10. Scheffer, R. P. and Livingston, R. S., *Phytopathology*, 1980, **70**, 400–404.
11. Knewsel, R. E., Schiltz, E. and Martem, U., *J. Biol. Chem.*, 1994, **269**, 3449–3456.
12. Ouchi, S., Toyoda, H., Otsumi, R., Hashimoto, H. and Hadama, T., in *Phytotoxins and Plant Pathogenesis* (ed. Graniti, A.), Springer-Verlag, Berlin, 1989.
13. Sriram, S., *et al.*, *Can. J. Microbiol.*, 2000, **46**, 520–524.
14. Ramesh Sundar, A., Viswanathan, R., Padmanaban, P. and Mohanraj, D., *Acta Phytopathol. Entomol.*, 1999, **34**, 211–217.
15. Velalazhan, R., Jeeva, M. L., Narayanaswamy, P. and Vidhyasekaran, P., *Indian Phytopathol.*, 1993, **46**, 178–180.
16. Damann, K. E., Gardner, J. M. and Scheffer, R. P., *Phytopathology*, 1974, **64**, 1003–1006.
17. Duke, S. L., Gohbara, M., Pauland, M. V. and Duke, M. V., *J. Phytopathol.*, 1992, **134**, 289–305.
18. Ransom, R. F., Hipskind, J., Leite, B., Nicholson, R. L. and Duncle, L. D., *Physiol. Mol. Plant Pathol.*, 1992, **4**, 75–84.
19. Gappilout, I., Milat, M. L. and Blein, J. P., *Eur. J. Plant Pathol.*, 1996, **162**, 127–132.
20. Viswanathan, R., Malathi, P., Padmanaban, P. and Mohanraj, D., Proceeding of Ecofriendly Approaches for Plant Diseases Management, University of Madras, Chennai, January 2001, pp. 22–34.
21. Zhang, L., Xu, L. and Birch, R. G., *Microbiology*, 1998, **144**, 555–559.
22. Dickman, M. B. and Mitra, A., *Physiol. Mol. Plant Pathol.*, 1993, **41**, 255–263.
23. Doumbou, C. L., Akimev, V. and Beaulieu, C., *Appl. Environ. Microbiol.*, 1998, **64**, 4313–4316.

ACKNOWLEDGEMENTS. We are grateful to the Director, Sugarcane Breeding Institute, Coimbatore for providing the necessary facilities and for encouragement.

Received 14 February 2002; revised accepted 26 July 2002

Genotypes of *Helicobacter pylori* isolated from various acid peptic diseases in and around Lucknow

K. K. Mishra, S. Srivastava, P. P. Dwivedi, K. N. Prasad and A. Ayyagari*

Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226 014, India

The vacuolating cytotoxin and the cytotoxin-associated protein, encoded by *vacA* and *cagA* respectively, are important virulence determinants of *Helicobacter pylori*. A total of 79 clinical isolates of *H. pylori* were characterized for *vacA* and *cagA* genes using specific primers. Fifty-five (69.6%) of 79 strains had *vacA* signal sequence genotype s1a, 13 (16.4%) had type s1b and 11 (14%) had type s2. The *vacA* middle-region types m1 and m2 were detected in 51 (64.6%) and 28 (35.4%) strains. The combinations s1a/m1 (42 [53.2%]), s1a/m2 (13 [16.5%]), s1b/m1 (9 [11.4%]), and s1b/m2 (4 [5.1%]) occurred more frequently than s2/m2 (11 [13.9%)]. No strains with s2/m1 were found ($P < 0.01$). Thirty-one (86.1%) of 36 patients with ulcers harboured type s1a strains, in contrast to 17 (68%) of 25 patients with gastritis. Moreover, all isolates from patients with ulcer, gastritis and gastric cancer were *cagA*$^+$, but only 10 (76.9%) of 13 isolates from patients with portal hypertension carried the *cagA* gene. Strains possessing *vacA* type s1 (37 [66.1%] of 56) were more frequently associated with vacuolating cytotoxicity than type s2 strains ($P < 0.01$). These results indicate that *cagA*$^+$, s1a/m1 type strains are associated with occurrence of peptic ulceration and cytotoxin activities common in and around Lucknow.

HELICOBACTER pylori is a Gram-negative, spiral, microaerophilic bacterium that chronically infects the gastric mucosa of more than half of the world population[1]. It has the unique property to colonize the gastric mucosa and persist in this niche for decades. *H. pylori* infection is a major cause of chronic active gastritis, peptic ulcer diseases[2,3] and an early risk factor for gastric cancer[4]. The pathogenesis of *H. pylori* is influenced by bacterial genotype as well as host response and environmental conditions.

There are specific virulence determinants in *H. pylori* strains, apart from immunological factors in the host, which influence the clinical outcome of infection. One important virulence factor is a vacuolating cytotoxin that induces the formation of intracellular vacuoles in mammalian cells *in vitro*, that leads to cell death[5]. Although the gene that encodes the cytotoxin, designated *vacA*, is present in nearly all strains[6–8], only about 50% of *H. pylori* strains can produce detectable amounts of this cytotoxin[9]. Analysis of *vacA* from different strains has shown that the gene differs in its signal sequence, which could be s1a, s1b, s1c or s2 and in its mid-region sequence, which could be, at least, m1 or m2 (refs 10 and 11).

Another virulence factor of *H. pylori* is cytotoxin-associated (*cagA*) gene; it produces the 128-kDa CagA protein[12]. The presence of *cagA* is associated with duodenal ulceration, gastric mucosal atrophy, and gastric cancer[13]. *cagA* is part of a larger genomic entity, designated the cag pathogenicity island (cag PAI)[14], which contains multiple genes that are related to the virulence and pathogenicity of the *H. pylori* strains. Therefore, the presence of *cagA* can be considered as a marker for this genomic PAI and is associated with more virulent *H. pylori* strains. Nearly all East Asian strains carry the cag PAI independent of disease status[15,16]. In contrast, only one half to two-thirds of the US and European strains carry the cag PAI.

Although *H. pylori* is cosmopolitan, little is known about the geographic distribution of specific *H. pylori* strains, especially in developing countries[17]. Also, data concerning the association between *vacA* genotypes and

*For correspondence. (e-mail: archana@sgpgi.ac.in)

disease in both developed and developing countries are scarce. Thus the relationship between ulcerogenesis and specific *vacA* alleles could not be assessed.

It is well established that *H. pylori* infection and peptic ulcer diseases are common in India. In this study, we aimed at assessing the *vacA* and *cagA* genotypes of *H. pylori* strains infecting patients in and around Lucknow and to correlate differences found within these genes with *vacA* and *cagA in vitro* cytotoxicity and clinical outcome.

Seventy-nine *H. pylori* strains were isolated from the gastric biopsy specimens of 79 patients attending the endoscopy units in the Gastroenterology Department at Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, for upper gastrointestinal complaints. Patients taking antibiotics, with bleeding ulcer or an acute haemorrhage from other sites in the upper gastrointestinal tract, and a patient with surgery of the stomach were excluded. All patients gave informed consent to be biopsied. The known strain, CCUG 17874 (provided by Dr B. Kaijser, University of Goteborg, Sweden) was used as a positive control in this study. Three gastric biopsy specimens were collected from a similar location in the antrum of the stomach from each patient. One piece each of the specimens was cultured for *H. pylori*, examined histologically by Giemsa and subjected to PCR analysis.

For bacterial culture, gastric biopsy sample was homogenized and cultured on Brucella chocolate agar containing antibiotic supplement (vancomycin 10 mg/l, polymyxin-B 2500 IU/l and amphotericin-B 5 mg/l) as described earlier, with 7% sheep's blood[18]. Plates were incubated at 37°C in microaerophilic atmosphere with CO_2 10%, O_2 5% and N_2 85%. Plates were opened after 72 h and later every 48 h, if no growth was obtained. Organisms were identified as *H. pylori* based on colony morphology, modified Gram staining, and positive oxidase, catalase and rapid urease tests[19]. Histological sections of formalin-fixed biopsy specimens were stained with haematoxylin–eosin to evaluate the presence of *H. pylori*.

Genomic DNA from *H. pylori* isolates was extracted and purified from freshly harvested bacterial cells by alkali lysis method[18] and dissolved in distilled water. On the basis of published sequence[10], oligonucleotides were prepared to amplify the *vacA* signal and the middle region. The oligonucleotide forward and reverse primers were synthesized (Genset, Lithuania). The primers used in this study are listed in Table 1.

To type the *vacA* signal and middle region, PCR was performed in 50 µl reaction mixture containing 100 ng of genomic DNA, 250 nM of each primer, 1X reaction buffer, 1.5 mM $MgCl_2$, 1 U of Taq-DNA polymerase and distilled water in a PCR system (Perkin–Elmer, Japan). After denaturation at 94°C for 5 min, amplification was carried out for 27 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s; the mixture was then cycled at 72°C for 7 min to complete the elongation step and was finally stored at 4°C. For identification of the amplified products, 10 µl of the PCR mixture was analysed by electrophoresis on 2% agarose gel (Sigma, USA), stained with ethidium bromide 0.5 µg/ml and read under UV illumination.

To determine whether the presence of *cagA* gene is associated with the s1 *vacA* signal sequence, the *cagA* gene was detected by Southern blot hybridization. Genomic DNA (1 µg) was digested with *Hin*dIII (Bangalore Genei, India) according to the manufacturer's instruction and was subjected to electrophoretic separation in 1% agarose gel. Genomic DNA was denaturated, transferred onto nitrocellulose and pre-hybridized at 42°C for 6 h in 50% formamide, 25 mM sodium phosphate (pH 6.5), 500 µg sonicated salmon testis DNA per ml, 5X in a hybridization oven (Amersham, UK). Filters were hybridized overnight in a solution containing 50% formamide, 10% dextran sulphate, 20 mM sodium phosphate (pH 6.5), 250 µg of sonicated salmon testis DNA per ml, 2X Denhardt's solution and *cagA* probe. The *cagA* probes were labelled with α-P^{32} dCTP using random primer kit (Banglore Genei) according to manufacturer's instructions. The *cagA* probe was a 320 bp PCR

Table 1. Oligonucleotide primers used in typing of *H. pylori vacA* alleles

Region amplified	Primer designation	Primer sequence	Site and location of PCR product
m1	VA3-F	5′ GGTCAAAATGCGGTCATGG 3′	2741–3030 (290 bp)
	VA3-R	5′ CCATTGGTACCTGTAGAAAC 3′	
m2	VA4-F	5′ GGAGCCCCAGGAAACATTG 3′	976–1327 (352 bp)
	VA4-R	5′ CATAACTAGCGCCTTGCAC 3′	
s1a	SS1-F	5′ GTCAGCATCACACCGCAAC 3′	866–1055 (190 bp)
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	
s1b	SS3-F	5′ AGCGCCATACCGCAAGAG 3′	869–1055 (187 bp)
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	
s2	SS2-F	5′ GCTTAACACGCCAAATGATCC 3′	371–569 (199 bp)
	VA1-R	5′ CTGCTTGAATGCGCCAAAC 3′	

Products amplified using these primer pairs were differentiated on the basis of their respective molecular size.

product (corresponding to nucleotides 1764 to 2083 of *H. pylori* CCUG 17874 *cagA*)[12] amplified by two *cagA* specific primers:

Forward, 5′-AGACAACTTGAGCGAGAAAG-3′,
Reverse, 5′-TATTGGGATTCTTGGAGGCG-3′.

PCR was performed using PCR-based typing of the *vacA* homologue at a modified annealing temperature of 55°C (ref. 20). The filters were rinsed several times at room temperature in a solution containing 0.1X SSC (1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.05% SDS to remove excess hybridization solution and were then washed at 52°C for 10 min in the same solution (0.1X SSC and 0.05% SDS).

A total of seventy-nine *H. pylori* isolates from patients with dyspeptic symptoms were characterized by PCR. Endoscopic and histological examination revealed gastritis in 25 patients, peptic ulcer in 36, and gastric carcinoma in 5 and control portal hypertension (PHT) in 13 patients. For each of these patients, both isolates showed identical features using PCR.

The *vacA* gene was detected in all 79 *H. pylori* isolates. By using the primers SS1-F, VA1-R; SS3-F, VA1-R; and SS2-F, VA1-R to amplify the *vacA* signal sequences (Table 1), the predicted PCR products of either 190, 187 or 199 bp were obtained from 79 (100%) of 79 isolates (Figure 1). Fifty-five (69.6%) of 79 strains yielded the 190 bp product representing genotype s1a, 13 (16.4%) yielded the 187 bp product representing genotype s1b and 11 (14%) yielded the 199 bp product representing genotype s2. All 79 strains yielded a PCR product of one of three sizes; none gave a product of any other size (Table 2).

All 79 *H. pylori* strains contained DNA, which was amplified either by the primers VA3-F and VA3-R, representing type m1, or by the primers VA4-F and VA4-R, specific for type m2 (Figure 2). Two different families of *vacA* allele m1 and m2 could be differentiated at the mid-region locus. Fifty-one (64.6%) isolates were classified as type m1 and twenty-eight (35.4%) were classified as type m2 (Table 2). None had DNA amplified by both primer sets or gave PCR product of sizes other than those predicted. PCR typing produced identical results for each of the 79 strains tested.

Among the 79 isolates studied, *vacA* homologues containing five of the six possible combinations of signal sequence and middle region (s1a/m1, s1a/m2, s1b/m1, s1b/m2, and s2/m2) types were identified. The s1a/m1 and s1a/m2 combinations were found in 42 (53.2%) and 13 (16.5%) strains, respectively ($P < 0.001$). The s1b/m1 and s1b/m2 combinations were found in 9 (11.4%) and 4 (5.1%) strains respectively, ($P > 0.01$); the differences were not significant. s2/m2 combinations were identified in 11 (13.9%) of 79 isolates. The s2/m1 combination was not detectable ($P < 0.01$) (Table 2).

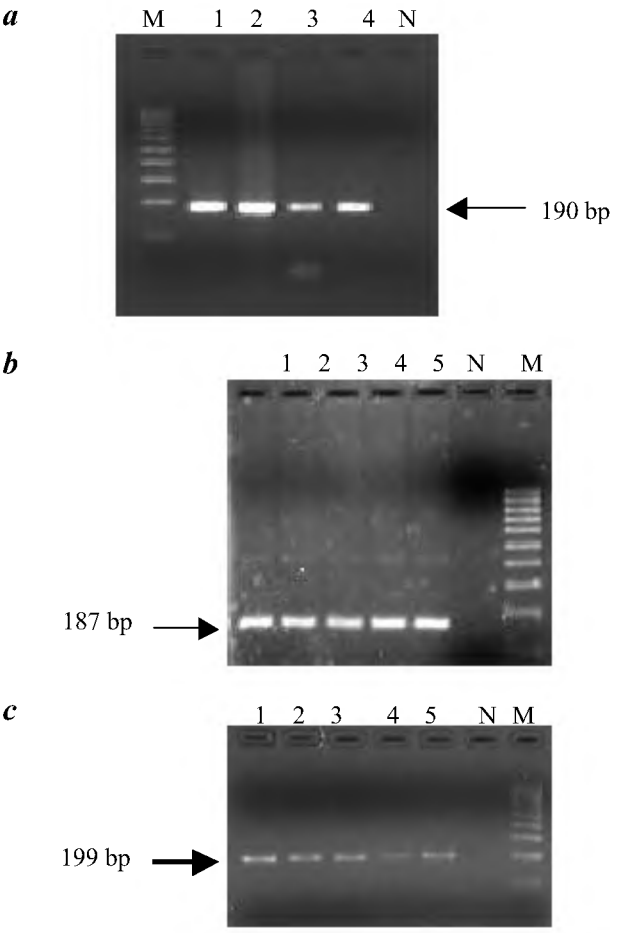

Figure 1. Representative sequence-specific *vacA* PCR tests. ***a***, s1a alleles using primers SS1-F and VA1-R, generating 190-bp product; ***b***, s1b alleles using primers SS3-F and VA1-R, generating 187-bp product; ***c***, s2 alleles using primers SS2-F and VA1-R, generating 199-bp product. Lanes 1–5, *H. pylori* strains; lane N, Negative control, and lane M, 100 bp DNA ladder.

Table 2. Relationship between mid-region and signal sequence typing of *vacA* alleles for 79 *H. pylori* isolates*

	Signal sequence type			
	s1a	s1b	s2	Total
Mid-region type				
m1	42	09	00	51
m2	13	04	11	28
Total	55	13	11	79

*Five of the six possible combinations of *vacA* signal sequence and mid-region types could be identified; No strain was found with the s2/m1 combination of signal sequence and mid-region types ($P < 0.01$).

We have determined whether particular *vacA* genotypes were associated with the occurrence of different gastrointestinal diseases. Infection with a type s1a strain was found in 31 (86.1%) of 36 patients with ulcer, compared to 17 (68%) of 25 subjects with gastritis ($P > 0.05$). Type s1b strains were found in 4 (11.1%) of 36 patients

with ulcer compared with 6 (24%) of 25 subjects with gastritis ($P > 0.1$). A single strain was isolated from patients with ulcer disease type s2 and two strains were isolated from patients with gastritis type s2. Thirty-one (86.1%) of 36 persons infected with type s1a strains had ulcer compared with four (11.1%) of 36 infected with type s1b strains, a significant difference ($P > 0.001$). The distribution of type s1a/m1 and type s1a/m2 strains was found in patients with ulcer (s1a/m1, 26; s1a/m2, 5) and distribution of type s1b/m1 and type s1b/m2 strains was found in patients with ulcer (s1b/m1, 3; s1b/m2, 1) and gastritis (s1a/m1, 11; s1a/m2, 6; s1b/m1, 5; s1b/m2, 1) (Table 3). Subgroup analysis showed that the *vacA* middle region types were not independently associated with the occurrence of ulcer and gastritis.

Almost seventy-six (96.2%) of 79 *H. pylori* isolates and reference strain were found to have *cagA* gene (*cagA*$^+$) by Southern blotting (Figure 3). All the isolates from patients with peptic ulcer disease 36 (100%) of 36, gastritis 25 (100%) of 25 and gastric cancer 5 (100%) of 5 were *cagA*$^+$, but only 10 (76.9%) of 13 isolates obtained from patients with PHT carried the *cagA* gene ($P < 0.05$).

When the *vacA* signal sequence type was compared with *cagA* status, 67 (98.5%) of 68 *vacA* type s1 strains were *cagA*$^+$ and nine (81.8%) of 11 *vacA* type s2 strains were *cagA*$^+$ ($P < 0.05$). Of the 76 *cagA*$^+$ strains, 67 (88.2%) were found to have the *vacA* genotype s1 and 9 (11.8%) were found to have the *vacA* genotype s2. This result suggests the close association between *cagA* status and the s1 signal sequence. A significant association also was found between *vacA* mid-region typing and *cagA* status. All the 51 m1-type strains were positive for *cagA* gene and 25 (89.3%) of 28 *vacA* type m2 strains were *cagA*$^+$ ($P < 0.05$). Thus, there was a significant association between the presence of *cagA* and the presence of type s1 *vacA* signal sequence; but subgroup analysis showed that only the association between signal sequence type and *cagA* status was independently significant.

Cytotoxin production by *H. pylori* was shown on the cell lines by development of intracytoplasmic vacuolization and the rounding of the cells noticed within 24–48 h. Of the 65 *H. pylori* strains tested, supernatants from 38 (58.5%) induced detectable vacuolation of HeLa cells (Tox$^+$). Thirty-one (73.8%) of 42 strains with the *vacA* genotype s1/m1 and 6 (42.9%) of 14 strains with genotype s1/m2 were Tox$^+$ ($P < 0.05$). In contrast, only one (11.1%) of nine strains with the genotype s2/m2 induced a weak vacuolation in HeLa cells. The strains possessing *vacA* type s1 (37 [66.1%] of 56) were more frequently associated with vacuolating cytotoxicity than those possessing type s2 (one [11.1%] of nine), the difference being significant ($P < 0.01$). The *vacA* genotype was highly associated with the level of *in vitro* cytotoxin activity. *H. pylori* strains isolated from patients with ulcer were more likely to be tox$^+$ *in vitro* than those obtained from patients with gastritis only; 21 (67.7%) of 31 pati

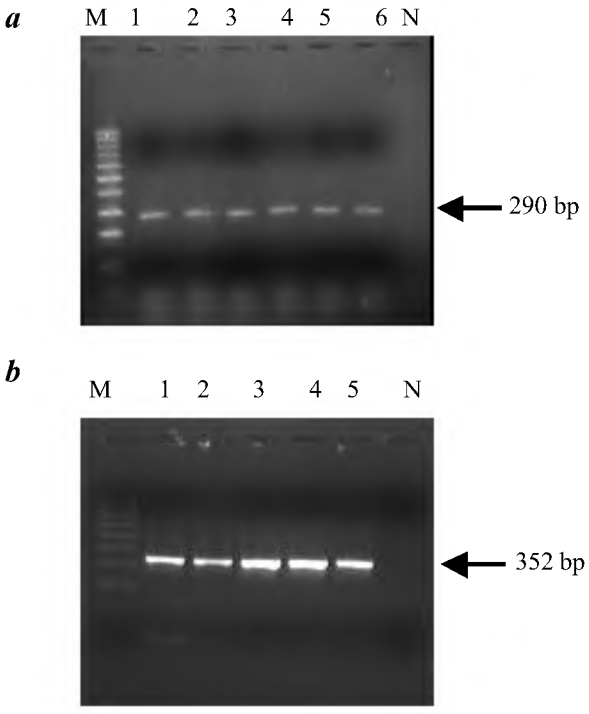

Figure 2. Representative sequence-specific *vacA* PCR tests. ***a***, *vacA* m1 using primers VA3-F and VA3-R, generating 290-bp product; ***b***, *vacA* m2 using primers VA4-F and VA4-R, generating 352-bp product. Lane N, Negative control; lane M, 100-bp DNA ladder; lanes 1–6, *H. pylori* strains.

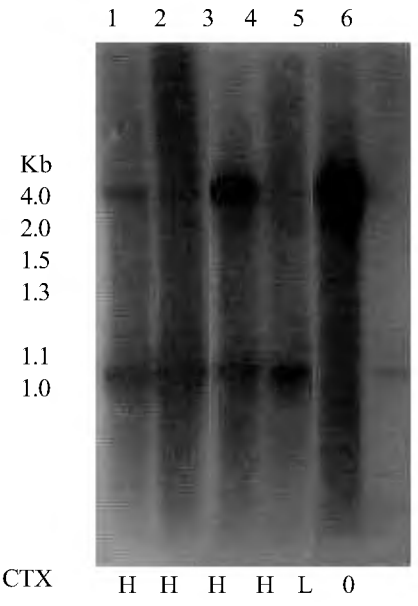

Figure 3. Southern blot of chromosomal DNA from *H. pylori* strains digested with *Hin*dIII. Restriction fragments were hybridized with *cagA* probe. The *cagA* gene is present in 96.2% isolates. There were several restriction fragment length polymorphism patterns (lanes 1–6). Molecular weight markers are shown on the left. H; High-grade tox$^+$, L; Low-grade tox$^+$, O, tox$^+$.

Table 3. Relationship between *H. pylori vacA* genotype and clinical diseases of stomach

Disease studied	No. of isolates	*vacA* subtypes				
		s1a/m1	s1a/m2	s1b/m1	s1b/m2	s2/m2
Ulcer	36	26	05	03	01	01
Gastritis	25	11	06	05	01	02
Carcinoma stomach	05	05	00	00	00	00
PHT	13	00	02	01	02	08
Total	79	42	13	09	04	11

vacA signal sequence s1a and s1b subgroups do not show significant association between ulcer and gastritis ($P > 0.1$).
In the case of ulcer, there is significant difference between *vacA* signal sequence s1a and s1b ($P < 0.001$).

ents with ulcers harboured tox$^+$ strains compared with 11 (55%) of 20 patients with gastritis ($P > 0.1$); the differences were not significant. All strains isolated from carcinoma-stomach patients were tox$^+$ (5 [100%] of 5) and only single strains were tox$^+$ *in vitro* (1 [11%] of 9) from patients with PHT.

Thirty-five (46.1%) of 76 *cagA*$^+$ strains and 3 (100%) of 3 *cagA*$^-$ strains induced vacuolation in HeLa cells ($P > 0.05$), demonstrating that *cagA* is not associated with cytotoxic activity.

The *vacA* genotypes have been considered markers of pathogenesis for individual *H. pylori* strains, at least in the US and in European countries, since *in vitro* production of cytotoxin, *in vivo* epithelial damage, and development of peptic ulcer disease or gastric adenocarcinoma are all related to *vacA*-specific genotypes[11,21]. However, studies on *H. pylori* virulence factors have been difficult to interpret because observations reported for one geographic region or ethnic group have not always been confirmed at different places[22–26]. In north India, where the prevalence of *H. pylori* infection is high, such studies are still scanty. This study provides a description of *vacA* genotyping of *H. pylori* strains from an adult population in and around Lucknow.

Our findings demonstrate that the *vacA* genotypes s1a, s1b, and s2 were identified in all *H. pylori* strains. For determination of the *vacA* mid-region genotypes, the published oligonucleotide primer VA4-F and VA4-R (Table 1) was allowed to characterize 100% of the *H. pylori* strains isolated from patients in and around Lucknow.

H. pylori strains with the *vacA* signal sequence type s1a were predominant in our series, whereas strains with type s1b and s2 rarely occurred. However, the percentage of strains with the *vacA* genotype s2 may have been underestimated, since the strains investigated in this study were obtained from dyspeptic patients. The *vacA* genotypes of our *H. pylori* isolates were identical to those reported from Kolkata[26]. However, in Kolkata, 7.2% strains had mixed s1 and s2 genotypes. In our study, s1b allele was present in 16.4% of s1 strains; almost similar prevalence of s1b had also been reported in other studies[27–29].

In this study, we found that our north Indian *H. pylori* isolates contained predominantly the s1a/m1 family of *vacA* alleles, which differ from the findings at Germany and Singapore[30,31], where the genotypes s1a/m2 are more common. It seems that there is less mosaicism in the *vacA* alleles of *H. pylori* in Germany and Singapore. The *vacA* genotype s2/m1 was not identified in our study. These data support the pioneering study of Atherton and co-workers, who also failed to detect the s2/m1 genotype, suggesting that strains with this genotype suffer from a selective disadvantage or are not viable. But there is one recent study by Letley *et al.*[32] in South Africa, where one isolate with *vacA* s2/m1 type was detected. However, *vacA* signal sequence type s1, particularly s1a, and genotype s1a/m1 appeared to occur more frequently in our study.

About 96.2% of isolates were *cagA*$^+$ by PCR, which is in agreement with the results of other studies[33–35], whereas Atherton and co-workers (see ref. 40) detected *cagA* gene only in 60% of *H. pylori* strains. All the strains (100%) from patients with ulcer, gastritis and carcinoma stomach were *cagA*$^+$, in contrast to strains isolated from patients with PHT only, where 76.9% of the *H. pylori* strains were *cagA*$^+$. It is unclear whether these differences in the *cagA* actually affect the clinical outcome of *H. pylori* infection or whether these results are identical. In a study from Kolkata, it had been reported that 90% of strains carried the *cag* PAI[26]. In both these Indian studies, prevalence of *cagA* was higher than that in the West and lower than that in East Asia.

Our study demonstrates a strong genetic association between of *cagA* and *vacA* signal sequence type s1. We observed 67 (98.5%) of 68 *vacA* type s1 strains were *cagA*$^+$ and of 76 *cagA*$^+$ strains, 67 (88.2%) were found to have the *vacA* genotype s1. Why two genetic elements without any physical linkage on the *H. pylori* chromosome should be so closely associated, is not clear. One hypothesis is that there are two clonal *H. pylori* populations (*vacA* s1/*cagA* and *vacA* s2/*cagA*), but evidence from analysis of other *H. pylori* genes fails to support it[36]. Another possibility is that there may be a functional linkage, whereby a selective advantage conferred by each

gene product is manifested only in the presence of the other. Our strains are similar to those studied in Kolkata for *vacA* s1 alleles where *vacA* s1 alleles along with *cagA* were present in almost equal proportion (80–90%), independent of the disease status.

In *H. pylori*-associated gastroduodenal disorders, the release of endotoxin and/or exotoxin may initiate an inflammatory response like many other bacteria[37]. Only 58.5% of *H. pylori* strains produced cytotoxin. Figura *et al.*[38] and Leunk *et al.*[5] have observed toxin production in 30–60% of *H. pylori* strains. In our study, toxin production is more common in the gastric carcinoma (100%) and ulcer group (67.7%) isolates compared to gastritis and PHT group, where 55% and 11% were positive for cytotoxin production. The finding corroborates the study of Figura *et al.*[38].

Furthermore, cytotoxin activity was observed almost exclusively in type s1 strains, which is similar to reports from China, Taiwan and Japan[39]. One of the striking findings of this study was that strains containing a type s2 *vacA* signal showed weak vacuolation of HeLa cells. However, strains with genotype s1 and lower cytotoxin activity may not have been identified[38]. In addition, the type of *vacA* middle region was independently associated with the level of cytotoxin activity produced by strains.

In our study and other previous studies[10,40], cytotoxic activity was found to be higher in s1a/m1 than in s1a/m2 strains and cytotoxicity seemed to be nearly absent in s2/m2 strains. It was proposed, as the most likely explanation for the high prevalence of the s1a genotype in the ulcer group, that the elevated toxicity of s1a/m1 strains might contribute to the development of ulceration.

A potentially important aspect of the *vacA* typing system presented here is its clinical relevance. In this and several previous studies[38,41,42], *H. pylori* isolates from patients with peptic ulcer disease expressed cytotoxin activity *in vitro* more commonly than isolates from patients with gastritis alone. The present investigation demonstrates a stronger link between the clinical status of patients and the *vacA* genotype strains. Why might the *vacA* genotype be a better predictor of a strain's ulcerogenic properties than direct testing of cytotoxin production *in vitro*? One possible explanation is that cytotoxin expression may occur at significantly higher levels *in vivo* than *in vitro*. Alternatively, induction of vacuolation in transformed cell lines by the cytotoxin may be only an imperfect marker for production of epithelial damage *in vivo*. The strong association between peptic ulcer disease and *vacA* type s1 strains is complemented by the equally important finding that *vacA* type s2 strains are rarely associated with peptic ulceration. The identification of *H. pylori* strains associated with different risks of ulcerogenicity may have clinical implications.

However, *cagA* was not associated with cytotoxicity on HeLa cells and only 46.1% of $cagA^+$ strains were cytotoxin-positive, and three $cagA^-$ strains also induced vacuolation in HeLa cells. These results are consistent with other reports that cytotoxin production is derived from the *vacA* gene, but not from the *cagA* gene[43,44]. We also found that all of our $cagA^+$ strains were not correlated with the grade of vacuolating cytotoxin activity, while previous studies indicated a close association between the CagA protein and vacuolating cytotoxin activity and concluded that the CagA protein regulates the production of vacuolating cytotoxin[12,45,46]. Tummuru *et al.*[46], however, reported that disruption of the *cagA* gene by shuttle mutation did not influence the production of the vacuolating cytotoxin.

In conclusion, *H. pylori* strains with the *vacA* signal sequence type s1a and middle region allele m1 were predominant in north India, in and around Lucknow. All combinations of these *vacA* alleles occurred, with the exception of s2/m1. Type s1 strains were associated with ulcer and carcinoma stomach, presence of the *cagA* gene and cytotoxin activity. The findings suggest that $cagA^+$ *H. pylori* strains with the *vacA* genotype s1 increase the risk for peptic ulcer diseases. Thus, *vacA* genotyping may allow identification of infected subjects at different risk levels. Among north Indian clinical isolates, there was a clustering of the $cagA^+$ strains with the s1a/m1 family of *vacA* alleles. Thus, *vacA* genotyping may allow identification of infected subjects at different risk levels.

1. Parsonnet, J., *Aliment. Pharmacol. Ther.*, 1995, **9**, 45–51.
2. NIH Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Diseases, *JAMA*, 1994, **272**, 65–69.
3. Westblom, T. U., Czinn, S. J. and Nedrud, J. G., *Curr. Top. Microbiol. Immunol.*, 1999, **241**, 301–308.
4. Parsonnet, J. (ed.), in *Microbes and Malignancy: Infection as a Cause of Cancers*, Oxford University Press, New York, 1999, pp. 372–408.
5. Leunk, R. D., Johnson, P. T., David, W. G. and Morgan, D. R., *J. Med. Microbiol.*, 1988, **26**, 93–99.
6. Cover, T. L., *Mol. Microbiol.*, 1990, **20**, 241–246.
7. Cover, T. L., Glupezynski, Lage, A. P., Burette, A. and Blaser, M. J., *J. Clin. Microbiol.*, 1995, **33**, 1496–1500.
8. Phadnis, S. H., Liver, D., Janzon, L. and Westblom, T. U., *Infect. Immunol.*, 1994, **62**, 1557–1565.
9. Cover, T. L. and Blaser, M. J., *J. Biol. Chem.*, 1992, **267**, 10570–10575.
10. Atherton, J. C., Cao, P. and Peek, R. M., *ibid*, 1995, **270**, 17771–17777.
11. Van Doorn, L. J., Figueiredo, C. and Sanna, R. J., *Clin. Microbiol.*, 1998, **36**, 2597–2603.
12. Covacci, A., Censini, S. and Bugnoli, M., *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 5791–5795.
13. Blaser, M. J., *Ann. Med.*, 1995, **27**, 559–563.
14. Censini, S., Lange, C. and Xiang, Z., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 14648–14653.
15. Ito, Y., Azuma, T. and Ito, S., *J. Clin. Microbiol.*, 1997, **35**, 1710–1714.
16. Jorgensen, M., Daskalopoulos, G. and Warburton, V., *J. Infect. Dis.*, 1996, **174**, 631–635.
17. Van Doorn, L. J., Figueiredo, C., Megraud, F. and Quint, W. G. V., *Gastroenterology*, 1999, **116**, 823–839.
18. Mishra, K. K., Srivastava, S., Dwivedi, P. P., Prasad, K. N. and Ayyagari, A., *Indian J. Pathol. Microbiol.*, 2002, **45**, 31–38.

19. Ayyagari, A., Ray, P. and Kocsr, R., *Indian J. Med. Res.*, 1990, **91**, 126–128.
20. Yoshiyuki Ito, Takeshi Azuma, Shigeji Ito, Hideki Miyaji and Masaru Kuriyama, *J. Clin. Microbiol.*, 1997, **35**, 1710–1714.
21. Evans, D. G., Queiroz, D. M. M. and Mendes, E. N., *ibid*, 1998, **36**, 3435–3437.
22. Go, M. F., Cissel, Fl. and Graham, D. Y., *Scand. J. Gastroenterol.*, 1998, **33**, 132–136.
23. Miehlke, S., Kibler, K. and Kim, J. G., *Am. J. Gastroenterol.*, 1996, **91**, 1322–1325.
24. Pan, Z. J., Berg, D. E. and van der Hulst, R. W. M., *J. Infect. Dis.*, 1998, **178**, 220–226.
25. Yamaoka, Y., Kodama, T. and Gutierrez, O., *J. Clin. Microbiol.*, 1999, **37**, 2274–2279.
26. Mukhomadhyay, A. K., Kersulyte, D. and Jeong, J. Y., *J. Bacteriol.*, 2000, **182**, 3219–3227.
27. Ito, Y., Azuma, T. and Ito, S., *J. Clin. Microbiol.*, 1997, **35**, 1710–1714.
28. Van Doorn, L. J., Figueiredo, C. and Rossau, R., *ibid*, 1998, **36**, 1271–1276.
29. Strovel, S., Vereswill, S. and Balig, P., *ibid*, 1998, **36**, 1285–1289.
30. Beji, A., Vincent, P. and Darchis, I., *Lancet*, 1989, **ii**, 1402–1403.
31. Zheng, P. Y., Hua, J. and Yeoh, K. G., *Gut*, 2000, **47**, 18–22.
32. Letley, D. P., Lastovica, A. and Louw, J. A., *J. Clin. Microbiol.*, 1999, **37**, 1203–1205.
33. Xiang, Z., Censini, S. and Bayelli, P. F., *Infect. Immunol.*, 1995, **63**, 94–98.
34. Crabtree, J. E., Figura, N. and Taylor, J. D., *J. Clin. Pathol.*, 1992, **45**, 73–734.
35. Xiang, Z., Bugnoli, M. and Ponzetto, A., *Eur. J. Clin. Microbiol. Infect. Dis.*, 1993, **12**, 739–745.
36. Garner, J. A. and Cover, T. L., *J. Infect. Dis.*, 1995, **172**, 290–293.
37. Marshall, B. J., *Am. J. Gastroenterol.*, 1994, **89**, S116–S128.
38. Figura, N., Gugliemetti, P. and Rossolini, A., *J. Clin. Microbiol.*, 1989, **27**, 225–227.
39. Wang, H. J., Kuo, C. H. and Yeh, A. A. M., *J. Infect. Dis.*, 1998, **178**, 207–212.
40. Atherton, J. C., Peek, R. M. and Tham, K. T., *Gastroenterology*, 1997, **112**, 95–97.
41. Tee, W., Lambert, J. and Dwyer, B., *J. Clin. Microbiol.*, 1995, **33**, 1203–1205.
42. Rautelein, H., Blomberg, B. and Jarnerot, G., *Scand. J. Gastroenterol.*, 1994, **29**, 128–132.
43. Weel, J. F. L., Van der Hulst, R. W. M. and Gerritis, Y., *J. Infect. Dis.*, 1996, **173**, 1175–1176.
44. Tummuru, M. K. R., Cover, T. L. and Blaser, M. J., *Infect. Immunol.*, 1994, **62**, 2609–2613.
45. Cover, T. L., Dooley, C. P. and Blaser, M. J., *ibid*, 1990, **267**, 10570–10575.
46. Tummuru, M. K. R., Cover, T. L. and Blaser, M. J., *ibid*, 1993, **61**, 1799–1809.

ACKNOWLEDGEMENTS. We thank Prof. S. R. Naik, Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow for extending clinical support for this study.

Received 20 February 2002; revised accepted 16 July 2002

FTIR investigation on the fluid inclusions in quartz veins of the Penakacherla Schist Belt

M. Ram Mohan and P. S. R. Prasad*
National Geophysical Research Institute, Hyderabad 500 007, India

Penakacherla Schist Belt is a part of the eastern Dharwar craton lying along the Ramagiri–Hungund arc. Fluids entrapped in quartz veins of two mafic volcanic rocks of this area are characterized by FTIR spectroscopy in the wave number region 2000–8000 cm^{-1}. The quartz–carbonate vein from the central part of the schist belt contains H_2O, CO_2 and aliphatic hydrocarbon inclusions, with bicarbonate in the form of nahcolite ($NaHCO_3$) coexisting with quartz, whereas, that from the nearby Bhadrampalli mine area is depleted in CO_2 and hydrocarbons, and enriched in H_2O inclusions. Distinctly clear signatures of silanol (Si-OH) group and the coexistence of calcite with quartz have also been observed in the latter quartz. These observations indicate that re-crystallization of quartz in these two similar rocks occurred in vastly different hydrothermal environments.

QUARTZ–gold veins in metamorphic rocks usually contain low to moderate saline, mostly immiscible H_2O and CO_2 fluid inclusions. There has been some debate over the nature of these inclusions, whether dominantly metamorphic, meteoric or magmatic[1–5]. The fluid inclusions, in general, are considered to preserve the conditions of mineral formation and are a measure of the genetic evolution[1]. However, in the recent past there has been a host of experimental and theoretical evidence against the 'closed' system behaviour of fluid inclusions[1,6–8]. Fluid inclusions are now considered to behave as an 'open' system and their contents may diffuse into or out of the host mineral or rock. Detailed characterization of interactions among fluid inclusions and the host mineral or rock, and formation of some daughter minerals are important to understand various metamorphic processes. Vibrational spectroscopy (IR and Raman) is a versatile and non-destructive analytical tool to characterize the physico-chemical conditions of fluid entrapment. Detailed structural variations due to environmental impact could be probed using this method[9,10]. Infrared absorption of hydroxyl ions is generally strong, and combined FTIR study covering both IR and NIR regimes is preferred to resolve the nature of these ions in minerals, namely hydrogen in the form of hydroxyl or water of crystallization[11,12]. On the contrary, Raman cross-sections for (OH) groups are less and, hence, IR spectroscopy is preferred

*For correspondence. (e-mail: psrprasad_6@rediffmail.com)