Neither gastric topological distribution nor principle virulence genes of *Helicobacter pylori* contributes to clinical outcomes

Yan Wing Ho, Khek Yu Ho, Felipe Ascencio, Bow Ho

**Abstract**

**AIM:** Studies on *Helicobacter pylori* (*H pylori*) and gastroduodenal diseases have focused mainly on the distal sites of the stomach, but relationship with the gastric cardia is lacking. The aim of this study is to determine if the gastric topology and genotypic distribution of *H pylori* were associated with different upper gastrointestinal pathologies in a multi-ethnic Asian population.

**METHODS:** Gastric biopsies from the cardia, body/corpus and antrum were endoscoped from a total of 155 patients with dyspepsia and/or reflux symptoms, with informed consent. *H pylori* isolates obtained were tested for the presence of 26kDa, ureC, cagA, vacA, iceAI, iceA2 and babyA2 genes using PCR while DNA fingerprints were generated using random amplification polymorphic DNA (RAPD).

**RESULTS:** *H pylori* was present in 51/155 (33%) of patients studied. Of these, 16, 15 and 20 were isolated from patients with peptic ulcer diseases, gastroesophageal reflux diseases and non-ulcer dyspepsia, respectively. Of the *H pylori* positive patients, 75% (38/51) had *H pylori* in all three gastric sites. The prevalence of various genes in the *H pylori* isolates was shown to be similar irrespective of their colonization sites as well as among the same site of different patients. The RAPD profiles of *H pylori* isolates from different gastric sites were highly similar among intra-patients but varied greatly between different patients.

**CONCLUSION:** Topographic colonization of *H pylori* and the virulence genes harboured by these isolates have no direct bearing to the clinical state of the patients. In multi-ethnic Singapore, the stomach of each patient is colonized by a predominant strain of *H pylori*, irrespective of the clinical diagnosis.

Ho YW, Ho KY, Ascencio F, Ho B. Neither gastric topological distribution nor principle virulence genes of *Helicobacter pylori* contributes to clinical outcomes. *World J Gastroenterol* 2004; 10(22): 3274-3277

http://www.wjgnet.com/1007-9327/10/3274.asp
Table 1 Primer sequences of genes of interest

| Region  | Primer | Nucleotide sequence (5’→3’) | PCR product (bp) | Reference |
|---------|--------|-----------------------------|------------------|-----------|
| 26kDa   | 26kDa-F| TGGCGGTGCTATGGACAGCGGAC     | 298              | 9         |
|         | 26kDa-R| CACTGCGGACATACTTCACAAGGCA   |                  |           |
| ureC    | ureC-F | AAGCTTTAAGGGTGTAGGGTTT      | 294              | 10        |
|         | ureC-R | AAGCTTGTTCTCAAACATACACCG    |                  |           |
| cagA    | cagA-F | AATACCAACAACGCCCTCAAGA      | 400              | 11        |
|         | cagA-R | TGGTGGCCGTTTGTCCCTC         |                  |           |
| vacA    | vacA-F | GCTTCTCTACCACATGC           | 1160             | 12        |
|         | vacA-R | TGGCGTGTCTATTGACAGCGAC      |                  |           |
| iceA1   | iceA1-F| GTTTTTTTAACCAAGATATCA       | 246              | 5         |
|         | iceA1-R| CTATAAGCAGCTCTTTTGC         |                  |           |
| iceA2   | iceA2-F| GTTGGGTTDTCACAAATTAT        | 229/334          | 5         |
|         | iceA2-R| TGCCCTTATTTTCTAGTAAGGT      |                  |           |
| babA2   | babA2-F| ATACCAAAAAAGGAGAAGATGAAA    | 831              | 4         |
|         | babA2-R| TGTTAGTGATTTCCGCTAGGACA     |                  |           |

F: forward primer R: reverse primer.

diagnosed upon endoscopy as suffering from gastric ulcers (ulcers at the corpus) or duodenal ulcers (ulcers at the antrum). A total of 465 biopsy specimens were obtained from the 155 patients.

Endoscopy
After an overnight or six hour fast, upper gastrointestinal endoscopy was performed according to standard technique. From each patient, one biopsy specimen was obtained using sterilized standard biopsy forceps from each of the three sites of the stomach: the cardia just below the z-line, the middle gastric corpus and the antrum within 2 cm of the pylorus, in that order. The biopsy forceps were thoroughly cleaned with alcohol swabs between biopsies to avoid contamination between specimens. The biopsies were transported in 0.85% sterile saline to the microbiological laboratory for processing within 6 h.

H pylori culture
Each biopsy specimen was homogenised aseptically in 500 µL of Brain Heart Infusion Broth (BHI, Oxoid Ltd., Basingstoke, UK) enriched with 4 g/L yeast extract (Oxoid Ltd., Basingstoke, UK). Approximately 100 µL homogenised specimens in BHI broth were inoculated onto H pylori selective chocolate blood agar plates and non-selective chocolate blood agar plates respectively. The selective blood chocolate agar was supplemented with 3 mg/mL vancomycin, 5 mg/mL trimethoprim, 10 mg/mL nalidixic acid and 2 mg/mL amphotericin B. All the antibiotics were from Sigma-Aldrich Chemie, Steinheim, Germany. The plates were incubated at 37 °C for up to 14 d in an incubator (Forma Scientific, USA) containing 50 mL/L CO2.

Aliquots of 50 µL of BHI-biopsy suspension were each inoculated into catalase reagent, oxidase reagent and 20 g/L urea solution for their respective testing. An isolate was identified as H pylori if minute (-1 mm in diameter) rounded translucent colonies with gram-negative S-shaped motile cells that exhibited positive catalase, oxidase and urease activities. For this study, a patient was considered positive for H pylori if the organism was isolated from any of the three gastric sites.

Genotyping of H pylori
The DNA of each 3-d old H pylori culture was extracted according to the method as described by Hua et al.[4]. A 50 ng working stock of DNA was used to amplify 26kDa[9], ureC[10], cagA[11], vacA[12], iceA[13], iceA2[14] and babA2[15] genes according to the protocol as described by Zheng et al.[16] using the specific forward and reverse primers for each of the corresponding genes (Table 1). The DNA fingerprint of the H pylori was obtained by PCR using the universal primer, 5’- AACGCCGCAA-3’ and amplified according to protocol as described by Hua et al.[13]. The PCR products obtained were electrophoresed and the ethidium bromide stained gels[13] were then photographed with filtered UV illumination on Chemi Genius2 (SynGene, Cambridge, UK).

Statistical Calculation
The significance of the results obtained was calculated using SPSS v.10 for Windows (SPSS, Chicago IL) to determine the Pearson chi-square whereby a P value <0.05 was considered to indicate statistical significance.

RESULTS

H pylori isolates in various clinical groups
Of the 155 patients studied, 51 (33%) were found to harbour H pylori in at least one of the 3 gastric biopsy sites. In all, 43, 47 and 44 isolates were obtained from gastric antrum, corpus and cardia respectively, giving a total of 134 isolates. H pylori was present in 16/36 (44%) PUD patients as compared with 15/50 (30%) GERD patients (P=0.169) and 20/69 (29%) NUD patients (P=0.113) (Table 2).

Table 2 Relationship between H pylori status and disease states

| Groups | No. of patients | No. of biopsies | No. of H pylori (+) | P     |
|--------|----------------|----------------|---------------------|-------|
| PUD    | 36             | 108            | 16 (44%)            | -     |
| GERD   | 50             | 150            | 15 (30%)            | 0.169 |
| NUD    | 69             | 207            | 20 (29%)            | 0.113 |

PUD, peptic ulcer disease; GERD, gastroesophageal reflux disease; NUD, non-ulcer dyspepsia. All test values were calculated with respect to PUD; P<0.05 indicates statistical significance.

Relationship between topographic distribution of H pylori isolates and clinical outcomes
Of the 51 H pylori positive patients, 38 (75%) showed the presence of H pylori in all the three gastric sites while 1 (1%), 2 (4%) and 4 (8%) had H pylori isolated from antrum & corpus, antrum & cardia, and corpus & cardia, respectively. H pylori
was isolated from a single site of the stomach in 6 (12%) patients, among which 2 isolates were from the antrum and 4 were from the corpus. This topographical pattern of *H pylori* colonization was observed in all the patients irrespective of the underlying clinical diagnosis.

**Relationship between topographic distribution of H pylori genes of interest and clinical outcomes**

All the *H pylori* isolates possessed the 26kDa gene and the *ureC* genes. The prevalence of virulence genes of interest were present in equal ratios in all the *H pylori* isolates obtained from all the 3 different gastric biopsy sites: 74-81% for *cagA* and 80-86% for *vacA*; 53-59% for *iceA1* and 36-42% for *babA2* regardless of the underlying clinical diagnosis. However, the *iceA2* gene was present less frequently, at 20-26% of the *H pylori* isolates. It is noted that the difference in gene frequency between the various sites was also not statistically significant (Table 3).

Similar observation was noted with respect to the distribution of virulence genes of *H pylori* isolated from the same site among the different disease groups. The prevalence for each virulence gene within the same site was highly similar. No significant association of the virulence gene was found to be associated with a particular biopsied site, regardless of the disease state, with the exception of *cagA* in isolates from the corpus of the stomach of GERD patients (Table 4).

**Table 3** Anatomical location of the 134 *H pylori* isolates and their virulence genes

| Clinical Diagnosis | Antrum (%) | Body/Corpus (%) | Cardia (%) |
|--------------------|------------|----------------|------------|
| PUD                | 12 (33)    | 15 (42)        | 12 (33)    |
| GERD               | 13 (26)    | 15 (30)        | 14 (28)    |
| NUD                | 18 (25)    | 17 (24)        | 18 (26)    |

**Genotype**

| 26kDa | ureC | cagA | vacA | iceA1 | iceA2 | babA2 |
|-------|------|------|------|-------|-------|-------|
| 43 (100) | 47 (100) | 44 (100) |       |       |       |       |
| 43 (100) | 47 (100) | 44 (100) |       |       |       |       |
| 35 (81)  | 35 (74)  | 33 (75)   |       |       |       |       |
| 37 (86)  | 38 (81)  | 35 (80)   |       |       |       |       |
| 25 (58)  | 25 (53)  | 26 (59)   |       |       |       |       |
| 10 (23)  | 12 (26)  | 9 (20)    |       |       |       |       |
| 18 (42)  | 20 (38)  | 16 (36)   |       |       |       |       |

PUD, peptic ulcer disease; GERD, gastroesophageal reflux disease; NUD, non-ulcer dyspepsia. All test values were calculated with respect to gastric antrum and none were significant.

**Relationship between topographic distribution of H pylori strain based on RAPD fingerprinting and clinical outcomes**

For comparison, differences in 2 or more bands of the RAPD profile are considered different while variations in band intensity were not taken into account. On this basis, the RAPD profiles of all the *H pylori* strains isolated showed an overall similarity in profiles within individual patients, with minor differences such as the presence or absence of a single band. However, distinct differences in the DNA profiles were observed between patients (Figure 1). In this study, no comparison could be made in 6 patients since *H pylori* was isolated from only one site of the stomach in these patients.

![Figure 1](image)

**DISCUSSION**

It is noted that 50% of the world’s population are infected with *H pylori* but only a small proportion manifest different gastroduodenal diseases[14]. One of the factors contributing to this phenomenon could be the patchy distribution of *H pylori* in different gastric sites of the stomach. As most of the earlier studies focused on *H pylori* isolated from the distal sites of the stomach[8,15], the present study shows that *H pylori* isolates obtained from all the 3 gastric sites, namely cardia, corpus and antrum, in 38/51 (75%) *H pylori* patients were similar genotypically. Care was taken in cleaning and disinfecting the biopsy forceps between biopsies to avoid contamination between specimens. The results imply that *H pylori* colonises the entire stomach instead of a predominant site in three quarters of our *H pylori* positive patients, irrespective of the underlying clinical diagnosis. The finding suggests that the site of *H pylori* colonization or topographic distribution does not contribute significantly to the outcome of the infection.

**Table 4** Distribution of virulence genes of 134 *H pylori* isolates from the same anatomical site of different patient groups

| Isolates | 26kDa (%) | ureC (%) | cagA (%) | vacA (%) | iceA1 (%) | iceA2 (%) | babA2 (%) |
|----------|-----------|----------|----------|----------|-----------|-----------|-----------|
| Antrum   | 12 (100)  | 12 (100) | 9 (75)   | 10 (83)  | 6 (50)    | 4 (33)    | 6 (50)    |
| PUD      | 13 (100)  | 13 (100) | 11 (85)  | 11 (85)  | 7 (54)    | 2 (15)    | 6 (46)    |
| GERD     | 18 (100)  | 18 (100) | 15 (83)  | 16 (89)  | 12 (67)   | 4 (22)    | 6 (33)    |
| Body/Corpus | 15 (100)  | 15 (100) | 9 (60)   | 10 (67)  | 6 (40)    | 5 (33)    | 7 (47)    |
| NUD      | 17 (100)  | 17 (100) | 14 (93)  | 14 (93)  | 8 (53)    | 3 (20)    | 8 (53)    |
| Cardia   | 12 (100)  | 12 (100) | 12 (71)  | 14 (82)  | 11 (65)   | 4 (24)    | 5 (29)    |

PUD, peptic ulcer disease; GERD, gastroesophageal reflux disease; NUD, non-ulcer dyspepsia. All test values were calculated with respect to PUD. 1Indicates statistical significance (P = 0.031).
While studies in Europe suggested that virulence genes, e.g., cagA and vacA affect the clinical outcome of H pylori infection\(^{[33]}\), the present study confirms previous studies carried out in Asian countries\(^{[6,15]}\) that showed a high prevalence of cagA and vacA genes regardless of the clinical outcome. This study comprised Singapore patients of various ethnicities (Chinese, Malays, Indians and other races), also shows that each of the virulence genes, i.e., cagA, vacA, iceA1, iceA2 and babA2 were equally distributed in H pylori isolates obtained from all the three anatomical sites studied regardless of the disease states. Similarly, comparisons of these genes from the same anatomical site of different patients showed no significant presence, except for cagA in the corpus of GERD isolates. However, it is important to point out that the number of isolates obtained from each is relatively low \((n \leq 18)\), regardless of the disease state. As such, the significant presence of cagA in the corpus of GERD patients needs further analysis with a larger pool of samples to confirm conclusively its contribution to the onset of GERD. The data therefore suggest that virulence genes and their topographic distribution do not contribute to the clinical status, at least in the Singapore population. This study supports the earlier reports of isolates from 2 sites (antrum & corpus) were identical. This finding is complemented by the similar status of presence of various virulence genes in these isolates obtained from the respective patients. As such, the isolation of a strain from any gastric site in a single patient could be taken as representative of H pylori infection present in the H pylori infected gastric environment.

This study, which included consecutive patients with dyspepsia and/or reflux symptoms showed a lower frequency of PUD as compared with that of GERD. H pylori was found in only 44% of patients with PUD. This seems to run counter to the generally held view that H pylori occurs frequently in Asians patients with PUD\(^{[33]}\). However, this finding is supported by an earlier study from the same unit\(^{[18]}\) showing the frequency of reflux oesophagitis was increasing while that of duodenal ulceration was decreasing in Singapore. The high frequency of patients with GERD in this study also relates to the fact that the endoscopist (KYH) sees most of the GERD patients in the hospital. The decreasing frequency of H pylori associated peptic ulcers was reported to be attributed to the increasing proportion of ulcers due to NSAID use\(^{[39]}\).

In summary, the present study shows that in Singapore, the topographic colonization of H pylori and their virulence genes within the host stomach do not play a significant role in the clinical manifestations of H pylori infection. This study also demonstrates that in Singapore, which has a multiethnic Asian population, the stomach of each patient with dyspeptic and/or reflux symptoms is colonized by a single predominant strain of H pylori, irrespective of the site of isolation and the clinical diagnosis of the patient. We suggest that the pathogenesis of H pylori induced gastroduodenal diseases is due to a more complex mechanism possibly involving host-pathogen interaction, environmental and dietary factors.

REFERENCES

1 Everhart JE. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am 2000; 29: 559-578

2 Go MF. Review article: natural history and epidemiology of Helicobacter pylori infection. Aliment Pharmacol Ther 2002; 16(Suppl 1): 3-15

3 Arents NLA, Van Zwei AA, Thijs JC, Kooistra-Samid AMD, van Slochteren R, Degener JE, Kleibeuker JH, van Doorn LJ. The importance of vacA, cagA and iceA genotypes of Helicobacter pylori infection in peptic ulcer disease and gastro-esophageal reflux disease. Am J Gastroenterol 2001; 96: 2603-2608

4 Gerhard M, Luhn N, Neumayer N, Boren T, Rad R, Schepp W, Mielhike S, Classen M, Prinz C. Clinical relevance of the Helicobacter pylori gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A 1999; 96: 12778-12783

5 van Doorn LJ, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, DeBoer W, Quint W. Clinical relevance of the cagA, vacA and iceA status of Helicobacter pylori. Gastroenterology 1998; 115: 58-66

6 Zheng PY, Hua J, Yeoh KG, Ho B. Association of peptic ulcer with increased expression of Lewis antigen but not cagA, iceA and vacA in Helicobacter pylori isolates in an Asian population. Gut 2000; 47: 18-22

7 Peek RM Jr. The biological impact of Helicobacter pylori colonization. Semin Gastrointest Dis 2001; 12: 151-166

8 Hua J, Ling KL, Ng HS, Ho B. Isolation of a single strain of Helicobacter pylori from the antrum and body of individual patients. Eur J Gastroenterol Hepatol 2000; 12: 1129-1134

9 Hammar M, Tyskieczewicz T, Wadstrom T, O’Toole PW. Rapid detection of Helicobacter pylori in gastric biopsy material by polymerase chain reaction. J Clin Microbiol 1992; 30: 54-58

10 Labigne A, Cussac V, Courcoux P. Shuttle cloning and nucleotide sequence of Helicobacter pylori genes responsible for urease activity. J Bacteriol 1991; 173: 1920-1931

11 Lap LF, Godfroid E, Faucconier A, Burette A, Butzler JP, Bollen A, Glupczynski Y. Diagnosis of Helicobacter pylori infection by PCR: comparison with other invasive techniques and detection of cagA found in gastric biopsy specimens. J Clin Microbiol 1995; 33: 2752-2756

12 Xiang Z, Censini S, Bayeli PF, Telford J, Figura N, Rappuoli R. Analysis of expression of CagA and VacA virulence factors in 43 strains of Helicobacter pylori reveals that clinical isolates can be divided into 2 major types and that CagA is not necessary for expression of the vacuolating cytotoxin. Infect Immun 1995; 63: 94-98

13 Hua J, Ho B. Is the coccoid form of Helicobacter pylori viable? Microbiol 1996; 87: 103-112

14 Hocker M, Hohenberger P. Helicobacter pylori virulence factors-1 part of a big picture. Lancet 2003; 362: 1231-1233

15 Kim SY, Woo CW, Lee YM, Son BR, Kim JW, Chae HB, Youn SJ, Park SM. Genotyping cagA, vacA subtype, iceA1, babA of Helicobacter pylori isolates from Korean patients, and their association with gastroduodenal diseases. J Korean Med Sci 2001; 16: 579-584

16 Kim JM, Kim JS, Jung HC, Song JS, Kim CY. Virulence factors of Helicobacter pylori in Korean isolates do not influence proinflammatory cytokine gene expression and apoptosis in human gastric epithelial cells, nor do these factors influence the clinical outcome. J Gastroenterol 2000; 35: 896-906

17 Park SM, Park J, Kim JG, Cho HD, Cho HJ, Lee DH, Cha YJ. Infection with Helicobacter pylori expressing the cagA gene is not associated with an increase risk of developing peptic ulcer disease in Korean patients. Scand J Gastroenterol 1998; 33: 923-927

18 Ho KY, Gwee KA, Yeoh KG, Lim SG, Kang JY. Increasing frequency of reflux oesophagitis in Asian patients. Gastroenterology 2000; 118: A1246

19 Ong TZ, Ho KY. The increasing frequency of non-Helicobacter pylori peptic ulcer disease in an Asian country is related to NSAID use. Gastrointestinal Endoscopy 2003; 57: AB153

Edited by Pan BR and Zhang JZ. Proofread by Xu FM.