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

Sahar A. Mandour[1*], Ahmed Shawkat Abdelmohsen[2], Mohammed A. Medhat[2], Doaa Abdeltawab[2], Ameer Elfarash[3], Abdulrahman K. Ahmed[4], Ahmed M. Galal[5] and Mohamed A. El-Mokhtar[6]

[1]*Department of Microbiology and Immunology, Faculty of Pharmacy, Deraya University, Minia 61111, Egypt*
[2]*Department of Tropical Medicine and Gastroenterology, Faculty of Medicine Assiut University, Assiut, Egypt*
[3]*Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt*
[4]*Medical Student, Faculty of Medicine, Assiut University, Assiut, Egypt*
[5]*Postgraduate Student, Department of Surgical Oncology, Sohag Cancer Center, Sohag, Egypt*
[6]*Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt*

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 monitory 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 stomach[1]. Twenty years ago, Barry Marshall and Robin Warren successfully isolated a spiral bacterial species' from the human's stomach recognized as *Helicobacter pylori*[2]. *H. pylori* is a Gram-negative, spiral shape, microaerophilic

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*Corresponding author: Sahar A. Mandour, E-mail: sahar.mandour@deraya.edu.eg

bacterium that commonly colonizes the human gastric mucosa during childhood and can persist for whole of life leading to inflammation of the gastric mucosa with no successful extermination therapy[2&3].

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[4]. Recently, animals such as cows, sheep, and goats were reported to participate in the transmission of *H. pylori* infections[5]. *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[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 g enes, 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 v*acA* gene consists of 5 polymorphic regions including signal regions (*s1* or *s2* type), middle regions (*m1/ m2*), intermediate (*i*1/*i*2), deletion (*d*1/*d*2) and (*c*1/*c*2)[5&13]. Vacuolating toxin *vacA* is generated by (*s*1/*m*1/*i*1) type strains, causing progressive vacuolation and gastric epithelium injury[1&3].

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 (o*ipA*) 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[8]. 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 gastro-intestinal 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-gastroduod-endoscopy. 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[2].

DNA extraction

Genomic DNA of *H. pylori* was extracted from the biopsy specimens using the QIAamp DNeasy 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[16]. 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 ul of extracted DNA, 0.5 ul 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.

Table 1: Primers used for amplification of virulence-related genes.

Target genes			Primer 5' 3' sequence	Annealing temp	Product size (bp)	Ref.
1,2	*vac A* allelic variants	*s1/ s2*	F: ATGGAAATACAACAAACACAC R: CTGCTTGAATGCGCCAAAC	45	259/286	18
3,4		*m1/ m2*	F: CAATCTGTCCAATCAAGCGAG R: GCGTCAAAATAATTCCAAGG	58	567/642	18
5		*i1*	F: GTTGGGATTGGGGGAATGCCG	58	426	19
6		*i2*	R: TTAATTTAACGCTGTTTGAAG		432	
7		*d1*	F: ACTAATATTGGCACACTGGATTTG	53	367-379	20
8		*d2*	R: CTCGCTTGATTGGACAGATTG		298	
9		*c1*	F: ATCATYSGTTATGRHAATGTT TCT R: TTATGCTCTAAACTGGCTA	55	600-700	20
10		*c2*	F: ATTATAATTTAGTAGGAGTGC AAG G R:TTA TGC TCT AAA CTG GCT A			
11	*cag A*		F: AATACACCAACGCCTCCAAG R: TTGTTGGCGCTTGCTCTC	55	400	3
12	*cag E*		F: TGAAAACTTCAAGGATAGGATAGAGC R: GCCTAGCGTAATATCACCATTACCC	54	508	5
13	*iceA1*		F: GTGTTTTTAACCAAAGTATC R: CTATAGCCASTYTCTTTGCA	55	247	5
14	*iceA2*		F: GTTGGGTATATCACAATTTAT R: TTRCCCTATTTTCTAGTAGGT	55	229/334	5
15	*oipA*		F: GTTTTTGATGCATGGGATTT R: GTGCATCTCTTATGGCTTT	55	401	5
16	*bab A2*		F: CCAAACGAAACAAAAAGCGT R: GCTTGTGTAAAAGCCGTCGT	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[17].

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).

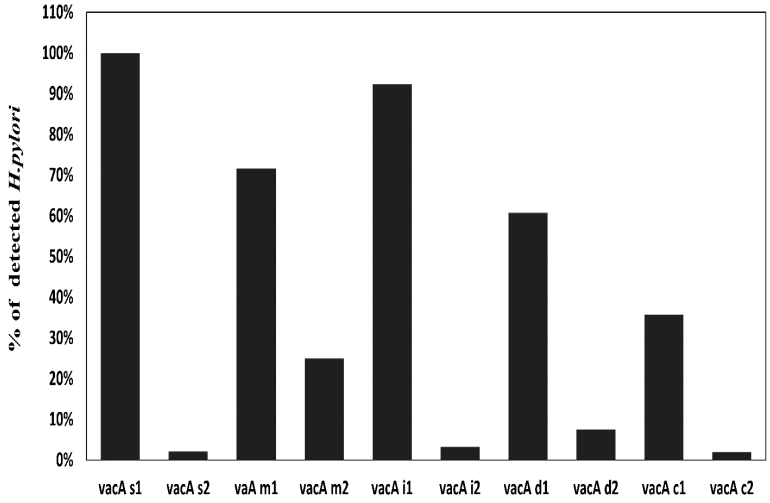


Fig. 1: The prevalence of the *vacA* alleles among detected *H. Pylori*.

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*		*i2*		P value
	No.	%	No.	%	
s1m1	61	66.3	3	32.6	*p*< 0.005
s1m2	18	19.5	0	0	
s2m1	0	0	0	0	
s2m2	2	2.1	0	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).

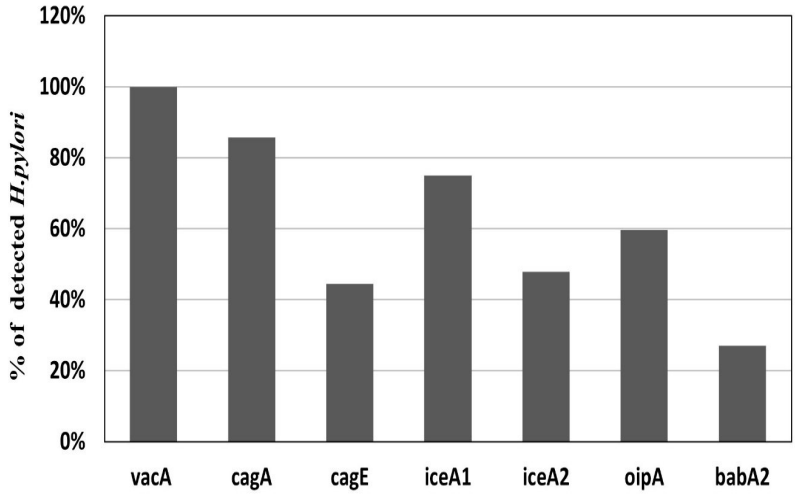
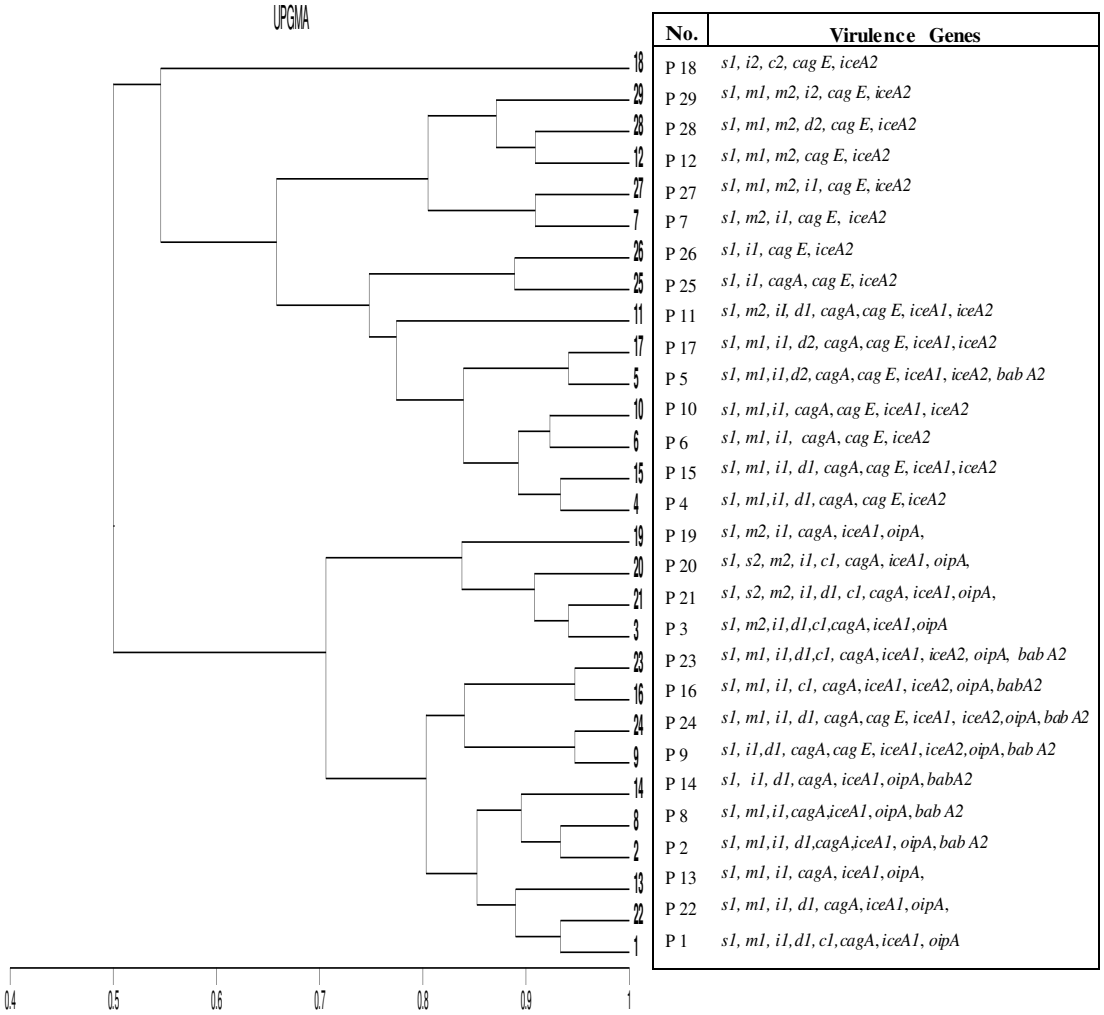


Fig. 2: Frequency of the different virulence-related genes among samples.

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, m1, i1,d1, c1,cagA*, *iceA1*, *oipA*	8	21	22.8
(Profile 2)	*s1, m1,i1, d1,cagA,iceA1*, *oipA*, *bab A2*	8	9	9.78
(Profile 3)	*s1, m2,i1,d1,c1,cagA*, *iceA1,oipA*	8	7	7.6
(Profile 4)	*s1, m1,i1, d1, cagA*, *cag E*, *iceA2*	7	5	5.43
(Profile 5)	*s1, m1,i1,d2, cagA*, *cag E*, *iceA1*, *iceA2, bab A2*	9	4	4.34
(Profile 6)	*s1, m1, i1, cagA*, *cag E*, *iceA2*	6	4	4.34
(Profile 7)	*s1, m2, i1, cag E*, *iceA2*	5	4	4.34
(Profile 8)	*s1, m1,i1,cagA,iceA1*, *oipA*, *bab A2*	7	3	3.26
(Profile 9)	*s1, i1,d1, cagA*, *cag E*, *iceA1*, *iceA2,oipA*, *bab A2*	9	3	3.26
(Profile 10)	*s1, m1,i1, cagA*, *cag E*, *iceA1*, *iceA2*	7	3	3.26
(Profile 11)	*s1, m2, iI, d1, cagA*, *cag E*, *iceA1*, *iceA2*	8	3	3.26
(Profile 12)	*s1, m1, m2, cag E*, *iceA2*	5	3	3.26
(Profile 13)	*s1, m1, i1, cagA*, *iceA1*, *oipA*,	6	2	2.17
(Profile 14)	*s1, i1, d1, cagA*, *iceA1*, *oipA*, *babA2*	7	2	2.17
(Profile 15)	*s1, m1, i1, d1, cagA*, *cag E*, *iceA1*, *iceA2*	8	2	2.17
(Profile 16)	*s1, m1, i1, c1, cagA*, *iceA1*, *iceA2, oipA*, *babA2*	9	2	2.17
(Profile 17)	*s1, m1, i1, d2, cagA*, *cag E*, *iceA1*, *iceA2*	8	2	2.17
(Profile 18)	*s1, i2, c2, cag E*, *iceA2*	5	2	2.17
(Profile 19)	*s1, m2, i1, cagA*, *iceA1*, *oipA*,	6	1	1.08
(Profile 20)	*s1, s2, m2, i1, c1, cagA*, *iceA1*, *oipA*,	8	1	1.08
(Profile 21)	*s1, s2, m2, i1, d1, c1, cagA*, *iceA1*, *oipA*,	9	1	1.08
(Profile 22)	*s1, m1, i1, d1, cagA*, *iceA1*, *oipA*,	7	1	1.08
(Profile 23)	*s1, m1, i1,d1,c1, cagA*, *iceA1*, *iceA2, oipA*, *bab A2*	10	1	1.08
(Profile 24)	*s1, m1, i1, d1,cagA*, *cag E*, *iceA1*,*iceA2*,*oipA*,*bab A2*	10	1	1.08
(Profile 25)	*s1, i1, cagA*, *cag E*, *iceA2*	5	1	1.08
(Profile 26)	*s1, i1, cag E*, *iceA2*	4	1	1.08
(Profile 27)	*s1, m1, m2, i1, cag E*, *iceA2*	6	1	1.08
(Profile 28)	*s1, m1, m2, d2, cag E*, *iceA2*	6	1	1.08
(Profile 29)	*s1, m1, m2, i2, cag E*, *iceA2*	6	1	1.08



No.	Virulence Genes
P 18	*s1, i2, c2, cag E, iceA2*
P 29	*s1, m1, m2, i2, cag E, iceA2*
P 28	*s1, m1, m2, d2, cag E, iceA2*
P 12	*s1, m1, m2, cag E, iceA2*
P 27	*s1, m1, m2, i1, cag E, iceA2*
P 7	*s1, m2, i1, cag E, iceA2*
P 26	*s1, i1, cag E, iceA2*
P 25	*s1, i1, cagA, cag E, iceA2*
P 11	*s1, m2, iI, d1, cagA, cag E, iceA1, iceA2*
P 17	*s1, m1, i1, d2, cagA, cag E, iceA1, iceA2*
P 5	*s1, m1,i1,d2, cagA, cag E, iceA1, iceA2, bab A2*
P 10	*s1, m1,i1, cagA, cag E, iceA1, iceA2*
P 6	*s1, m1, i1, cagA, cag E, iceA2*
P 15	*s1, m1, i1, d1, cagA, cag E, iceA1, iceA2*
P 4	*s1, m1,i1, d1, cagA, cag E, iceA2*
P 19	*s1, m2, i1, cagA, iceA1, oipA,*
P 20	*s1, s2, m2, i1, c1, cagA, iceA1, oipA,*
P 21	*s1, s2, m2, i1, d1, c1, cagA, iceA1, oipA,*
P 3	*s1, m2,i1,d1,c1,cagA, iceA1,oipA*
P 23	*s1, m1, i1,d1,c1, cagA, iceA1, iceA2, oipA, bab A2*
P 16	*s1, m1, i1, c1, cagA, iceA1, iceA2, oipA, babA2*
P 24	*s1, m1, i1, d1, cagA, cag E, iceA1, iceA2, oipA, bab A2*
P 9	*s1, i1,d1, cagA, cag E, iceA1, iceA2, oipA, bab A2*
P 14	*s1, i1, d1, cagA, iceA1, oipA, babA2*
P 8	*s1, m1,i1,cagA,iceA1, oipA, bab A2*
P 2	*s1, m1,i1, d1,cagA,iceA1, oipA, bab A2*
P 13	*s1, m1, i1, cagA, iceA1, oipA,*
P 22	*s1, m1, i1, d1, cagA, iceA1, oipA,*
P 1	*s1, m1, i1,d1, c1,cagA, iceA1, oipA*

Fig. 3: Dendrogram illustrating the genetic relationship among the 29 genetic profiles of the detected *H. pylori*.

Discussion

Previous studies concluded that an invasive test should be used for diagnosis and confirmation of *H. pylori* infections[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[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[8]. Every *H. pylori* -positive person has a different strains 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[18].

The virulence factors of *H. pylori* have been shown to play a crucial role in determining the course of its associated diseases[19&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*[8].

Abu-Taleb, *et al.*[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

the growth of *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 H. pylori in such cases[18]. In the current study, the prevalence of *H. pylori* infection was 92/120 (76.6%) of the collected biopsy specimens in Upper Egypt. This result agrees with the finding of Essawi, *et al.*[3], who observed that in some developing countries, the prevalence of *H. pylori* infection may surpass 70% which could be explain by the involvement of microbial factors contributing to the pathogenicity of the organism.

VacA gene is a secreted pore-forming toxin that was detected in 92 (100%) in *H. pylori* and used for detection of the bacteria in our study. Our results are consistent with other reports[9,21&22] that showed that the distribution rate of *vacA* was 100% in all *H. pylori* positive gastric biopsies. In contrast, another study showed that the prevalence of the *vacA* gene was 40.9% among positive-*H. pylori*[1]. VacA plays an important role in the pathogenesis of *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[5,23&24]. This toxin causes injury to gastric epithelium following progressive vacuolation[3].

In this study, we analyzed the frequency of the 5 common alleles of the *vacA* region *s*, *m*, *I, d* and, *c* in *H pylori* positive samples. All isolates showed mixed genotypes. Korona-Glowniak, *et al.*[18] reported that mixed *vacA* gene types were found in 8% of the samples that colonize gastric mucosa. In our investigation, the distribution *vacA* subtypes *s1/m1*, *s1/m2* and *s2/m2* were 66 (71.7%), 23 (25%) and 2 (2.1%), respectively. This is in agreement with other previous studies[5&21], that reported that the most predominant genotype was *vacA s1/m1* in 57.7%, followed by *s2/m2, s1/m2* or *s2/m1*[18]. A previous findings detected *(s1m0)* in 4.8% of samples[18]. The difference in distribution of the *vacA* subtypes is explained by the regional and ethnic differences[24]. This analysis detected the distribution rate of *cagA, cagE, iceA1,* and *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, *et al.*[22], investigated that the distribution of *cagA, cagE, iceA1, and iceA2,* virulence factors were 87.3%, 48.5%, 47% and 47% respectively[11&22]. *H. pylori* travel from the gastric mucosal epithelium layer to the basal layer, the *oipA* gene plays a key role in the colonization of this pathogen in the gastric mucosa[5]. The frequency of *oipA* was 59.7%. In contrast, *oipA* was detected in 15.2% in a previous study[5&22]. *OipA* has been significantly associated with increased IL-8 levels[8&25].

Regarding the main allelic antigens of *iceA*, the distribution of *iceA1* and *iceA2* were comparable in 32.3% and 35.5% respectively[18]. In the current study we observed that, *babA2* gene was detected in 25 (27.1%) of *H. pylori*. A similar rate was also shown in other studies[21&22]. There is a relationship between adhesiveness of *H. pylori* to the glycosylated mucins via the blood group antigen-binding adhesion *babA* and associated structures in the gastric mucosa[8&26].

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

Geno-typing using the pattern of virulence markers is one of the best tools to study the correlations between *H. pylori* obtained from different samples[5]. The different types of these gene regions are associated with variability in vacuolation, specificity and clinical symptoms[24]. This investigation detected that the frequency of different alternative combinations of the 10 virulence markers *vacA* alleles with *cagA*, *cagE, iceA1, iceA2, oipA* and *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 *s1/i1/m1* was the most prevalent genotype and this result is in agreement with other study by Erzin, *et al.*[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 *s*2, *m*2, or *i*2 types that they are considerably less active and are substantially nontoxic[24]. In our results, the prevalence of *vacA i1* was 85 (92.3%) which in agreement with Subsomwong, *et al.*[1], 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.*[1], who concluded that the *s1/m1/i1/c1/d1* 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 *vacAm1, i1, d1* with *cagA* genotypes may play a significant role in determining *H.pylori*-related outcomes of clinical data[19&29]. In addition, *H. pylori vacA s1a*, *cagA*, and *cagE* genotypes have remarkable correlations with the presence of duodenal ulcer or gastric carcinoma[28].

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 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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ملامح أوجه شراسة الهيليكوباكتر بيلوري التي تم اكتشافها في خزعات المعدة للمرضى الذين يخضعون للتنظير في صعيد مصر

سحر أحمد مندور[١] – أحمد شوكت عبد المحسن[٢] – محمد أحمد مدحت[٢] – دعاء عبد التواب[٢] – أمير الفراش[٣] – عبد الرحمن خليفة أحمد[٤] – أحمد محمود جلال[٥] – محمد أحمد المختار[٦]

[١]قسم الميكروبيولوجيا والمناعة ، كلية الصيدلة ، جامعة دراية ، المنيا ٦١١١١ ، مصر

[٢]قسم طب المناطق الحارة والجهاز الهضمي ، كلية الطب ، جامعة أسيوط ، أسيوط ، مصر

[٣]قسم الوراثة ، كلية الزراعة ، جامعة أسيوط ، أسيوط ، مصر

[٤]طالب طب ، كلية الطب ، جامعة أسيوط ، أسيوط ، مصر

[٥]طالب دراسات عليا ، قسم جراحة الأورام ، مركز أورام سوهاج ، سوهاج ، مصر

[٦]قسم الميكروبيولوجيا والمناعة ، كلية الطب ، جامعة أسيوط ، أسيوط ، مصر

الخلفية: ميكروب الهيليكوباكتر بيلوري او جرثومه المعده هي سبب شائع لقرحة المعدة وهي عامل خطر لسرطان المعدة. هناك القليل من البيانات المتاحة عن سمات جرثومة هيليكوباكتر بيلوري المسببة للعدوى في المرضى من صعيد مصر. لذلك ، كان الهدف من هذه الدراسة هو التحقيق في وتيرة الإصابة بالبكتيريا الحلزونية في المرضى المصريين الذين يخضعون للتنظير الداخلي والشكوى من أعراض الجهاز الهضمي العلوي المستمرة مباشرة في عينات الخزعة باستخدام تقنية تفاعل البوليميراز المتسلسل ودراسة الجينات المرتبطة باوجه الشراسه في هيليكوباكتر بيلوري الموجبة.

المواد والطرق: تم جمع مائة وعشرين عينة من خزعة المعدة من المرضى المصريين الذين تم إدخالهم إلى مستشفى جامعة أسيوط والذين يشكون من أعراض مزمنة في الجهاز الهضمي العلوي. تم استخراج الحمض النووي الحلزونية البوابية للتعرف الجزيئي بواسطة تفاعل البوليميراز المتسلسل. تم تحليل العينات الإيجابية كذلك للتحقق من وجود جينات وتوليفات جينية مختلفة مرتبطة باوجه الشراسه.

النتائج: تم اكتشاف الحلزونية البوابية في ١٢٠/٩٢ (٧٦,٦٪) من الخزعات التي تم جمعها. حملت جميع السلالات النوع الفرعي *vacA s1*. كان انتشار جينات الفوعة *cagA* و *cagE* و *iceA1* و iceA2 و *oipA* 79 (٨٤,٨٪) و ٤١ (٤٤,٥٪) و ٦٩ (٧٥٪) و ٤٤ (٤٧,٨٪) و ٥٥ (٥٩,٧٪) من الحلزونية البوابية عينات إيجابية ، على التوالي. أظهر التحليل الجيني أن الحلزونية البوابية تم تصنيفها في ٢٩ تركيبة وراثية مختلفة. كان النمط الجيني *s1 / m1 / i1 / d1 / c1 / cagA / iceA1 / oipA* هو الأكثر انتشارًا.

الخلاصة: أظهرت نتائجنا ارتفاع وتيرة الإصابة بالبكتيريا الحلزونية بين المرضى المصريين الذين يعانون من أعراض الجهاز الهضمي. الهجين *vacA s1 / m1 / i1* هو النوع الفرعي الأكثر انتشارًا. يُظهر توزيع الجينات المختلفة المرتبطة بالفوعة معدلات مثيرة للقلق من *cagA* و *cagE* و *iceA1* و *iceA2* و *oipA*. يجب إجراء مراقبة دقيقة لعدوى الحلزونية البوابية والتحقيق في خصائصها الوراثية للسيطرة على انتشار السلالات الخبيثة.