VIRULENCE PROFILE OF HELICOBACTER PYLORI DETECTED IN GASTRIC BIOPSIES OF PATIENTS UNDERGOING ENDOSCOPY IN UPPER EGYPT

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Background: Helicobacter pylori (H. pylori) is a common cause of gastric ulcers and is a risk factor for gastric carcinoma. Little data are available about the characters of H. pylori causing infections in patients from Upper Egypt. Therefore, the aim of this study was to investigate the frequency of H. pylori infection in Egyptian patients undergoing endoscopy and complaining of persistent upper gastrointestinal symptoms directly in biopsy specimens using PCR technique and to study the associated virulence-related genes in H. pylori positive samples.

Materials and Methods: One hundred and twenty gastric biopsy specimens were collected from Egyptian patients admitted to Assiut University Hospital and complaining of persistent upper gastrointestinal symptoms. H. pylori DNA was extracted for molecular identification by PCR. Positive samples were further analyzed to investigate the presence of different virulence-related genes and gene combinations. Results: H. pylori was detected in 92/120 (76.6%) of collected biopsies. All strains carried the vacA s1 subtype. The prevalence of the virulence genes cagA, cagE, iceA1, iceA2 and oipA were 79 (84.8%), 41 (44.5%), 69 (75%), 44 (47.8%), and 55 (59.7%) of the H. pylori positive samples, respectively. Genetic analysis showed that H. pylori were grouped into 29 different genotype combinations. The s1/m1/i1/d1/c1/cagA/iceA1/oipA genotype was the most predominant.

Conclusion: Our results show a high frequency of H. pylori infections among Egyptian patients with gastrointestinal symptoms. The s1/m1/i1 vacA hybrid is the most prevalent subtype. The distribution of the different virulence-related genes shows alarming rates of cagA, cagE, iceA1, iceA2 and oipA. Careful monitoring of H. pylori infections and investigation of their genetic characters should be carried out to control the spread of the virulent strains.

INTRODUCTION

It was concluded more than a century ago that there is a certain type of bacteria that could be detected in the human's stomach1. Twenty years ago, Barry Marshall and Robin Warren successfully isolated a spiral bacterial species from the human's stomach recognized as Helicobacter pylori2. H. pylori is a Gram-negative, spiral shape, microaerophilic
bacterium that commonly colonizes the human gastric mucosa during childhood and can persist for whole of life leading to inflammation of the gastric mucosa without successful extermination therapy[5,6,7].

About half of the world’s population are estimated to be colonized with H. pylori, the major modes of transmission of infection are fecal-oral or oral-oral; however, there is another indirect mode of transmission through drinking water and other environmental sources[3]. Recently, animals such as cows, sheep, and goats were reported to participate in the transmission of H. pylori infections[3]. H. pylori is responsible for many gastro-duodenal diseases with distinct clinical symptoms, including dyspepsia, gastric atrophy, gastritis and peptic ulcer, gastric adenocarcinoma in addition to primary gastric B-cell lymphoma[3,4,6,7].

The virulence factors of H. pylori bacteria are the main factor of such diseases[8,9]. The presence of a bundle of pathogenic genes in H. pylori of the stomach biopsies, provides conditions for oncological processes in this area[10]. In gastric epithelial cells, the ability of the bacteria to colonize, adhere, and invade the mucosa are supported by the presence of different virulence-related genes[5].

There are two important novel virulence genes, vacuolating cytotoxin A (vacA) and cytotoxin-associated gene A (cagA), which are associated with severe clinical manifestations such as gastro-duodenal ulcers and gastric adenocarcinoma[3,5,11]. They are considered as factors affecting colonization efficiency[8]. Moreover, the cagA gene encoding a protein involved in the expression of interleukin 8 in gastric epithelial cells[12]. Also, it influences the host cell in many ways, such as the formation of gastric epithelium cell, cytoskeleton transition, and influencing cell proliferation[5].

The vacA gene consists of 5 polymorphic regions including signal regions (s1 or s2 type), middle regions (m1/m2), intermediate (i1/i2), deletion (d1/d2) and (c1/c2)[3,5,11]. Vacuolating toxin vacA is generated by (s1/m1/i1) type strains, causing progressive vacuolation and gastric epithelium injury[3,5,13].

There are two main allelic variants of the epithelium antigen (iceA) gene: iceA1 and iceA2[5]. These genes are induced by contact with the epithelium antigen[5,13,14]. Outer inflammatory protein (oipA) induced by contact with blood group antigen-binding adhesion (babA) and the epithelium antigen (iceA)[5]. BabA gene enhances the IL-8 response, leading to increased inflammation of the mucosa[1]. Ilver, et al.[15] identified this gene as it is involved in the binding activity between bacterial adhesion and Lewis b blood group antigens on the gastric epithelium of human host cells, as well as related with duodenal ulcer. This study aimed to investigate the frequency of H. pylori infections in Egyptian patients undergoing endoscopy and complaining of persistent upper gastrointestinal symptoms directly in biopsy specimens using PCR technique and to study the associated virulence-related genes in H. pylori positive samples.

**MATERIALS AND METHODS**

**Patients selection**

Biopsy specimens were obtained from 120 patients who performed esophago-gastroduodenoscopy. Of them 74 (61.6%) showed H. pylori-positive stool antigen test. They were complaining of persistent upper gastrointestinal symptoms (GIT) e.g. epigastric pain, early satiety, postprandial upper abdominal distension, nausea, and repeated vomiting at GI endoscopy unit in Al-Rajhi University Hospital, Assiut, Upper Egypt.

The following patients were excluded from the study; pregnant women, children under 18 years, patients with previous upper GI surgery, severely sick individuals, those who have had gastrointestinal bleeding in the past month, and those who refused to sign the consent form voluntarily.

**Specimen collection**

Two gastric biopsies, approximately 2±3 cm (one biopsy from the antrum, and the other from the greater curvature of the corpus) were collected and underwent endoscopy examination and rapid urease test (RUT) after evaluation by experienced gastroenterologists at Al-Rajhi University Hospital, Assiut University, Upper Egypt in the period between January and November 2019.
Processing of the gastric biopsies

All gastric biopsy samples were analyzed routinely by RUT as part of the gastroenterology outpatient service of the hospital. Care was taken to ensure that the patients did not receive eradication therapy for the *H. pylori* in the last two months, antibiotics, antisecretory drugs, especially proton pump inhibitors (PPI) in the last two weeks or receiving bismuth-containing regimens. Biopsy specimens were used for molecular examination.

DNA extraction

Genomic DNA of *H. pylori* was extracted from the biopsy specimens using the QIAamp DNAeasy Blood and Tissue Kits (Qiagen, Germany) according to the instructions of the manufacturer and stored at -20°C. Extracted DNA was used for subsequent PCR experiments.

Detection of *H. pylori* and analysis of the virulence-related genes

PCR amplification of the *vacA* gene was used for the detection of *H. pylori* in biopsy samples[10]. In addition, the various subtypes of *vacA* alleles (s, m, i, d, c regions) were determined. The presence of different virulence-related genes: *cagA*, *cagE*, main allelic variants of *iceA* gene are *iceA1* and *iceA2*, *oipA* as well as *babA2* was examined. Generally, PCR reaction conditions were carried out using the Dream Taq PCR Master Mix (Thermo Fisher Scientific, USA) in a final volume of 25 µL containing 5 µl of extracted DNA, 0.5 µl of 10 mM of each primer. The reaction conditions were as follow: 95°C for 4 minutes, 40 cycles of 95°C for 60 second, annealing for 45 second and according to temperatures reported in (Table 1), extension at 72°C for 30 second, and a final extension at 72°C for 10 minutes.

| Target genes       | Primer 5' 3' sequence | Annealing temp | Product size (bp) | Ref. |
|--------------------|------------------------|----------------|------------------|-----|
| 1, 2               | s1/s2                  | *F*: ATGGAAATACAAACAAACAC<br>  *R*: CTGCTTTGAATGCGCCAAAC | 45    | 259/286 | 18   |
| 3, 4               | m1/m2                  | *F*: CAATCTGCTCAATCAAGCGAG<br>  *R*: GCCGCAAATAATCTCAGGAG | 58    | 567/642 | 18   |
| 5                  | i1                     | *F*: GTTGGGATGGGGAATGCGG | 58    | 426    | 19   |
| 6                  | i2                     | *R*: TTAATTTAAGCTTTCAAGAG | 58    | 432    |       |
| 7                  | d1                     | *F*: ACTAATATTTGGAACACTGGATTG<br>  *R*: CTGCTTTGATGGACAGATTG | 53    | 367-379 | 20   |
| 8                  | d2                     | *F*: ATCATYSGTTATGRHAATGTT<br>  *R*: TTAATTTAAGCTTTCAAGAG | 55    | 298    |       |
| 9                  | c1                     | *F*: ATCATGTTGTTAGGATAGG<br>  *R*: TTATGCTTCAAACTGGCTA | 55    | 600-700 | 20   |
| 10                 | c2                     | *F*: ATTATAATTTAAGGATGG<br>  *R*: TTAATTTAAGGATGGTTG | 55    | 700-900 |       |
| 11                 | cag A                   | *F*: AATACACCAACGCTCAAG<br>  *R*: TTGTGGGCTGCTTCCTC | 55    | 400    | 3     |
| 12                 | cag E                   | *F*: TGAAAACTTCAAGGATAGG<br>  *R*: GCCCTAGCTTAATACCAATTACCC | 54    | 508    | 5     |
| 13                 | iceA1                  | *F*: GTGTTTTTAAACCAAGAT<br>  *R*: CTATAGGCCASTYTCTTGGCA | 55    | 247    | 5     |
| 14                 | iceA2                  | *F*: GGTGGGTATATCACATTTAT<br>  *R*: TTRCCCTATTTTCTAGTATGGT | 55    | 229/334 | 5     |
| 15                 | oipA                    | *F*: GTTTTTTGATGCCATGG<br>  *R*: GTGACATCTCTTATGGCTTT | 55    | 401    | 5     |
| 16                 | babA2                   | *F*: CCAAAAGCGAAAAAAGAC<br>  *R*: GCTTGGTGAATGCGCTG | 55    | 271    | 5     |

Abbreviations: *(vacA)*: (vacuolating cytotoxin or pore forming toxin), *vacA* alleles: signal regions (*s1* and *s2*), mid-region (*m1* and *m2*) alleles, intermediate region (*i1*, *i2*), deletion (*d1*,*d2*), (*c1*,*c2*) indicates no deletion, *cagA*: (cytotoxin-associated gene), *cagE*: (encoding a protein involved in the process of interleukin 8 expression in gastric epithelial cells), *iceA1*, (*iceA2*): (epithelium antigen) main allelic variants of *iceA* gene. *oipA*: (outer inflammatory protein), *babA2*: (blood group antigen-binding adhesion).
The amplified products were visualized by electrophoresis in 1.5% agarose gels stained with Gel Red™. Table 1 summarizes the list of the used primers and their sequences. Positive and negative controls were evaluated with test samples. Phylogenetic grouping profile of the isolates was done by using the software package MVSP and genetic similarities were computed using the Dice coefficient of similarity of Nei and Li\(^7\).

**Statistical Analysis**

The results are presented as frequency with percentage. We tested for differences between study groups using the Mann-Whitney \(U\) test, Wilcoxon test, and one-way ANOVA using SPSS (IBM SPSS Statistics for Windows, Version 22, NY, USA). A p value of <0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION**

**Results**

**Identification of \( H. \) pylori**

During the study period, 120 patients were enrolled. The presence of \( H. \) pylori was determined by detection of the \( vacA \) gene by PCR directly from the biopsy specimens. A total of 92/120 (76.6%) samples tested positive for \( H. \) pylori. Patient age ranged from 18 to 67 years old.

**Distribution of the \( vacA \) allelic subtypes**

Various subtypes of the \( vacA \) gene alleles (\( s1, \ s2, m1, m2, i1, i2, d1, d2, c1, \) and \( c2 \)) were observed using PCR assay. All samples 92 (100%) carried the \( s1 \) genotype of \( vacA \). Similarly, \( vacA i1 \) of the \( i \) region and \( vacA m1 \) genotype of the \( m \) region, were the most predominant genotypes; they were detected in 85 (92.4%) and 66 (71.7%) of the positive samples, respectively (Table 2).

**Table 2:** The distribution rate of the \( vacA \) alleles among \( H. \) pylori positive samples.

| \( vacA \) genotype | \( H. \) pylori positive cases |
|---------------------|-----------------------------|
|                      | N= 92                       | (100%)                     |
| \( vacA s1 \)       | 92                          | 100                         |
| \( vacA s2 \)       | 2                           | 2.2                         |
| \( vaA m1 \)        | 66                          | 71.7                        |
| \( vacA m2 \)       | 23                          | 25                          |
| \( vacA i1 \)       | 85                          | 92.4                        |
| \( vacA i2 \)       | 3                           | 3.3                         |
| \( vacA d1 \)       | 56                          | 60.8                        |
| \( vacA d2 \)       | 7                           | 7.6                         |
| \( vacA c1 \)       | 33                          | 35.8                        |
| \( vacA c2 \)       | 2                           | 2.1                         |

The genotypes of \( vacA \) were evaluated on the basis of five polymorphic regions: the \( s, \ i, \ m, \ d \) and \( c \) regions. The total \( vacA \) (\( s1, s2 \), (\( m1, m2 \), (\( i1, i2 \), (\( d1,d2 \), and (\( c1, c2 \)), regions were amplified in 92 (100%), 83 (90.2%), 88 (95.6%), 63 (68.4%) and 35 (38%) in the \( H. \) pylori positive specimens, respectively (Fig. 1).

![Fig. 1: The prevalence of the \( vacA \) alleles among detected \( H. \) Pylori.](image-url)
Analysis of all vacA subtypes showed that the s1/m1/i1 hybrid was the most prevalent subtype 61 (66.3%) (Table 3). The distribution rate of the vacA i subtype among the s and m regions were 61 (66.3%), 18 (19.5%) and 2 (2.1%) of vacA s1/m1, vacA s1/m2, and vacA s2/m2 genotype strains respectively (Table 3, Fig. 1). However, none of the examined strains exhibited the s2/m1 vacA genotype.

Table 3: Association between the i region of vacA among the s and m regions of vacA alleles.

| vacA subtype | i1 No. | i2 No. | % | % | P value |
|--------------|--------|--------|----|----|---------|
| s1/m1        | 61     | 3      | 66.3 | 32.6 | p<0.005 |
| s1/m2        | 18     | 0      | 19.5 | 0   |         |
| s2/m1        | 0      | 0      | 0   | 0   |         |
| s2/m2        | 2      | 0      | 2.1 | 0   |         |

Prevalence of different virulence-related genes

The distribution of different virulence genes among the H. pylori positive gastric biopsy samples showed highest levels for cagA, cagE, iceA1, iceA2 and oipA. They were detected in 79 (85.8%), 41 (44.5%), 69 (75%), 44 (47.8%), and 55 (59.7%) of H. pylori positive gastric biopsy samples, respectively. On the other hand, babA2 was the least frequently detected gene 25 (27.1%), as shown in (Fig. 2).

Moreover, we compared the genetic profile of the virulence-related genes among the positive samples and our results showed the predominance of Profile 1 (observed in 21 samples) which carries 8 genes; s1/m1/i1/d1/c1/cagA/iceA1/oipA, followed by profile 2 (observed in 9 samples) which also carries a similar set of 8 genes; s1, m1,i1, d1,cagA,iceA1, oipA, babA2. In contrast, the least prevalent genotypes were "Profile 19" to "Profile 29" which were recorded in only one sample. Two profiles (Profile 23 and Profile 24) carried 10 mixed virulence genes. Other profiles showed variable frequencies and are listed in (Table 4). The genetic relatedness among the different 29 profiles based on the pattern of the presence of the different combinations of the virulence-related genes shows different clusters (Fig. 3).

![Fig. 2: Frequency of the different virulence-related genes among samples.](image-url)
Table 4: Profiles of the different combinations of the virulence genes of *H. pylori* positive specimens.

| Profile No. | Virulence Genes                                      | No. of genes | No. of detected *H. pylori* | %   |
|-------------|------------------------------------------------------|--------------|----------------------------|-----|
| (Profile 1) | s1, ml, il, d1, c1, cagA, iceA1, oipA               | 8            | 21                         | 22.8|
| (Profile 2) | s1, ml, il, d1, cagA, iceA1, oipA, bab A2           | 8            | 9                          | 9.78|
| (Profile 3) | s1, ml, il, d1, c1, cagA, iceA1, oipA              | 8            | 7                          | 7.6 |
| (Profile 4) | s1, m2, il, d1, cagA, cag E, iceA2                  | 7            | 5                          | 5.43|
| (Profile 5) | s1, ml, il, d2, cagA, cag E, iceA1, iceA2, bab A2   | 9            | 4                          | 4.34|
| (Profile 6) | s1, ml, il, cagA, cag E, iceA2                      | 6            | 4                          | 4.34|
| (Profile 7) | s1, ml, il, cag E, iceA2                            | 5            | 4                          | 4.34|
| (Profile 8) | s1, ml, il, cagA, iceA1, oipA, bab A2               | 7            | 3                          | 3.26|
| (Profile 9) | s1, il, d1, cagA, cag E, iceA1, iceA2, oipA, bab A2 | 9            | 3                          | 3.26|
| (Profile 10)| s1, ml, il, cagA, cag E, iceA1, iceA2              | 7            | 3                          | 3.26|
| (Profile 11)| s1, m2, il, d1, cagA, cag E, iceA1, iceA2           | 8            | 3                          | 3.26|
| (Profile 12)| s1, ml, m2, cag E, iceA2                            | 5            | 3                          | 3.26|
| (Profile 13)| s1, ml, il, cagA, iceA1, oipA                       | 6            | 2                          | 2.17|
| (Profile 14)| s1, il, d1, cagA, iceA1, oipA, bab A2               | 7            | 2                          | 2.17|
| (Profile 15)| s1, ml, il, d1, cagA, cag E, iceA1, iceA2           | 8            | 2                          | 2.17|
| (Profile 16)| s1, ml, il, c1, cagA, iceA1, iceA2, oipA, bab A2    | 9            | 2                          | 2.17|
| (Profile 17)| s1, ml, il, d2, cagA, cag E, iceA1, iceA2           | 8            | 2                          | 2.17|
| (Profile 18)| s1, il, c2, c2, cag E, iceA2                        | 5            | 2                          | 2.17|
| (Profile 19)| s1, ml, il, cagA, iceA1, oipA                       | 6            | 1                          | 1.08|
| (Profile 20)| s1, s2, m2, Il, c1, cagA, iceA1, oipA               | 8            | 1                          | 1.08|
| (Profile 21)| s1, s2, m2, il, d1, c1, cagA, iceA1, oipA           | 9            | 1                          | 1.08|
| (Profile 22)| s1, ml, il, d1, cagA, iceA1, oipA                   | 7            | 1                          | 1.08|
| (Profile 23)| s1, ml, il, d1, c1, cagA, iceA1, iceA2, oipA, bab A2| 10           | 1                          | 1.08|
| (Profile 24)| s1, ml, il, d1, cagA, cag E, iceA1, iceA2, oipA, bab A2|10            | 1                          | 1.08|
| (Profile 25)| s1, il, cagA, cag E, iceA2                          | 5            | 1                          | 1.08|
| (Profile 26)| s1, il, cag E, iceA2                                 | 4            | 1                          | 1.08|
| (Profile 27)| s1, ml, m2, il, cag E, iceA2                        | 6            | 1                          | 1.08|
| (Profile 28)| s1, ml, m2, d2, cag E, iceA2                        | 6            | 1                          | 1.08|
| (Profile 29)| s1, ml, m2, il2, cag E, iceA2                       | 6            | 1                          | 1.08|
Discussion

Previous studies concluded that an invasive test should be used for diagnosis and confirmation of *H. pylori* infections\(^\text{16}\). Invasive upper endoscopy is recommended in the assessment of all patients presenting with dyspepsia with alarm symptoms, including gastrointestinal bleeding, dysphagia, sudden weight loss, as well as unexplained iron-deficiency anemia\(^\text{7}\). The symptoms of *H. pylori* infection are affected by different factors, including genetic factors of the host, bacterial virulence determinants and environmental components such as smoking, salt intake, and living conditions\(^\text{8}\). Every *H. pylori* -positive person has a different strain due to the genetic heterogeneity of *H. pylori*, depending on the ability of this microorganism to adapt to its host's gastric conditions, in addition to the different patterns of the host's immune response to *H. pylori* infection\(^\text{18}\).

The virulence factors of *H. pylori* have been shown to play a crucial role in determining the course of its associated diseases\(^\text{19}\&\text{20}\). A more detailed insight into the pathogenesis of *H. pylori* infection can be provided by understanding the mechanisms of these virulence factors and heterogeneity in biological characteristics of infection with *H. pylori*\(^\text{8}\).

Abu-Taleb, *et al.*\(^\text{11}\) analyzed *H. pylori* virulence genes from gastric biopsies and their interaction with the clinical symptoms. The low bacterial load, presence of non-culturable coccoid forms, antibiotic therapy, or contamination by other bacteria that suppress

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**Fig. 3:** Dendrogram illustrating the genetic relationship among the 29 genetic profiles of the detected *H. pylori*.
the growth of \textit{H. pylori} lead to a low detection rate using the culture method (26.5\%) and false-negative results. Therefore, PCR is a very useful tool for detection of \textit{H. pylori} in such cases\textsuperscript{18}. In the current study, the prevalence of \textit{H. pylori} infection was 92/120 (76.6\%) of the collected biopsy specimens in Upper Egypt. This result agrees with the finding of Essawi, \textit{et al.}\textsuperscript{3}, who observed that in some developing countries, the prevalence of \textit{H. pylori} infection may surpass 70\% which could be explain by the involvement of microbial factors contributing to the pathogenicity of the organism.

\textit{VacA} gene is a secreted pore-forming toxin that was detected in 92 (100\%) in \textit{H. pylori} and used for detection of the bacteria in our study. Our results are consistent with other reports\textsuperscript{9,21&22} that showed that the distribution rate of \textit{vacA} was 100\% in all \textit{H. pylori} positive gastric biopsies. In contrast, another study showed that the prevalence of the \textit{vacA} gene was 40.9\% among positive-\textit{H. pylori}\textsuperscript{1}. \textit{VacA} plays an important role in the pathogenesis of \textit{H. pylori} infections. It acts as exotoxin protein and that can induce multiple cellular activities, including membrane channel formation, the release of mitochondrial cytochrome C following pro-inflammatory response activation leading to programmed cell death and binding to cell membrane receptors\textsuperscript{5,23&24}. This toxin causes injury to gastric epithelium following progressive vacuolation\textsuperscript{5}.

In this study, we analyzed the frequency of the 5 common alleles of the \textit{vacA} region \textit{s}, \textit{m}, \textit{l}, \textit{d} and, \textit{c} in \textit{H pylori} positive samples. All isolates showed mixed genotypes. Korona-Glowniak, \textit{et al.}\textsuperscript{18} reported that mixed \textit{vacA} gene types were found in 8\% of the samples that colonize gastric mucosa. In our investigation, the distribution \textit{vacA} subtypes \textit{s1/m1}, \textit{s1/m2} and \textit{s2/m2} were 66 (71.7\%), 23 (25\%) and 2 (2.1\%), respectively. This is in agreement with other previous studies\textsuperscript{5&21}, that reported that the most predominant genotype was \textit{vacA s1/m1} in 57.7\%, followed by \textit{s2/m2}, \textit{s1/m2} or \textit{s2/m1}\textsuperscript{18}. A previous findings detected \textit{(s1/m0)} in 4.8\% of samples\textsuperscript{18}. The difference in distribution of the \textit{vacA} subtypes is explained by the regional and ethnic differences\textsuperscript{23}. This analysis detected the distribution rate of \textit{cagA}, \textit{cagE}, \textit{iceA1}, and \textit{iceA2} virulence genes were expressed at a markedly high rate 79 (85.8\%), 41 (44.5\%), 69 (75\%) and 44 (47.8\%) respectively. This result mostly similar to Hemmatinezhad, \textit{et al.}\textsuperscript{22}, investigated that the distribution of \textit{cagA}, \textit{cagE}, \textit{iceA1}, and \textit{iceA2}, virulence factors were 87.3\%, 48.5\%, 47\% and 47\% respectively\textsuperscript{11&22}. \textit{H. pylori} travel from the gastric mucosal epithelium layer to the basal layer, the \textit{oipA} gene plays a key role in the colonization of this pathogen in the gastric mucosa\textsuperscript{5}. The frequency of \textit{oipA} was 59.7\%. In contrast, \textit{oipA} was detected in 15.2\% in a previous study\textsuperscript{5&22}. \textit{OipA} has been significantly associated with increased IL-8 level\textsuperscript{5&25}.

Regarding the main allelic antigens of \textit{iceA}, the distribution of \textit{iceA1} and \textit{iceA2} were comparable in 32.3\% and 35.5\% respectively\textsuperscript{18}. In the current study we observed that, \textit{babA2} gene was detected in 25 (27.1\%) of \textit{H. pylori}. A similar rate was also shown in other studies\textsuperscript{21&22}. There is a relationship between adhesiveness of \textit{H. pylori} to the glycosylated mucins via the blood group antigen-binding adhesion \textit{babA} and associated structures in the gastric mucosa\textsuperscript{5&26}.

Our results showed that the most predominant genotype combination was \textit{cagA} with \textit{vacAs1} This result is consistent with other studies carried out on strains in Europe and in Asian countries\textsuperscript{11}. However, a lower rate (62\%) was reported by Garcia, \textit{et al.}\textsuperscript{21}. \textit{cagA} and \textit{vacA} are strongly associated with gastric ulcers, inflammatory effects, IL-8 production, and gastric cancer\textsuperscript{22&27}.

Geno-typing using the pattern of virulence markers is one of the best tools to study the correlations between \textit{H. pylori} obtained from different samples. The different types of these gene regions are associated with variability in vacuolation, specificity and clinical symptoms\textsuperscript{24}. This investigation detected that the frequency of different alternative combinations of the 10 virulence markers \textit{vacA} alleles with \textit{cagA}, \textit{cagE}, \textit{iceA1}, \textit{iceA2}, \textit{oipA} and \textit{babA2} were grouped into 29 different genotypes. Each of these strains carrying mixed virulence genes ranging from at least 4 genes as represented in "Profile 26" to 10 virulence genes that were detected in only 2 samples (Profiles 23 and 24).

We noticed that \textit{s1/m1/m1} was the most prevalent genotype and this result is in agreement with other study by Erzin, \textit{et al.}\textsuperscript{28} who reported different involving combination
of vacA gene such as s1/i1/m1, s1/i1/m2, s1/i2/m2 and s2/i2/m2 types. The s1, m1, and i1 types are associated with a higher risk of gastric carcinoma development than the s2, m2, or i2 types that are considerably less active and are substantially nontoxic. In our results, the prevalence of vacA i1 was 85 (92.3%) which in agreement with Subsomwong, et al., who found that the predominant genotype was vacA i1 (98.6%). Among our isolates the most predominant profile was "Profile 1" (s1/ m1/ i1/ d1/ c1/ cagA/ iceA1/ oipA) represented in 21 (22.8%) of the H. pylori positive samples with 8 mixed virulence-related genes. This is in agreement with Subsomwong, et al., who concluded that the s1m1i1c1d1 genotype was the predominant vacA type 32.4%. Analysis of combined genotypes of the vacA alleles and cagA status in addition to their combination with vacA n1, i1, d1 with cagA genotypes may play a significant role in determining H. pylori-related outcomes of clinical data. In addition, H. pylori vacA s1a, cagA, and cagE genotypes have remarkable correlations with the presence of duodenal ulcer or gastric carcinoma.

Conclusion
Our results show a high frequency of H. pylori infections among Egyptian patients with gastrointestinal symptoms. The s1m1i1 vacA hybrid is the most prevalent subtype. The distribution of the different virulence-related genes shows alarming rates of cagA, cagE, iceA1, iceA2 and oipA. Careful monitory of H. pylori infections and investigation of their genetic characters should be carried out to control the spread of the virulent strains.

Ethical approval
A written patient’s consent was obtained prior to registration from all participants in this study. The study protocol was in agreement with the Helsinki Declaration and was approved by the Ethics Committee in Human Research of Faculty of Medicine, Assiut University, Egypt (IRB No 17300569).

Competing interests
The authors declare that they have no competing interest "None declared".

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نشرة العلوم الصيدلية
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الخلاصة: أظهرت نتائجنا ارتفاع وتيرة الإصابة بالبكتيريا الحزلية لدى المرضى المصريين الذين يعانون من أعراض الجهاز الهضمي وذلك بسبب بكتيريا هيكلوباكتر. لم تُظهر الدراسات السابقة تفاعلاً بوليوميتابراكستية في هذه الدراسة. فالخلاصة النهائية بين البكتيريا والحمضية تعتمد على الخصائص البيولوجية للمريض. 

المقاطع: تشير نتائجنا إلى أن البكتيريا هيكلوباكتر هي المسبب الوحيد للمرض. وào لم تظهر أي تفاعلات بوليوميتابراكستية في هذه الدراسة. وào يرجى الانتباه إلى خصائص البكتيريا الحزلية في هذه الدراسة.

الخلاصة: تشير نتائجنا إلى أن البكتيريا هيكلوباكتر هي المسبب الوحيد للمرض. وào لم تظهر أي تفاعلات بوليوميتابراكستية في هذه الدراسة. وào يرجى الانتباه إلى خصائص البكتيريا الحزلية في هذه الدراسة.