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ORIGINAL ARTICLE

Different Detection Methods of Virulent *Helicobacter pylori* in Gastric Biopsies

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ABSTRACT

Background: *The global allocation and high level of frequency with the consequence of related pathologies make the eradication of Helicobacter pylori is a very useful advance to test and treat. In addition, the determination of the genotype of H. pylori isolates helps us to comprehend the correlation between assumed virulence genes and clinical disease out come.* ***Objectives****: To determine the prevalence of H. pylori infections in patients complaining of gastric disorders, the best phenotypic method for detection of H. pylori and the antimicrobial susceptibility patterns among the isolated strains. To compare between phenotypic and genotypic detection methods and to evaluate the frequency of vacA, cagA and iceA genotypes with their clinical outcomes.* ***Methodology****: This study was carried out by collecting gastric biopsy endoscopic specimens from 92 participants admitted to Internal Medicine Endoscopy Unit, Faculty of medicine, Menoufia University. Direct detection of H. pylori in gastric biopsy specimens was done by polymerase chain reaction (ureA gene), microaeroplillic culturing, histological examination and Campylobacter like organism (CLO) rapid urease test. Antimicrobial susceptibility patterns among the isolated strains were determined by disc diffusion method. Some virulence genes (cytotoxin-associated gene (cagA), vaculating cytotoxin (vacA) alleles; vacAs1, vacAs2 and vacAm, also induced by contact epithelium (iceA)) were determined using multiplex PCR.* ***Results:*** *H.pylori genome (UreA) detection by conventional PCR was used as the confirmatory diagnostic tool with 70 PCR positive isolates from 92 participants totally by 76.1%. Histo-pathological examination by both H&E and Giemsa stain detected H. pylori in 68 cases (73.9%). CLO rapid urease test detected H.pylori urease activity in 64 cases (69.6%). Microaerophilic culturing detected H. pylori growth in only 32 cases (34.8%). About 100%, 68.8%, 81.3%, 68.8% and 12.5% of isolates were resistant to metronidazole, amoxicillin, tetracycline, clarithromycin and ciprofloxacin respectively. CagA was identified in 58 isolates (82.9%), iceA in 38 (54.3%), vacAs1 in 22 (31.4%), vacAs2 in 10 (14.3%), vacAm in 32 (45.7%). CagA and cagA+vacAs1m1+IceA were the most prevalent genotypes.* ***Conclusion:*** *Egypt is among the countries that reported high prevalence rate of H.pylori infections mainly with antibiotic resistant virulent strains.*

INTRODUCTION

Helicobacter pylori is a micro-aerophilic Gram-negative fastidious human pathogen. *H. pylori* is formerly documented as one of the most frequent chronic bacterial infections all over the world **,** and classified as class I carcinogen by the World Health Organization [1.] *H. pylori* infection is responsible for gastritis, peptic and duodenal ulcers, gastric mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma [2].

For epidemiology, *H. pylori* infection is more obvious in the developing countries (80%) than in developed ones (25%). In Egypt, the prevalence of *H. pylori* infection is 50% or more. Globally, this prevalence variation is assumed to be socioeconomically obsessed and related to the first 5 years of life acquisition [3].

Since the discovery of *H. pylori* in 1983, multiple methods for bacterium detection have been developed. Currently, histology, culture, rapid urease test and polymerase chain reaction (PCR) are the tests that performed on gastric biopsies. Isolation of *H. pylori* is necessary to study its growth requirements, antibiotic susceptibility testing, its virulence factor for vaccine developement and numerous explorations. PCR based

diagnosis may be considered as gold standard by designing primers particularly specific to *H. pylori* and targeting at least more than one conserved gene [4].

Disease outcome varies according to different bacterial virulence genes; cytotoxin-associated gene A (*CagA*), and the vacuolating cytotoxin A (*VacA*) are the mostly investigated [5]. The *CagA* gene is the first virulence factor detected in *H. pylori* strains and it is one of several genes in a pathogenicity island known as cagPAI. This gene encodes a protein that is associated with an increase in intensity of gastric inflammation and, consequently, with peptic ulcers & gastric cancer [6].

VacA toxin encoded by *VacA* gene induces cytoplasmic vacuoles, increases permeability and leads to the damage of gastric epithelial cells. The *VacA* gene exhibits significant allelic variation in the signal (s) and middle (m) regions. The s-region consists of two major subtypes' s1 and s2, whereas m-region designates m1 and m2 subtypes. A pleomorphic combination of s and m regions affects the vacuolating activity of *VacA* gene. Different genotypic combination of *VacA* region results in different pathogenicity level [4]. The *IceA* gene encoding a CATG-specific restriction endonuclease which is regulated by the contact of *H. pylori* with the human gastric epithelial cells has also been studied [7].

One of the major cause of *H. pylori* eradication failure is antibiotic resistance that often parallels to the antibiotic consumption patterns, and varies within patient groups according to geographic region, patient age and sex and the presence of other infections [3]. The high prevalence of *H. pylori* antimicrobial resistance leads to difficulty in testing and treating infected persons. Therefore, it is significant to discover higher risk persons for severe disease to allow the best clinical management and intensive follow-up [8].

METHODOLOGY

Collection of samples and identification of *H. pylori* isolates:

This study was performed in Microbiology & Immunology Department, Faculty of Medicine, Menoufia University during the period from March 2016 to August 2017. The study included 92 participants complained of gastrointestinal disorers (abdominal pain, nausea, vomiting, bloating or black stool). All participants were presented to Internal Medicine Endoscopy Unit, 38 had gastritis, 34 had gastric ulcer, and 20 showed normal gastric mucosa. Patients who underwent sclerotherapy or band ligation of oesophageal varices, those with coagulation disorders, portal or hepatic vien thrombosis, were excluded from this study. Full history was taken including age, sex, residence, socioeconomic status, smoking history, associated comorbidities, drug history (aspirin, NSAID or antibiotics), dietary habits and hygienic practice. Informed consents were obtained from all participants in this study.

Six gastric biopsies; 3 antral and 3 corpal were collected from each participant; A set of one antral and one corpal biopsies for rapid urease test, the second set was dispatched in 10% buffered formalin for histopathologic examination using Hematoxyline-Eosin (H&E) (Fig 1A) and Giemsa stains (Fig 1B) and the third set (for PCR & culture) was transferred immediately to the Microbiology laboratory into sterile tubes containing Brain Heart Infusion broth supplemented by 30% sterile glycerol.

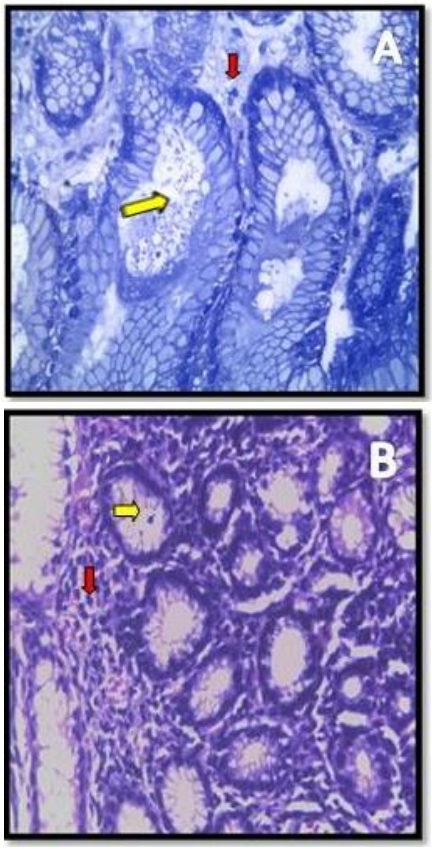

Fig. 1: *Hellobacter. Polyri* detection by hisopathology staining methods. A) Numerous *H. pylori* bacilli were observed in the lumen of a gastric pit (yellow arrow) with predominant lymphocytosis (red arrow) by Giemsa stain (x400). B) *H. pylori* bacilli seen by H&E stain in the lumen of a chronic inflamed gastric titssue (yellow arrow) with predominant leukocytosis (red arrow) (x400)

Cultures were performed on both selective (Campylobacter skirrows media as Brain heart infusion agar supplemented with 8% defibrinated sheep blood) & non selective (chocolate agar) media for isolation of *H.Pylori*. Skirrow supplements in the form of vancomycin 10 mg/L, trimethoprim 5 mg/L & polymyxin B 2500 IU/L, were added to inhibit growth

of associated bacterial pathogens. Plates were incubated under humid microaerophilic conditions for 3–7 days at 37 ºC**.** Colonies were identified by their morphology on culture plates, Gram staining and positive biochemical reactions (urease, oxidase and catalase) [9].

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing against 5 antimicrobial agents {amoxacillin (10ug), tetracycline (30ug), ciprofloxacin (5ug), metronidazole (5ug) & clarithromycin (15ug)} was done for all *H. pylori* isolates by disk diffusion method. The results were categorized as sensitive or resistant on the basis of the Psychological, Health and Learning Sciences (PHLS) Helicobacter working group guidelines and previous studies [10&11].

Detection of *ureA* gene, and virulence markers; (*cagA, iceA, vacAs1, vacAs2, vacAm1*) genes:

- *DNA extraction:* Total genomic DNA was extracted from all biopsies using Quick-g DNA ™ Miniprep Kit, USA according to the Manufacturer's instructions. Extracted DNA was stored at -80°C until used for identification of *ureA* gene, and virulence markers (*cagA*, *iceA*, *vacAs1*, *vacAs2*, *vacAm1*).
- Conventional PCR reaction was performed to detect *ureA* gene (Fig 2A).
- Multiplex PCR reaction was performed to detect *cagA* & *iceA* genes (Fig 2B) and other to detect *vacAs1*, *vacAs2*& *vacAm* genes (Fig 2C).

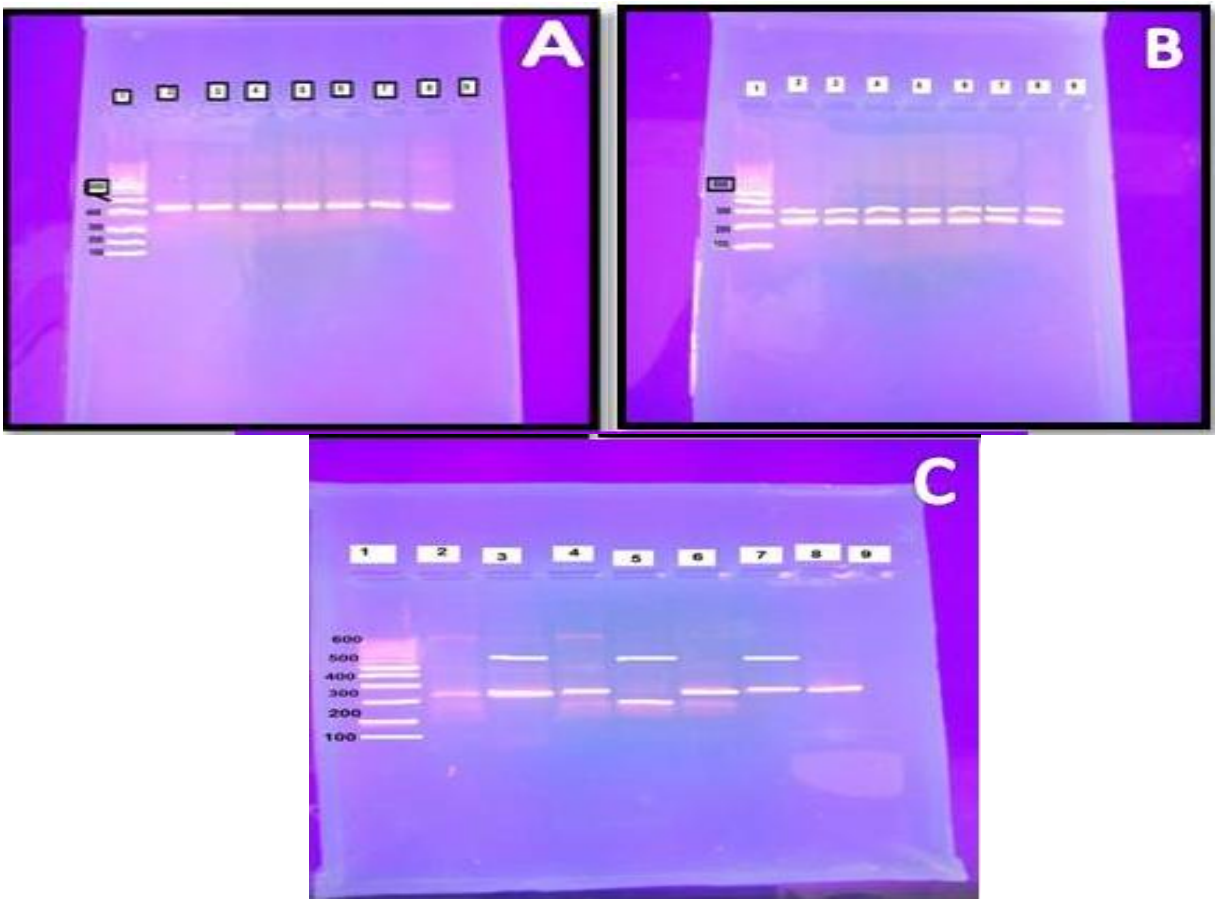

Fig. 2: A- Agarose gel electrophoresis of conventional PCR amplification product of *H. pylori ureA* gene (411bp): Lane 1; DNA ladder (100-1000 bp), lanes 2,3,4,5,6,7& 8 were positive for *ureA* gene. **B:** Agarose gel electrophoresis of multiplex PCR Agarose gel electrophoresis of *H. pylori* virulence genes *cagA* (298bp) & *iceA* (246bp): Lane 1; DNA ladder (100-1000 bp). Lanes 2,3,4,5,6,7&8 were positive for both genes. **C:** Agarose gel electrophoresis of multiplex PCR Agarose gel electrophoresis of *H. pylori* virulence genes *vacA s1*(338bp), *vacA s2*(286bp), *vacA m* (600bp). Lane 1; DNA ladder (100-1000 bp). *VacA* s1(338bp) was positive in lanes 3,4 ,6,7&8.*VacA* s2(286bp) was positive in lane 5. *VacA* m (600bp) was positive in lanes 3,5&7.

The oligonucleotide primers, sequence and size along with the thermocycling conditions used for PCR amplification of ureA gene, and virulence markers; (*cagA, iceA, vacAs1, vacAs2, vacAm*) genes.

Sequence (5'-3')	Primer name & size	Thermocycling conditions
5'-GCCAATGGTAAATTAGTT-5' 5'-CTCCTTAATTGTTTTTAC-3'	***ureA*** (411bp)	one pre-incubation cycle at 94°C for 5 min followed by 35 cycles at 94°C for 1 min, 45 °C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 7 min (conventional PCR)
5'-ATAATGCTAAATTAGACAACTTGAG-3' 5'-AGAAACAAAAGCAATACGATCATT-3'	***Cag A*** (298bp)	one pre-incubation cycle at 94°C for 2 min followed by 35 cycles at 95°C for 1 min, 50 °C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 5 min (Multiplex PCR)
5'-GTGTTTTTAACCAAAGTATC-3' 5'-CTATAGCCATTATCTTTGCA-3'	***ice A*** (246bp)	
5'-ATGGAAATACAACAAACACACCG-3' 5'-CAACCTCCATCAATCTTACTGGA-3'	***vacAs1*** (338bp)	one pre-incubation cycle at 94°C for 1 min followed by 35 cycles at 95°C for 1 min, 53 °C for 1 min, and 72°C for 1 min, with a final extension step of 72°C for 7 min(Multiplex PCR).
5'-ATGGAAATACAACAAACACAC-3' 5'-CTGCTTGAATGCGCCAAAC-3'	***vac As2*** (286bp)	
5'-CAATCTGTCCAATCAAGCGAG-3' 5'-GCGTCAAAATAATTCCAAGG-3'	***vac Am*** (600bp)	

Amplification cycles were performed with Thermocycler apparatus (***Biometra, Germany).*** Synthesized DNA fragments were detected on 1.5% agarose gels by ethidium bromide staining. A DNA ladder (100-1000 bp) was used to estimate allele sizes in base pairs (bp) for the gel [2&12].

Statistical analysis

It was performed using a Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, USA). Chi-square ($\chi 2$), Fisher exact and Kruskal-Wallis tests were used. The Roc curves were used to determine the sensitivity, specificity, positive and negative predictive values, and accuracy. Statistical significance was set at p value <0.05.

RESULTS

Ninty two participants were included in this study(54 male and 38 female), their mean age was 47.2 ± 16.6 years old and were classified into three endoscopic groups; normal mucosa group 20/92(21.7%), gastritis group 38/92(41.3%) and ulcer group 34/92(37.1%).

About 70/92(76.1%) of participants had PCR confirmed *H. pylori* infections, in comparison to 68/70(73.9%) for histopathology, 64/70(69.6%) for CLO test and 32/70(34.8%) for microaerophillic culturing. PCR & Histopathological detection methods almost had the same results in the different endoscopic groups without statistically significant difference. However, CLO test and the culture gave positive results with 76.5% and 35.3% of ulcer group in comparison to 50% and 10 % of normal mucosa group respectively with statistically significant difference for both (table 1). Variable diagnostic validities were recorded for different used methods in comparison to the gold standard PCR diagnostic tool; histopathology was the most accurate (98%)followed by CLO test (91%), while culture (59%) was the least dependable diagnostic tool (table 2). Old age, smoking, low socioeconomic status, bad hygienic practice and bad dietary habits were the risk factors for symptomatic *H. pylori* infections.

Table 1: Distribution of *H. pylori* by different detection methods (PCR, histopathology, culture and CLO test) among the studied groups (N=92)

Studied variables	Studied groups						Total (N=92)		χ^2	P value
	Group I Normal (N=20)		Group II Gastritis (N=38)		Group III Ulcer (N=34)					
	No	%	No	%	No	%	No	%		
PCR										
▪ Positive	12	60.0	30	78.9	28	82.4	70	76.1	2.35	P1:0.925
▪ Negative	8	40.0	8	21.1	6	17.6	22	23.9	3.28	P2:0.070
									0.13	P3:0.715
Histopathology										
▪ Bacteria	12	60.0	30	78.9	26	76.5	68	73.9	2.35	P1:0.925
▪ No bacteria	8	40.0	8	21.1	8	23.5	24	26.1	1.64	P2:0.200
									0.06	P3:0.801
CLO test										
▪ Positive	10	50.0	28	73.7	26	76.5	64	69.6	3.25	P1:0.071
▪ Negative	10	50.0	10	26.3	8	23.5	28	30.4	3.97	**P2:0.046***
									0.07	P3:0.785
Culture										
▪ Positive	2	10.0	18	47.4	12	35.3	32	34.8	2.44	P1:0.118
▪ Negative	18	90.0	20	52.6	22	64.7	60	65.2	4.20	**P2:0.040***
									1.08	P3:0.299

**significant*

P1: Between group I and group II P2: Between group I and group III P3: Between group II and group III

Table 2: Comparison between PCR and other *H. pylori* detection methods (histopathology, rapid urease test & culture) among the studied group (N=92) with evaluation of their diagnostic validity:

***H. pylori* detection method**	**PCR**				**Total**	
	Positive (70)		**Negative (22)**		**(92)**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Histopathology						
▪ Bacteria	68	97.1	0	0.00	68	73.9
▪ No bacteria	2	2.90	22	100	24	26.1
Rapid urease test						
▪ Positive	62	88.6	2	9.10	64	69.6
▪ Negative	8	11.4	20	90.9	28	30.4
Culture						
▪ Positive	32	45.7	0	0.00	32	34.8
▪ Negative	38	54.3	22	100	60	65.2
Diagnostic validity	**Sensitivity (%)**	**Specificity (%)**		**PPV (%)**	**NPV (%)**	**Accuracy (%)**
Histopathology	97%	100%		100%	92%	98%
Rapid urease test	91%	91%		97%	77%	91%
Culture	46%	100%		100%	37%	59%

Regarding antimicrobial susceptibility testing, about 100%, 68.8%, 81.3%, 68.8% and 12.5% of *H.pylori* isolates were resistant to metronidazole, amoxicillin, tetracycline, clarithromycin and ciprofloxacin respectively (fig 3).

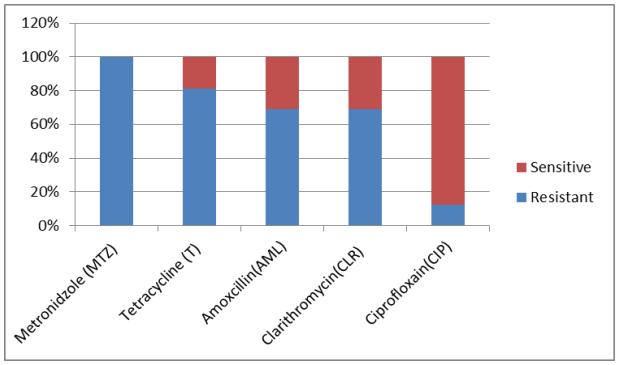

Fig. 3: Antimicrobial susceptibility patterns of *32 H. pylori* isolates

Variable distributions of different virulence genes detected by multiplex PCR were recorded. About 58/70 (82.9%), 38/70 (54.3%), 22/70 (31.4%), 10/70 (14.3%) and 32/70 (45.7%) of all patients had ***cagA, iceA1, vacAs1***, ***vacAs2*** and ***vacAm*** virulence genes respectively. Both *cagA* and *iceA*1 genotypes were significantly associated with ulcer and gastritis. Various genetic combinations were recorded with predominance of *CagA* & *IceA* combined genotype and *VacAs1m* & *CagA* & *IceA* combined genotype by 22.9% of PCR proved *H. pylori* strains for each. The combined genotype *CagA* & *IceA* was detected by 26.7% and 28.6% in gastritis and ulcer groups respectively. Also, *VacAs1m* & *CagA* &*IceA* combined genotype was reported by 20% and 28.6% in gastritis and ulcer groups respectively as shown in table 3

Table 3: Distribution of PCR detected virulence genes (*Cag A, Ice A1, Vac A s1, Vac A s2 and Vac A m)* among the PCR positive groups (N=70)

Virulence genes	Positive PCR(*ureA*)						Total (N=70)		χ^2	P value
	Group I Normal (N=12)		Group II Gastritis (N=30)		Group III Ulcer (N=28)					
	No.	%	No.	%	No.	%	No.	%		
Cag A									0.19	P1:0.665
▪ Present	8	66.7	22	73.3	28	100.0	58	82.9	10.4	**P2:0.001****
▪ Absent	4	33.3	8	26.7	0	0.00	12	17.1	8.66	**P3:0.003****
Ice A1									6.45	**P1: 0.011***
▪ Present	2	16.7	18	60.0	18	64.3	38	54.3	7.62	**P2:0.005****
▪ Absent	10	83.3	12	40.0	10	35.7	32	45.7	0.11	P3:0.736
Vac A s1									1.17	P1:0.280
▪ Present	2	16.7	10	33.3	10	35.7	22	31.4	1.45	P2:0.228
▪ Absent	10	83.3	20	66.7	18	64.3	48	68.6	0.04	P3:0.848
Vac A s2									0.06	P1:0.803
▪ Present	2	16.7	6	20.0	2	7.10	10	14.3	0.84	P2:0.357
▪ Absent	10	83.3	24	80.0	26	92.9	60	85.7	2.01	P3:0.155
Vac A m									1.37	P1:0.241
▪ Present	4	33.3	16	53.3	12	42.9	32	45.7	0.32	P2:0.573
▪ Absent	8	66.7	14	46.7	16	57.1	38	54.3	0.64	P3:0.424
Single gene										
CagA1 alone	6	50.0	4	13.3	8	28.6	18	25.7	-----	----------
Combined genes										
CagA1 &IceA1	0	0.00	8	26.7	8	28.6	16	22.9	-----	----------
VacAs2&VacAm	2	16.7	2	6.70	0	0.00	4	5.70	-----	----------
VacAs1,VacAm& CagA	0	0.00	2	6.70	2	7.10	4	5.70	-----	----------
VacAs1,Vac Am& IceA1	0	0.00	2	6.70	0	0.00	2	2.90	-----	----------
VacAs2,VacAm&CagA	0	0.00	2	6.70	0	0.00	2	2.90	-----	----------
VacAs2,VacAm&IceA1	0	0.00	2	6.70	0	0.00	2	2.90	-----	----------
VacAs1,VacAm,CagA&IceA1	2	16.7	6	20.0	8	28.6	16	22.9	-----	----------
VacAs2,VacAm,CagA&IceA1	0	0.00	0	0.00	2	7.10	2	2.90	-----	----------
None	2	16.7	2	6.70	0	0.00	4	5.70	-----	----------

The correlation between different *H.pylori* detection methods (histpathology, microaerophillic culturing and rapid urease test) and different virulence genes (*Cag A, Ice A1, Vac A s1, Vac A s2 and Vac A m1)* among 70 PCR positive cases showed non significant data except that microaerophilic culturing was the least accurate diagnostic tool significantly with *vacAs1* and *vacAm* genotypes (table 4).

Table 4: Correlation between *H.pylori* detection methods (histpathology, culture and rapid urease test) and virulence genes (*Cag A , Ice A1, Vac A s1, Vac A s2 and Vac A m1)* among PCR positive cases (N=70):

Studied variables	PCR positive cases(70)		Histopathology				Culture				Rapid urease test			
			+ve		-ve		+ve		-ve		+ve		-ve	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Vac A S1														
▪ Present	22	31.4	20	29.4	2	100	14	43.8	8	21.1	18	29.0	4	50.0
▪ Absent	48	68.6	48	70.6	0	0.00	18	56.2	30	78.9	44	71.0	4	50.0
Test			FE=4.49				X2 =4.15				FE=1.44			
P value			0.096				0.042*				0.249			
Vac A S2														
▪ Present	10	14.3	10	14.7	0	0.00	6	18.8	4	10.5	8	12.9	2	25.0
▪ Absent	60	85.7	58	85.3	2	100	26	81.2	34	89.5	54	87.1	6	75.
Test			FE=0.343				FE=0.959				FE=0.847			
P value			0.733				0.495				0.320			
Vac A m														
▪ Present	32	45.7	30	44.1	2	100	20	62.5	12	31.6	26	41.9	6	75.0
▪ Absent	38	54.3	38	55.9	0	0.00	12	37.5	26	68.4	36	58.1	2	25.0
Test			FE=2.44				X2 =6.69				FE=3.12			
P value			0.205				0.010**				0.077			
Cag A														
▪ Present	58	82.9	56	82.4	2	100	24	75.0	34	89.5	52	83.9	6	75.0
▪ Absent	12	17.1	12	17.6	0	0.00	8	25.0	4	10.5	10	16.1	2	25.0
Test			FE=0.426				X2=2.56				FE=0.393			
P value			0.684				0.109				0.618			
Ice A1														
▪ Present	38	54.3	36	52.9	2	100	20	62.5	18	47.4	36	58.1	2	25.0
▪ Absent	32	45.7	32	47.1	0	0.00	12	37.5	20	52.6	26	41.9	6	75.0
Test			FE=1.73				X2=1.60				FE=3.12			
P value			0.496				0.206				0.130			

Thirty two *H.pylori* isolates of different virulence genotypes (*Vac A s1, Vac A s2, Vac A m, Cag A & Ice A1)* showed 100% resistance for metronidazole. About 57- 80% of different virulence genotypes positive isolates were resistant to amoxicillin, clarithromycin and tetracycline, while 8.3- 33.3% of *H. pylori* isolates of different genotypes showed resistance for ciprofloxacin with 8.3% recorded for *cagA* and 33.3% for *vacAs*2 (table 5).

Table 5: Correlation between PCR detected virulence genes (*Vac A s1, Vac A s2, Vac A m, Cag A & Ice A1)* and antimicrobial susceptibility pattern of 32 *H. pylori* isolates by disc diffusion method:

Studied variables	*CagA* (N=24)		*IceA1* (N=20)		*VacA S1* (N=14)		*Vac A S2* (N=6)		*Vac A m* (N=20)	
	No.	%	No.	%	No	%	No.	%	No	%
Metronidzole										
▪ Resistant	24	100	20	100	14	100	6	100	20	100
▪ Sensitive	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Amoxcillin										
▪ Resistant	18	75.0	16	80.0	8	57.1	4	66.7	12	60.0
▪ Sensitive	6	25.0	4	20.0	6	42.9	2	33.3	8	40.0
Ciprofloxain										
▪ Resistant	2	8.3	2	10.0	2	14.3	2	33.3	2	10.0
▪ Sensitive	22	91.7	18	90.0	12	85.7	4	66.7	18	90.0
Clarithromycin										
▪ Resistant	18	75.0	12	60.0	8	57.1	4	66.7	12	60.0
▪ Sensitive	6	25.0	8	40.0	6	42.9	2	33.3	8	40.0
Tetracycline										
▪ Resistant	18	75.0	14	70.0	10	71.4	6	100	16	80.0
▪ Sensitive	6	25.0	6	30.0	4	28.6	0	0.00	4	20.0

DISCUSSION

Helicobacter pylori is currently recognized as one of the most common chronic bacterial infections worldwide and classified as class I carcinogen by the World Health Organization. *H. pylori* virulence genes have significant genetic heterogenicity associated by variable clinical outcome[13].

In the present study, different methods were used for detection of *H. pylori* in gastric biopsy specimens obtained by endoscopy. Polymerase chain reaction was the gold standard diagnostic tool with 100% accuracy, followed by histopathology (98%) then rapid urease test (91%), while culture was the least accurate diagnostic method (59%). These findings were agreed with *Miftahussurur & Yamoka* [14] and *Quach et al*[15].

Each diagnostic method has its merits and drawbacks. Histology enables identification of bacteria and evaluation of the intensity of gastric mucosa inflammation. However, false negative results maybe secondary to imperfect specimen preparation, presence of non typical bacterial morphology and/or low bacterial count *Yang*[16]. For rapid urease test, it is easy to perform but, false-negative results may occur because of low bacterial count or the use of antimicrobials or proton pump inhibitors *Ramis et al.* [17]. The culture is a highly specific diagnostic method, it allows the testing of antibiotic sensitivity but is more time-consuming. The false-negative results of this method may occur due to the absence or low density of bacterium in the biopsy specimens, use of antimicrobials and PPIs, inappropriate conditions of transport or loss of viability of the microorganism due oxygen exposure[18].

In the current study, *H. pylori* prevalence detected by conventional PCR was 76.1% which was the results obtained by [1, 2, 6]. Prevalence of infection had been reported to vary (0–100%) depending on geographical region, age of bacterial acquisition, period of persistent infection, hygiene practices, socioeconomic status and rate of eradication as seen with other communicable infectious diseases[19].

In this work, *H. pylori* isolates showed resistance by 100%, 68.8%, 81.3%, 68.8% and 12.5% to metronidazole, amoxicillin, tetracycline, clarithromycin and ciprofloxacin respectively. Similar resistance rates were recorded by *Vagarali et al* [20] and *Fathi et al.* [5]. However, lower resistance rates were obtained by *Mergraud* [21]. *H. pylori* high resistance to most used antimicrobial agents could be explained by the high prevalence of *H. pylori* infection with the high consumption pressure of these antibiotics. Resistance to fluoroquinolones is very low worldwide. Ciprofloxacin has low MICs against *H. pylori* and is highly active *in vitro* in eradicating the organism[10].

In the present study, only 8.3% of *CagA* positive *H.pylori* isolates were resistant to ciprofloxacin in comparison to 33.3% of *VacAs2* positive isolates. The underlying phenomenon could be explained by *cagA* negative strains maintained their mutation frequency in the increasing concentrations of ciprofloxacin whereas a sharp decline was observed in the development of resistant mutants of *cagA* positive strains. However, the opposite actions occur with *vacAs2* genotype [22].

In this study, *CagA, IceA& VacAs1m1* were the most prevalent genotypes among gastritis & ulcer patients by 82.9%, 54.3% and 31.4% respectively. Also, *CagA+ IceA* and *VacAs1m + CagA +IceA* were the commonest virulence genes combinations detected among studied patients by 22.9% for each. These results matched those obtained by many studies [2, 7 & 14]. *CagA* was detected by most gastritis and ulcer group patients. Also *IceA1* was detected in 64.3% of ulcer group in the same line with findings concluded by many studies [12&21]. It is important to note that not only *cagA* or *iceA* contributes to heterogeneity and correlation of gastric pathology, but also other factors like *vacA* and *babA* genotypes. The association and interaction of different virulence factors may increase the risk of the developing gastric lesions. Therefore, the study of these genes in combination is mandatory.

In the present study, most PCR proved patients of different virulence genotypes were males of ≥ 55 years old. This finding came in a line with results obtained by *Feliciano et al*[7] and *Yakoob et al* [23]. Most males spend most day time outdoors eating spicy or improperly prepared food. More than 60% of patients were smokers (especially with *vacAs2* genotype) with low socioeconomic status, bad dietary habits and bad hygienic practice matching the demographic and clinical characters of *H. pylori* infected patients obtained by many studies [1, 2&14]. In this research, *H. pylori* infection revealed a significant relationship with rural localities where poor medical facilities and lack of health education programs by 65.7 %. These data were matched with those obtained by *Contreras et al.* [24] in Venezuela and *El Shenawy et al* [25] in Egypt.

Conclusions & recommendations:

PCR is the gold standard diagnostic method detecting the presence of the *H. pylori* and their virulence genes. *H. pylori* is highly resistant to most antibiotics used as treatment options. It is mandatory to detect *H. pylori* antimicrobial susceptibility patterns prior to launching therapy to minimize antibiotics resistance rates. Ciprofloxacin revealed excellent activity against *H.pylori* isolates. The association and interaction of different virulence factors can increase the risk of the developing gastric lesions. Further studies concerned with various *H.pylori* virulence genes and its antimicrobial resistance patterns are recommended.

More health education programs should be carried out in rural regions.

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