.

A previous report demonstrated a higher prevalence of resistance to metronidazole than to other antibiotics in *H. pylori* isolates from Malaysia[14]. In the current study, we attempted to further relate the metronidazole susceptibility of *H. pylori* to patient ethnicity and to correlate this susceptibility with the *cag*PAI and *vacA* genotypes. We noted a significant association between the metronidazole-sensitive *H. pylori* strains and Chinese ethnicity, whereas the Malay and Indian patients were more likely to be infected with the metronidazole-resistant strains. This finding may reflect differences in metronidazole use between different ethnic populations. The pattern of metronidazole susceptibility in different ethnicities paralleled the specific genotypes of the infecting strains. For example, the strains of the specific *cag*PAI genotype and *vacA* allele isolated from the Chinese patients were more sensitive to metronidazole.

The presence of frameshift and nonsense mutations in the antibiotic-resistant *H. pylori* strains suggested that these mutations confer resistance to metronidazole. However, more than half of the resistant strains exhibited missense mutations that were also detected in the antibiotic-sensitive strains. This finding implied that *rdxA* is not the only gene involved in the metronidazole-resistant phenotype and thus is not a reliable epidemiological marker. Rather, other genes or mechanisms may be implicated in the generation of resistance[38-40].

We observed no association between the specific *H. pylori* genotypes and the strains' antibiotic susceptibilities

in severe disease. These results may be complicated by the fact that most of the patients in our study population had gastritis. Although the *cag*PAI and *vacA* alleles are important virulence factors in infection, the development of disease is likely to involve a highly complex interplay of many bacterial and/or host factors.

The distribution of the *vacA* s1m2 genotype was broad, as this genotype was detected in the majority of the strains with intact *cag*PAI regions or *cag*PAI rearrangement of type C (positive for more *cag*PAI genes than other types) and the metronidazole-resistant strains. This finding may indicate that the *vacA* s1m2 strains have variable pathogenic properties when combined with other genotypic characteristics. The isolates with the deleted *cag*PAI regions were also primarily linked to metronidazole resistance. As these isolates may induce less inflammation in the host gastric epithelia, their genotypic characteristics may reduce antibiotic delivery and thus hinder the eradication of *H. pylori*. In contrast, the *vacA* s1m2 genotype could not contribute to metronidazole resistance, as this genotype was distributed evenly between the antibiotic-sensitive and -resistant strains[41,42].

In conclusion, we report a large proportion of *H. pylori* isolates harbouring *cag*PAI rearrangement and demonstrate that metronidazole susceptibility varies with patient ethnicity. The distinct distribution of the *H. pylori* *cag*PAI genotypes, *vacA* alleles and metronidazole susceptibilities in the different ethnicities of Malaysia may contribute to varying risk of gastroduodenal diseases. These distinct, patient ethnicity-associated *H. pylori* genotypes may have important clinical and epidemiological implications. Finally, the present study of *H. pylori*-specific genotypes from different host genetic backgrounds enhances our understanding of bacterium-host interactions and bacterial ecology in various niches. Further information on the characteristics of *H. pylori* will allow a more precise identification of virulent strains and a better definition of risk factors in susceptible hosts.

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COMMENTS

Background

The prevalence of *Helicobacter pylori* (*H. pylori*) infection in Malaysia consistently revealed ethnic differences, with Indians exhibiting higher infection rates than the Chinese and Malays. In contrast, peptic-ulcer disease and gastric carcinoma are known to be more prevalent in the Chinese and lowest in the Indians. The great genetic diversity of *H. pylori* may play an important role in the consequences of infection in different hosts. The *cag*PAI and *vacA* genes are well-established *H. pylori* virulence factors that interact with the host cells and disrupt downstream signalling pathways. The *cag*PAI structures, *vacA* alleles and metronidazole susceptibilities of different strains in hosts of varying genetic makeup may enhance the knowledge of bacterium-host interactions in *H. pylori* infections in multiethnic populations.

Research frontiers

The specific genotypes stemming from the *cag*PAI and *vacA* alleles were identified in *H. pylori* strains isolated from different ethnic groups. The strains isolated from the Chinese and peptic-ulcer disease (PUD) patients were more sensitive to antibiotics, indicating a selective advantage that occurred during early infection and persisted in chronic infection. In the present study, however, no association between the specific *H. pylori* genotypes and PUD could be determined due to the small number of PUD cases. Consistent with this observation, previous epidemiological studies have reported that less than 20% of patients infected with *H. pylori* are diagnosed as PUD.

Innovations and breakthroughs

Past reports have highlighted the importance of *H. pylori* genetic diversity in chronic infection. The current study emphasises and adds to findings from the same institution, demonstrating that the genetic background of the *H. pylori* strains may play an important role in the risk of gastroduodenal disease in different ethnic groups.

Applications

The results of this study provide insight into the effects of the genomic diversity of *H. pylori* on hosts of different genetic backgrounds. Therefore, these findings can be used in the development of advanced screening tools for diagnosing and determining the prognosis of *H. pylori* infection.

Terminology

The term *cag*PAI is defined as the *cag* pathogenicity island, a common gene sequence believed to be responsible for pathogenesis. This sequence contains approximately 40 kbp of nucleotides encoding over 40 genes. The pathogenicity island is typically absent from the *H. pylori* strains isolated from human carriers of *H. pylori* who remain asymptomatic.

Peer review

The authors investigated the diversity of the *H. pylori* virulence factors (the *cag*PAI and *vacA* alleles) and metronidazole susceptibilities of strains isolated from patients of different ethnicities. The association between specific *H. pylori* genotypes and patient ethnicity provides insight into the pathogenesis of *H. pylori* infection in different hosts and possibly the different risk factors in *H. pylori* infection.

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