

Original Article

# Detection of *vacA* gene alleles frequency in *Helicobacter pylori* strains from patients with gastric diseases in Zliten city Libya

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## Abstract

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*Helicobacter pylori* (*H. pylori*) has worldwide distribution, leading to various gastric diseases, including chronic gastritis, peptic ulcer and gastric cancer. A *vacA* gene, which encodes a vacuolating cytotoxin is one of the most known virulence gene of the bacterium. The aim of this study was to evaluate the most common *vacA* alleles (*s1* and *s2*) in *H. pylori* strains isolated from Libyan patients and its relationship with ages and gastritis lesions. Gastric biopsies were obtained from patients for DNA extraction. *vacA* genotypes were analyzed by PCR and agarose electrophoresis. *s1* and *s2* genotypes were also confirmed by DNA sequencing. The allele *s2* occurred in 81% of the all examined group, which represent the most frequently observed of the signal encoding region. Whereas *s1* genotype had the lowest frequency 19%. Statistically significant differences in *s1* and *s2* alleles in relation to a ages were not detected. This study showed that there was a relationship between the presence of *vacA* gene and progression of gastritis. the predominant *vacA* gene alleles in Zliten city is *s2* allele.

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**Key words:** *H. pylori*, *vacA* alleles (*s1* and *s2*), Libya

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## Introduction

*H. pylori* has worldwide distribution, leading to various gastric diseases, including chronic gastritis, peptic ulcer and gastric cancer[1]. Approximately 50% of world population are infected with *H. pylori*. Moreover, the rate of colonization in developing countries is higher than western countries. In Africa, the prevalence rate reach up 90% of populations, and the majority of infection with this bacterium occurs in childhood ages[2,3].

In recent years, *H. pylori*-associated with gastric diseases are related to a number of virulence factors such as the cytotoxin-associated gene A (cagA), vacuolating cytotoxin gene A (vacA) and duodenal ulcer promoting gene A (dupA)[4,5]. VacA gene is one of the most essential virulence factors of this bacterium. vacA gene has both conserved and variable allelic sequences. These variable sequences are found in different regions at N-terminal side, including signal sequence (s1 and s2) region and mid (m1 and m2) region. The influence of cytotoxicity is depended on genetic diversity among genotypes. The both s1 and m1 regions subdivided to several subtypes, such as s1a, s1b, s1c, m1a, m1b and m1c[6,7]. The cytotoxicity power of this antigens occurs through induction of cytoplasmic vacuolation and leading cause of death of infected gastric epithelial cells[8]. Some studies confirmed that there is a strong relationship between the presence of vacA gene and progression of peptic ulcer and gastric cancer[9]. However, a number of studies have also rejected this finding [10].

In vitro study showed that non-cytotoxic (Tox-) strains lack a short fragment in the s region in (Tox-) strains. This fragment inhibits the splitting of vacuolating toxin into two subunits and reduces its activity. The presence of s1 alleles in s region is characteristic for (Tox+) strains while s2 occurs in (Tox-) strains[11]. Another in vitro study conducted on Hela cells reported that the type s1a is more active than type s1b. Whereas, cytotoxic activity of s2 was not observed [12].

No study has been carried out on *H. Pylori* strains in Libya. Therefore the aim of the present study to evaluate frequency of vacA (s1 and s2) alleles in *H. pylori* strains and also their relationship with age and developing of chronic gastritis in Zliten city, Libya .

## Methods

### Collection of biopsies

Forty patients who were positive for IgG antibodies against *H. Pylori* (AccuBio tech) and suffering from dyspeptic symptoms underwent gastroscopy at Zliten hospital, Libya. Gastric biopsies were transferred into vials containing distilled water and stored at -20°C. In this study, the patients were divided into two groups; adult patients below 60 years and elderly patients equal or above 60 years.

### DNA extraction

The DNA was extracted from biopsies using a commercial kit (QIAamp DNA Mini Kit;

Qiagen, Hilden, Germany), according to the manufacturer's instructions. Briefly, biopsies were lysed in 180 µl of ATL buffer and 20 µl of proteinase K at 56 °C and incubated for overnight. Two hundred microliter of AL buffer was added to the lysate and samples were incubated for 10 min at 70 °C. After the addition of 200 µl absolute ethanol, lysates were purified over a QIAamp column as specified by the manufacturer. The column was washed step wisely with 500 µl buffer AW1 and buffer AW2, after which an ultra-pure DNA product was eluted for PCR assay.

**Molecular confirmation of *H. pylori***

The extracted DNA from biopsies was amplified using specific primers for *H. pylori*, glmM PCR test (detection of housekeeping genes). The PCR was carried out under the following conditions: Initial denaturation of 95 °C for 5 mins and 35 cycles of 95 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s and a final extension time of 72 °C for 10 min. The PCR

amplification was performed using a thermocycler system (Bio-Rad Thermal cycler). Each 25 µl PCR reaction mixture contained 12.5 µl PCR master mix (Promega, GoTag® Green Master Mix, USA), 0.5 µl each of primer (Metabion, Germany), 5 µl of template DNA and 6.5 µl of PCR grade water. To detect the amplified product, 5 µl of amplicons was visualized by electrophoresis through a 1.5% agarose gel (Merck, SA) at 100 V for 40 min in 1X TAE buffer and stained with ethidium bromide (500 ng/ml) (Sigma-Aldrich, USA) using the gel documentation system (Alliance 4.7, France). Identification of DNA bands was established by comparison of the band sizes with molecular weight markers of 100-bp (MaestroGen). The glmM and vacA (subtypes: s1 and s2) were detected using PCR specific primers (Table 1). Amplification PCR products of VacA (S1 and S2) were sent to Carthagénomics lab -Tunisia for sequencing.

**Table 1. Primer sequences for PCR detection of glmM and vacA genes.**

Target gene	Primer pair (5'- 3')	Amplicon (bp)	References
<b>glmM</b>	F5'- AAGCTTTTAGGGGTGTTAGGGGTT-3' R5'- AAGCTTACTTTCTAACACTAACGC-3'	294	[13]
<b>VacA</b>	F5'- ATGGAAATACAACAAACACAC-3' R5'- CTGCTTGAATGCGCAAAC-3'	S1: 259 S2: 286	[14]

**Statistical analysis**

The results of collected data were analysed by SPSS Software, Version 22.  $P \leq 0.05$  were considered statistically significant.

### Results

18 patient's biopsies samples out of 40 were positive to glmM PCR test (housekeeping gene; 294 bp), which followed by PCR amplification for s1 and s2 alleles on vacA gene. 16 PCR products were positive to s1 or s2 alleles out of 18 glmM amplify. The presence of allele s1(259 bp) and allele s2(286 bp) of *H. Pylori* vacA gene in the infect patients, was observed on electrophorized PCR product (Figure 1). Further identification of the s1 and s2 alleles was confirmed by DNA sequencing. The DNA sequences were aligned to the sequences that available at the GenBank. There was a high homology between the compared sequences (Figure 2).

and allele s2 (286 bp) of *H. pylori vacA* gene - the analysis of PCR product with the use of the electrophoretic technique in agarose gel.

Helicobacter pylori strain MT5135 chromosome, complete genome  
Sequence ID: [NZ\\_CP071982.1](#) Length: 1615199 Number of Matches: 1

Range 1: 903694 to 903887 [GenBank](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
315 bits(170)	2e-82	186/194(96%)	0/194(0%)	Plus/Plus
Query 1	TTTAGCAGGAGCGTTGATTAGTGCCATACCGCAAGAAAGTCATGCCCTTTTTCACGAC	60		
Sbjct 903694	TTTAGCAGGAGCGTTGATTAGTGCCATACCGCAAGAAAGTCATGCCCTTTTTCACGAC	903753		
Query 61	CGTGATCATTCCAGCCATTGTTGGGGGTATCGCCACAGGCACCGCTGTAGGAACGGTCTA	120		
Sbjct 903754	CGTGATCATTCCAGCCATTGTTGGGGGTATCGCCACAGGCACCGCTGTAGGAACGGTCTC	903813		
Query 121	CGGGCTTCTTAGTTGGGGATCCAACAAGCCGAAGAAGCGAATAAAACCCAGACAAACC	180		
Sbjct 903814	AGGGCTTCTTAGTTGGGGATCCAACAAGCCGAAGAAGCGAATAAAACCCAGATAAAC	903873		
Query 181	CGATAAAGTTTGGC	194		
Sbjct 903874	CGATAAAGTTTGGC	903887		

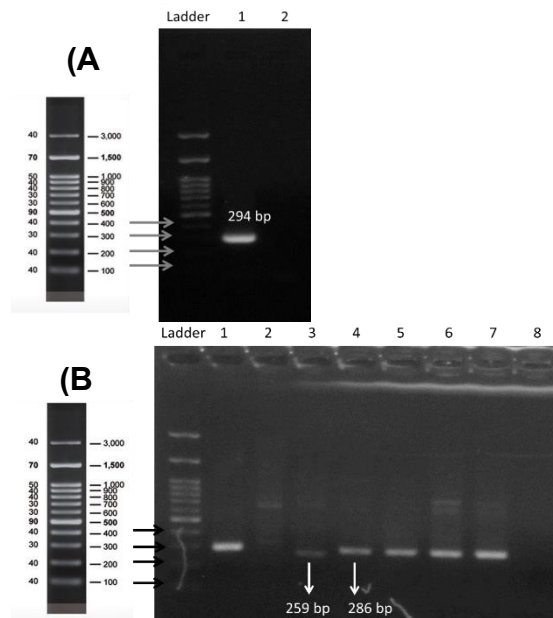


Figure1 (A). Agarose gel electrophoresis of PCR products of the amplified 294bpglmM gene. (B) The presence of allele s1 (259 bp)

Figure 2. (A, B) Alignment of amino acid sequences corresponding to the amplified s1 and s2 vacA allele genes respectively with other representative sequences obtained from the GenBank.

The most frequently observed *H. pylori vacA* gene signal region allele, in all group of patients, was the s2 allele. Allele s2 of the region of the examined gene was determined in 13 out of 16 (81%), whereas allele s1 in 3 out of 16 (19%) as shown in figure 3. Among adult analyzed: 1 out of 9(11%) were infected with vacA s1 allele *H. Pylori* strains and 8 out of 9 (89%) had s2 allele form of bacteria. In elderly patients: 2 out of 7 (29%) were infected with vacA s1 allele *H. pylori* strains, and 5 out of 7 (71%) had s2 allele form of bacteria Figure 4. There were no statistically significant differences

found in particular alleles frequencies in the examined adult and elderly patients.

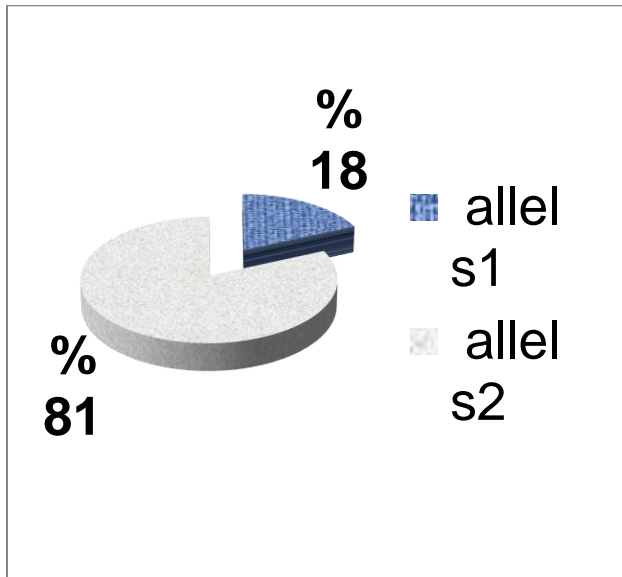


Figure 3. Frequency of *H. pylori* gene vacA signal sequence (s1 and s2) in total group of patients

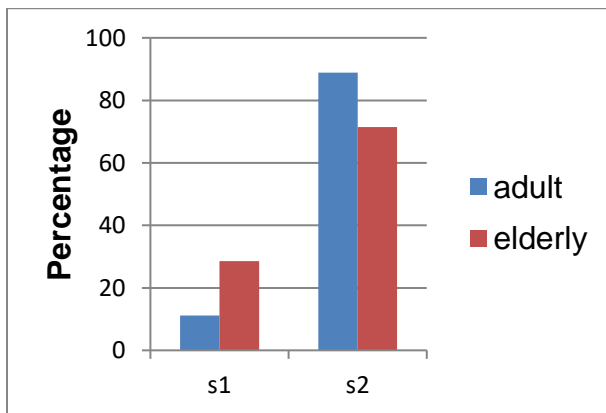


Figure 4 The frequency of alleles of the s region (s1 and s2) in adult and elderly patients.

Correlation of vacA gene s1 and s2 alleles of *H. Pylori* and endoscopy finding. The s2 alleles were more common in patients with normal mucosa ( $P < 0.05$ ). Whereas, s1 and s2 has a less homogeneous distribution among patients with chronic gastritis (pan gastritis, peptic ulcer and gastric erosion), without reaching statistical significance (Figure 5).

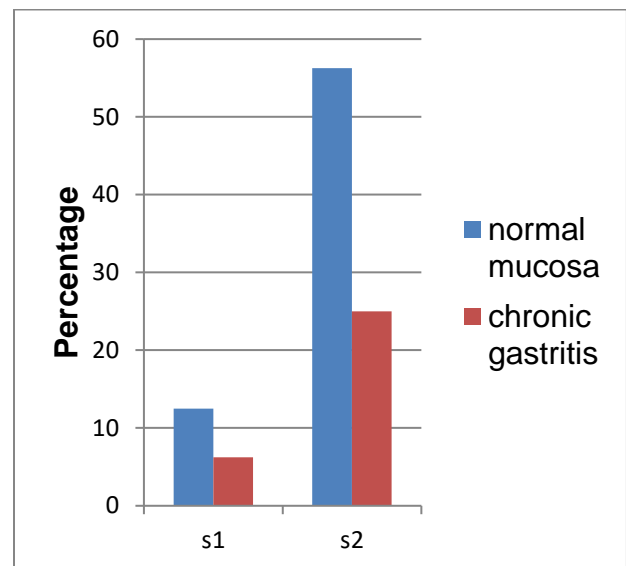


Figure 5 Frequency of isolated *H. pylori vacA* alleles s1 and s2 from patients with normal gastric mucosa and chronic gastritis.

### Discussion

The examination of vacA genotype showed that the prevalence of *H. pylori* strains containing alleles s1 or s2 is various in different parts of the world. In our first study in Libya showed vacA s2 to be predominant, which is similar to findings reported elsewhere, such as Chilean city-china[15]. Moreover, the most of the Israeli population had the vacA s2 genotype [16]. Van Doorn reported that the low prevalence of the vacA s1 and m1 genotypes in strains from Egypt

was 42.9% and 14.3%, respectively[17]. Saudi Arabia was predisposed to be equally infected with the vacA s1 and s2 alleles[18]. On the other hand, Many studies concerning the geographic distribution of *H. pylori* strains with subtypes of the allele s1 (s1a, s1b, s1c) revealed that 89% of the subtype s1a are present in the Northern and Eastern Europe[19]. In addition the studies in Africa[20], Thailand[21] Indian[22] and Morocco[23] showed the predominance of vacA genes alleles is s1.

The allele s2 of the single region was the one most frequently observed in current study (89% in adult and 71% in elderly patients). The allele s1 occurred in 29% of adult, 11% of elderly. This study had shown no significant relationship between vacA genotypes and ages, this could be due to low sample power.

In the present study, the frequency of chronic gastritis was approximately 31% in Zliten patients who were infected with virulence strain of *H. pylori*. Furthermore, this study confirmed that there is a strong relationship between the presence of vacA gene and progression of chronic gastritis, which in agreement with other studies that reported vacA gene is essential in developing of peptic ulcer and gastric cancer[24]. The vacA s1/m1 alleles are the most virulent, while the s1/m2, s2/m1 and s2/m2 genotypes demonstrate little to no pathogenicity[25]. In our finding s1 showed more virulent than s2. Further study should be conduct to

determination the frequency of subtypes vacA (s1, s2, m1, m2, s1m1, s1m2, s2m1 and s2m2) alleles in Libya.

## Conclusions

The study of *H. pylori* virulence genes alleles s1 and s2 in Libya could be essential for epidemiological survey to evaluate risk factors associated with gastric cancer and peptic ulcer development. This study showed that there is a relationship between vacA genotypes s1 and s2 and development of infections caused by *H. pylori* to chronic gastric disease. There were no statistically significant differences in relation to the occurrence of particular alleles in the examined adult and elderly patients. There were some limitations in this study including; few samples number, study was conducted in zliten region only, did not involve different group ages such as children, no information about other subtypes of vacA genes alleles. Comprehensive studies should be performed under the appropriate study design using many individuals among different Libyan cities with several vacA genotypes (s1a, m1, m2, s1m1, s1m2, s2m1 and s2m2), in parallel with serology and culture techniques.

Conflict of interest: The authors declare that they have no conflict of interest.

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