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Prevalence of *Helicobacter pylori vacA*, *cagA and iceA* genotypes in South African patients with upper gastrointestinal diseases

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ABSTRACT

Clinical response to *Helicobacter pylori* infection may be determined by specific virulence-associated genotypes which varies geographically. The aim of this study was to investigate the diversity of putative virulence markers of *H. pylori*; *cagA*, *vacA and iceA* in the Eastern Cape Province of South Africa. One hundred *H. pylori* strains obtained from dyspeptic patients were used. Gastric biopsies were obtained from 254 dyspeptic patients. *H. pylori* was cultured and strains were studied. Bacterial genotypes *cagA*, *vacA* (s and m subtypes) and *iceA* were analysed by PCR using specific primers. *CagA* was identified in 90% of the strains investigated. Fifty-eight of the 100 strains had the *vacA* signal sequence genotype s1 and 26 had subtype s2. Combined *vacA* s1/s2 was detected in 16 of the strains. *VacA* middle region analysis showed that 8 (8%) strains were m1 while 50 were m2. Combined *vacA* m1/m2 was detected in 36 of the strains. s1m2 (20%) and s2m2 (20%) genotypes were the most common allelic combinations of the *vacA* gene among the strains. Multiple *vacA* genotypes were detected in this study. Twenty-six percent of the strains identified had both *iceA1* and *iceA2*. All our strains tested positive for the *ureC (glmM)* gene. This study reveals a high prevalence of *vacA*, *cagA* and *iceA2*.

1. Introduction

Helicobacter pylori is a Gram-negative curve rod that inhabits the gastric mucosa of the human stomach. It chronically infects billions of people worldwide, is one of the most genetically diverse of bacterial species, and is a major cause of peptic ulcer disease and gastric cancer in many populations (Rudi et al., 1999; Andreson et al., 2002; Cover and Blanke, 2005; Ndip et al., 2008). Several potential markers related to risk of gastroduodenal diseases with *H. pylori* infection *vacA*, *cagA* and several other 'housekeeping genes' such as *ureA* and *ureC* (van Doorn et al., 1998; Letley et al., 1999; Smith et al., 2002) which might not be directly linked to virulence of the strain have been identified.

The vacuolating cytotoxin (*vacA*) gene is present in virtually all *H. pylori* strains and contains two variable regions; the signal (s) region, which encodes the signal peptide, and the middle (m) region (Arents et al., 2001; Smith et al., 2002). The s region has two subtypes, s1 and s2 alleles. The s1 exists as an s1a, s1b and s1c. The m region also has two subtypes, m1 and m2 which occurs as, m2a, or m2b (Bravo et al., 2002; Datta et al., 2003; Wang et al., 2003; Asrat et al., 2004).

H. pylori vacA type s1 strains appear to be more virulent than type s2 strains and are associated with higher risks for peptic ulcer disease, gastric atrophy, and gastric carcinoma (van Doorn et al., 1998; Letley et al., 1999; Figueiredo et al., 2002). The mosaic combination of s- and m-region allelic types determines the production of the cytotoxin and thereby associated with pathogenicity of the bacterium (Asrat et al., 2004). The *vacA* s1m1 strains produce large amounts of toxin and are strongly associated with a higher degree of inflammation and epithelial damage in the gastric mucosa; s1m2 strains produce moderate amounts of toxin while the s2m1 strains produce very little or no toxin (van Doorn et al., 1998; Figueiredo et al., 2002; Kusters et al., 2006; Ko et al., 2008).

The cytotoxin-associated gene (*cagA*) is a marker for a genomic pathogenicity island of 40 kb (Akopyanz et al., 1992). Several genes of this cag island, such as *picB*, encode proteins that enhance the virulence of the strains, by increasing the production of interleukin 8 (IL-8) by gastric epithelial cells amongst others (van Doorn et al.,

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1998). The *cagA* gene is present in 60–70% of *H. pylori* strains and encodes a high molecular weight antigenic protein (120–140 kDa) (Kidd et al., 1999).

Another virulence associated gene designated *iceA* (induced by contact with epithelium) has been identified. There are two main allelic variants of the gene: *iceA1* and *iceA2*. The function of *iceA1* is not yet clear but there is significant homology to a type 11 restriction endonuclease. The expression of *iceA1* is upregulated on contact between *H. pylori* and human epithelial cells and maybe associated with peptic ulcer disease (van Doorn et al., 1998) and enhanced acute neutrophilic infiltration. However, linkage between the *iceA1* genotype and ulcer disease is not universal (Kidd et al., 2001). Only *iceA1* RNA is induced following adherence *in vitro* (Kidd et al., 2001).

Sequence typing has indicated that different *H. pylori* genotypes predominate in different human populations. In particular, African strains seem to be distinct from those of other continents (Falush et al., 2003). Many studies have shown geographic differences in predominant *H. pylori* genotypes, based either on virulence associated genes such as *vacA* and *cagA* or "housekeeping genes" that are present in all *H. pylori* strains and not particularly linked to virulence (van Doorn et al., 1998; Bravo et al., 2002).

H. pylori colonisation is very common in South Africa, as in other developing countries (Pelsar et al., 1997; Mosane et al., 2004; Samie et al., 2007; Tanih et al., 2010). In a Sowetan study of asymptomatic children aged 6–15 years, 86.5% were infected with a *vacA* positive strain and 87% with a *cagA*-positive strain. The majority of *cagA*-positive strains carried *vacA* s1 allele while most *cagA*-negatives carried the *vacA* s2 allele (Ally et al., 1999). In a separate study, *vacA* diversity was demonstrated among South African *H. pylori* strains; interestingly, no strains with the *vacA* s1a genotype were found among the isolates from black or mixed-race South Africans (Letley et al., 1999). In another study by Kidd et al. (1999); *vacA* s1 genotype, and a fragment length of the 3′ region of *cagA* were identified and were associated with significant clinical disease. The aim of this study was to examine the genotypes of *H. pylori* strains, *vacA*, *cagA* and *iceA*, using PCR based methods, in patients from the Eastern Cape Province of South Africa, which is a predominantly rural region, a predisposing factor for *H. pylori* acquisition. Furthermore, this study is expected to provide baseline information for future clinical-epidemiological studies of this population.

2. Materials and methods

2.1. Study subjects

We evaluated 254 consecutive patients referred for endoscopy at the Livingston Hospital, Port Elizabeth, South Africa between May and December 2008. Livingston hospital is a tertiary level 1000 bed teaching and referral hospital with a wide referral base in the Eastern Cape. The patient base consists largely of those of the low and middle class mostly the coloured and blacks. The nature and purpose of the study was explained to the patients until fully understood. We enrolled only patients who gave informed consent. The study was approved by the institutional review board of the University of Fort Hare and the Eastern Cape Department of Health (Protocol number EcDoH-Res 0002).

2.2. Culture and identification

Antral and corpus gastric mucosal biopsy samples were taken from each dyspeptic patient. The biopsies were immediately placed in sterile bijou bottles containing 0.2 g/L of cysteine and 20% glycerol in Brain heart infusion (BHI) broth and transported in ice to the laboratory within 2 h of collection for culture. *H. pylori* was cultured using standard method and isolates were identified based on colony morphology, Gram staining, oxidase, catalase, and urease tests (Ndip et al., 2008). Isolates were further confirmed by amplification of the *glmM* gene. Confirmed isolates were suspended in 20% glycerol and BHI and stored at −80 °C (Sanyo, Japan) until genotyping was performed. *H. pylori* reference strain NCTC 11638 was included in all experiments.

2.3. Genomic DNA extraction

DNA was extracted from one hundred *H. pylori* strains. Isolates were centrifuged at 10,000 × *g* for 5 min, and DNA extracted from the pellets by use of the QIAamp DNA kit (Qiagen DNA extraction kit, SA) according to the manufacturer's recommendations and stored at −20 °C until analysis. DNA extraction negative controls were performed in parallel by including sterile tubes without samples to check for contamination of the DNA extraction reagents.

2.4. Polymerase chain reaction (PCR)

PCR analysis of the targeted genes was performed using Thermostat Taq DNA polymerase (ABgene, UK) and manufacturer-provided reaction buffer. Five microlitre of DNA were added to 50 μl of reaction mixture containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM (each) dNTPs (ABgene, UK) and 0.5 μM of respective oligonucleotide primers. Thermo-stat Taq DNA polymerase (ABgene, UK) 1.25 U/μl was added to each tube. PCR was performed with a thermal cycler (MJ Research, USA). The amplification cycles consisted of an initial denaturation of target DNA at 95 °C for 15 min and then denaturation at 94 °C for 1 min, primer annealing at 60 °C, 56 °C, and 50 °C for *cagA*, (*vacA* m1, m2, s1/s2 and *iceA*), and *glmM* respectively for 1 min and extension at 72 °C for 1 min. All reactions were performed through (35 cycles) except *glmM* (40 cycles). The final cycle included an extension step for 5 min.

The primers used to amplify the targeted genes are summarised in Table 1. Negative controls were added to each PCR run including all reagents except template DNA which was substituted with ultra pure water (Sigma–Aldrich, UK). Amplification of DNA was analysed by agarose gel electrophoresis using standard procedures (van Doorn et al., 1998). Briefly, aliquots of amplified samples (5 μl) were electrophoresed on 2% high-resolution agarose gel in TAE buffer. The gel was stained with ethidium bromide 0.5 μg/ml. The amplified bands were visualised under ultraviolet light and photographed.

2.5. Statistical analysis

Epi info version 2000 (Center for Disease Control and Prevention, Atlanta, Ga.) was used for statistical analysis. Chi square or fischer exact test was applied to test whether differences in prevalence between values of the various genotypes or disease conditions were significant at P-value < 0.05.

3. Results

3.1. Distribution of genotypes of vacA, cagA and iceA

H. pylori DNA was successfully extracted from 100 strains. DNA integrity and specificity was confirmed by *glmM* amplification, which rendered the expected band size of all isolates (data not shown). All our samples were positive for the *glmM* (100/100).

Most of our samples were positive for *vacA* at least for one of the alleles (the s-region or the m-region). The most virulent *vacA*s1 allele reported in most study was predominantly present in our

Table 1
PCR primers for amplification of *cagA*, *vacA*, *iceA* and *glmM* sequences.

Gene	Primer sequence	PCR product (bp)	Reference
cagA	5′-TTGACCAACAACCACAAACCGAAG-3′ 3′-CTTCCCTTAATTGCGAGATTCC-5′	183	Smith et al. (2002)
vacA s1/s2	5′-ATGGAAATACAACAAACACAC-3′ 3′-CTGCTTGAATGCGCCAAAC-5′	259/286	Smith et al. (2002)
vacA m1	5′-GTCAAAATGCGGTCATGG-3′ 3′-CCATTGGTACCTGTAGAAAC-5′	290	Smith et al. (2002)
vacA m2	5′-GGAGCCCCAGGAAACATTG-3′ 3′-CATAACTAGCGCCTTGCAC-5′	290	Smith et al. (2002)
IceA1	5′-GTTGGGTAAGCGTTACAGAATTT-3′ 3′-CATTGTATATCCTATCATTAC-5′	567	Smith et al. (2002)
IceA2	5′-GTTGGGTATATCACAATTTAT-3′ 3′-TTRCCCTATTTTCTAGTAGGT-5′	229 or 334	Smith et al. (2002)
glmM	5′-TTTGGGACTGATGGCGTGAGGGGTAA-3′ 3′-GGACATTCAAATTCACCAGGTTTTGAG-5′	1142	Burucoa et al. (1999)

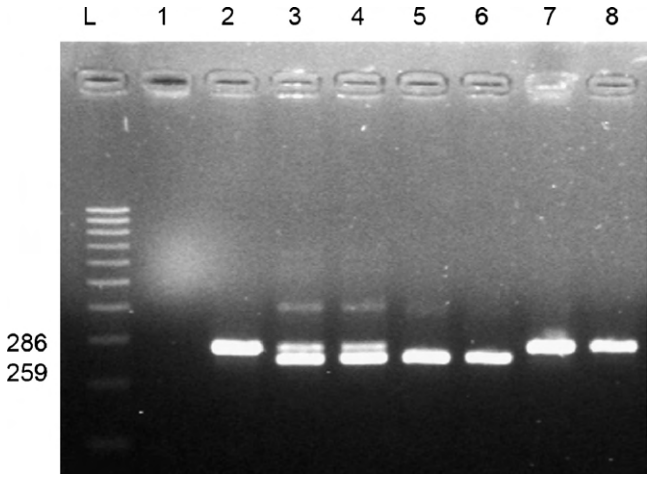

Fig. 1. Detection of *s1* and *s2* alleles of the *vacA* gene. Lane L, marker; lane 1 negative control; lanes 2, 7 and 8 s2 allele; lanes 5 and 6 *s1* allele; lanes 3 and 4 *s1*/*s2* alleles. Numbers on the left indicate molecular size (in base pairs).

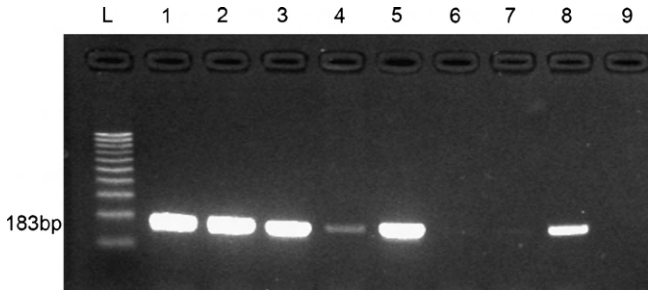

Fig. 2. Agarose 2% gel electrophoresis of PCR products for *cag A* detection. Lanes 1, 2, 3, 4, 5 and 8, *cagA*+; lanes 6, 7 and 9, *cagA*−; lane l, marker.

strains 58/100 (58%), and was visualised as a band of 259 bp on agarose gel electrophoresis (Fig. 1), whereas 26/100 (26%) of the isolates had the *vacA*s2 genotype. Combined *vacA* s1/s2 was detected in 16 (16%) of our strains. The middle region of the *vacA* gene was detected in 94/100 (94%) of our strains. *vacA* m1 was detected in 8 (8%) strains while m2 was found in 50 (50%) of the strains. Combined *vacA* m1/m2 was detected in 36 (36%) of our strains. The s genotypes were more equally distributed than m1 and m2 genotypes. Genotyping of the middle region failed in 6/100 (6%) of our strains. s1m2 and s2m2 genotype were the most common allelic combinations of the *vacA* gene among our strains. Eight percent of our strains harboured s1m1 genotype. The genotype s2m1 was not identified in this study.

Multiple *vacA* genotypes were detected in this study 46/100 (46%) with *vacA* s1m1m2 genotype being the most prevalent allelic combination 28 (28%) (Table 2) indicating the presence of mixed infection. Multiple *vacA* genotypes appeared to be more prevalent in patients with non ulcer dyspepsia (20/40, 50%) than in those with the other disease conditions enrolled in this study, although the difference was not statistically significant ($P > 0.05$).

Amplification of the *cagA* gene was visualised as a band of 183 bp (Fig. 2) and was present in 90% of our strains (Table 3). *IceA1* was detected in 2 (2%) and *iceA2* was found in 58 (58%) cases. Mixed *iceA* (*iceA1* + *iceA2*) genotypes were found in 26 (26%) of our strains. *IceA* genotype was completely absent in 14 (14%) (Table 2).

Table 2
Association of *vacA* with *cagA* and *iceA* genotypes.

vacA	*cagA*+ (%)	*cagA*− (%)	*iceA1* (%)	*iceA2* (%)	*iceA*− (%)
s-region					
s1	56 (96.6)	2 (3.4)	12 (22.2)	52 (73.3)	4 (4.4)
s2	24 (92.3)	2 (7.7)	8 (23.5)	22 (64.7)	4 (11.7)
s1s2	12 (75)	4 (25)	2 (14.3)	12 (85.7)	4 (14.3)
m-region					
m1	8 (100)	0 (0)	4 (8.7)	42 (91.3)	0 (0)
m2	46 (92)	4 (8)	24 (21.4)	80 (71.4)	10 (11.1)
m1m2	34 (94.4)	2 (5.6)	8 (18.18)	36 (81.81)	0 (0)
s\m region					
s1m1	8 (100)	0 (0)	2 (20)	8 (80)	0 (0)
s1m2	18 (90)	2 (10)	10 (35.7)	16 (57.1)	2 (7.1)
s2m2	18 (90)	2 (10)	4 (20)	12 (60)	4 (20)
Multiple vac	40 (86.9)	6 (13.1)	10 (17.9)	44 (78.6)	2 (3.8)
Incomplete vac	6 (100)	0 (0)	2 (33.3)	2 (33.3)	2 (33.3)

Table 3
vacA, *cagA*, and *iceA* status of *Helicobacter pylori* from 100 strains.

Genotype status	PUD $n=30$ (%)	GERD $n=10$ (%)	NUD $n=40$ (%)	GS $n=8$ (%)	OTHERS $n=12$ (%)	Total $n=100$ (%)
vacA						
s1m1	0(0)	2(20)	6(15)	0(0)	0(0)	8(8)
s1m2	8(26.7)	2(20)	2(5)	4(50)	4(33.3)	20(20)
s2m2	6(20)	2(20)	8(20)	0(0)	4(33.3)	20(20)
s1m1m2	6(20)	2(20)	16(40)	2(25)	2(16.6)	28(28)
s2mIm2	0(0)	2(20)	0(0)	0(0)	2(16.6)	4(4)
s1s2m2	4(13.3)	0(0)	4(10)	2(25)	0(0)	10(10)
s1s2m1m2	4(13.3)	0(0)	0(0)	0(0)	0(0)	4(4)
Incomplete *vac s* or *m*	2(6.7)	0(0)	4(10)	0(0)	0(0)	6(6)
cagA						
cagA+	24(80)	10(100)	36(90)	8(100)	12(100)	90(90)
cagA−	6(20)	0(0)	4(10)	0(0)	0(0)	10(10)
IceA						
IceA1	0(0)	0(0)	2(5)	0(0)	0(0)	2(2)
IceA2	18(60)	8(80)	22(55)	6(75)	4(33.3)	58(58)
IceA−	10(33.3)	0(0)	4(10)	0(0)	0(0)	14(14)
IceA1 + IceA2	4(13.3)	2(20)	10(25)	2(25)	8(66.6)	26(26)

3.2. Association amongst the genotypes

In this investigation, *vacA* (s and m region) strains had a higher prevalence for both *cagA* positive and *iceA2* positive than with the negatives. Fifty-six (89.2%) of our *vacA*s1 strains were *cagA* positive. The majority of our *vacA*s1 strains were *iceA2* positive, 52 (73.3%) while 12 (22.2%) of *vacA*s1 were *iceA1* positive. Four strains which were positive for *vacA*s1 and s2 respectively were negative for the *iceA* gene.

Most of our s1m2 carried a *cagA* positive (90%) and *iceA2*-positive (57.1%) allele while s2m2 represented 90% of our *cagA* positive strains and 60% *iceA*-positive. Examination of the various combinations (s1m1, s1m2, s2m2) revealed a higher prevalence of *iceA2* than *iceA1*.

3.3. Relationship between genotypes and gastroduodenal diseases

Of the 100 strains studied, 40 were from patients diagnosed with non ulcer dyspepsia (NUD), 30 with peptic ulcer disease (duodenal ulcer and gastric ulcer), 10 gastro-oesophageal reflux disease (GERD), 8 gastritis (GS) and 12 had signs and symptoms of gastroduodenal disease and not a particular disease condition per se (Table 3). *vacA* s1m2 genotype was detected in all the disease conditions mentioned above but at a higher frequency in strains from patients with GS (4/8, 50%) and peptic ulcer disease (8/30, 27%). s2m2 was completely absent in patients presenting with gastritis but had the highest prevalence in patients with NUD (8/40, 20%). Most of the strains with the combination s1m1 were from patients with NUD. Multiple *vacA* genotypes were detected in this study with s1m1m2 being the most prevalent with the highest frequency in patients with NUD (16/40, 40%) however, there was no statistical correlation of the combinations of *vacA* genotypes with the various disease status ($P > 0.05$) (Table 2).

4. Discussion

H. pylori colonisation is associated with a spectrum of gastroduodenal pathologies. Although infection is universally associated with gastritis, the development of clinically significant disease seems to depend on a number of factors, including the virulence of the infecting strain, the susceptibility of the host and environmental cofactors, which varies geographically.

Molecular studies on *H. pylori* have resulted in the identification of a number of non-conserved candidate virulence markers and their association with the clinical outcome of disease. Kidd et al. (2001) and some other authors (Ally et al., 1999; Letley et al., 1999) have carried out a number of studies in South Africa to determine the genotype of *H. pylori* circulating in their study area (predominantly affluence), but the present study is the first in the Eastern Cape Province (predominantly rural with deprived living conditions) aimed at investigating the prevalence of various virulence factors (*vacA*, *cagA* and *iceA*) using PCR based methods and their relationship with the clinical outcome of disease.

Vacuolating cytotoxin has been implicated in the pathogenesis of peptic ulceration and a number of gastroduodenal pathologies. *VacA* signal type s1a are often associated with peptic ulceration, while *vacA* s2 strains are usually found in patients with no ulcers, *vacA* slb strains seem to be intermediately ulcerogenic. The *vacA* s1 and s2 leader sequences are different in a small insert, totalling 27 bp, carried by the *vacA*s2 allele which has a reduced capacity to secrete *vacA* toxin. South African *H. pylori* isolates were previously characterised by the universal presence of *cagA* but have differences in vacuolating cytotoxin gene (*vacA*) alleles (Kidd et al., 2001).

According to our results, the most virulent *vacA*s1 allele was predominant in our study population 58 (58%) a finding which has also been observed in other studies in South Africa and the world (Letley et al., 1999; Bravo et al., 2002; Smith et al., 2002). Ally et al. (1999) reported a high prevalence of *vacAs1 H. pylori* strains in their study in South Africa. Asrat et al. (2004) and Smith et al. (2002) also demonstrated a high prevalence of *vacA*s1 in their study in Ethiopia and Nigeria, respectively. Our results are however contrary to the findings noted in African Arabs who are predominantly infected with the s2 type allele (Al Qabandi et al., 2005).

In this study, the prevalence of *vacA* m2 (50%) was higher than *vacA*m1 (8%) (Table 2). Our results are similar to the findings of Wong et al. (2001) who reported a higher prevalence of m2 in their study; however, some authors have documented a higher prevalence of m1 in their study area (Smith et al., 2002; Asrat et al., 2004; Ko et al., 2008). Our results portray a high prevalence of *vacA*m1/m2 (36%).

Meanwhile, s1m2 and s2m2 were the most common combinations of the *vacA* gene in our study population. The genotype s1m1 was detected in 8% of all the strains analysed. Our results are in accordance with the findings of Wong et al. (2001) that delineated a high prevalence of s1m2 and a low prevalence of s1m1. However, the frequency of *vacA* s1m1 allelic type in our study is low than those reported from the Netherland (36%), Hong Kong (26–31%) and Nigeria (24%) (van Doorn et al., 1998; Wong et al., 2001; Smith et al., 2002) which may be a reflection of the great heterogeneity exhibited by this organism.

In the present investigation, the rare s2m1 allele was also not detected. This finding is in line with several studies in different parts of the world but contrary to the finding of Asrat et al. (2004) and Smith et al. (2002) who reported the presence of this allele though in low percentages; 2% and 6.7% respectively in their various investigations. However, the fact that the s2m1 allele was not found in this study cannot be completely ruled out for we detected multiple *vacA* genotypes (s2m1m2) of which s2m1 was a makeup. The allele s2m1 has been noted to suffer from a selective disadvantage (Letley et al., 1999).

Multiple *H. pylori* genotypes were detected in 46% (46/100) of the strains. The presence of multiple *vacA* genotypes in this study ties with the finding of other studies both in Africa and the world (Morales-Espinosa et al., 1999; Wong et al., 2001; Arents et al., 2001; Andreson et al., 2002; Asrat et al., 2004). However, this is the first study in South Africa to report the detection of multiple *vacA* genotypes. Infection with these strains has been associated with a higher degree of inflammation and gastroduodenal lesions. Our data did not indicate that multiple strain infection increases the risk of developing NUD ($P > 0.05$). Furthermore, the middle region of *vacA* was not detected in six of our strains. Genotyping of the *vacA* middle region failed in four strains, probably due to heterogeneity in the *vacA* gene, a finding reported previously (Asrat et al., 2004). *vacA s*2m2 genotype was noted at a higher frequency in NUD; but no significant correlation was observed between s2m2 genotypes and the appearance of NUD. Generally, there was no statistically significant difference between specific genotypes and disease conditions which is in agreement with previous reports (Park et al., 1998; Wong et al., 2001).

H. pylori cagA positive strains have been associated with more severe gastroduodenal diseases (van Doorn et al., 1998). Of the one hundred strains examined in this study, the *cagA* gene was detected in 90%, prevalence similar to that reported in many African countries and other parts of the world (Wong et al., 2001; Smith et al., 2002; Asrat et al., 2004). Our results are also consistent with those of a Sowetan study (South Africa) of asymptomatic children aged 6–15 years, in which 87% were *cagA*-positive strains (Ally et al., 1999). In our study, *cagA* positivity was not associated with the presence of a specific disease condition ($P > 0.05$).

The present study showed that *iceA2* (58%) allele was predominant among our strains. This corroborates the findings of Yamaoko et al. (1999) in the USA and a study among Jordanian patients by Nimri et al. (2006) where *iceA2* was predominant, but however different from the strains reported in Hong Kong, Japan, Korea and Nigeria where *iceA1* allele was predominant (Yamaoko et al., 1999; Nimri et al., 2006). Twenty-six percent of the strains had both *iceA1* and *iceA2* genotypes. Our results corroborates the findings of a study in South Africa by Kidd et al. (2001) who demonstrated the presence of *iceA1* and *iceA2* in 68% and 80% respectively of their isolates examined; and also detected combined *iceA* (*iceA1*, *iceA2*) in approximately 40% of patients. *IceA* positivity was not associated with the presence of a specific disease condition in our study ($P > 0.05$). On comparing the various *vacA* genotypes and the presence of *iceA2* allele, we observed that there was a significant correlation between *vacA* genotype and *iceA*2 ($P < 0.05$).

We studied the association of *cagA*, *iceA* status and *vacA*s1 genotype which is commonly linked to an increase in *H. pylori* virulence (Arents et al., 2001). There was no association between *cagA*, *iceA* status and *vacAs1* genotype ($P > 0.05$). Previous studies have shown a high association between *cagA* positive genotype and the appearance of PUD (Park et al., 1998; Wong et al., 2001). In this study, however, no correlation was observed between *cagA* status and PUD. However, a high frequency of cag-positive strains was observed in PUD patients (Table 3), which may indicate that a statistical association could be reached by increasing the number of patients in future studies.

5. Conclusion

We are led to conclude that the absence of a correlation between the virulence genes analysed and the development of gastroduodenal disease might be due to the small number of patients with each disease condition in our study, even though several others have not found any correlation between the presence of *H. pylori* main virulent factor (alone or in combination) and gastroduodenal diseases. It may be that a large population of patients must be studied to reveal statistically significant relations between *H. pylori* virulence genes and patterns of clinical disease.

This study has shown a relatively high prevalence of the main virulence factor (*vacA*, *cagA* and *iceA* genes) in South Africa *H. pylori* isolates. It also demonstrates that *vacA* s1, *iceA2* and *cagA*+ are common genotypes of *H. pylori* in our study group. Multiple *vacA* genotypes were found, and the presence of such combined genotypes in infected patients may increase the risk of development of clinical conditions such as peptic ulceration and gastric cancer. We advocate further genetic analysis to determine the homology of *H. pylori* genome in members of the same family in order to study the transmission of the organism from person to person and their clinical implications in our environments.

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