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Seroprevalence of *Helicobacter pylori* infection among students of the Higher Institute of Technical Sciences, Msallata, Libya

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ABSTRACT

Objective: Seroprevalence of *Helicobacter pylori* (*H. pylori*) infection among undergraduate students at Higher Institute of Science and Technology, Msallata. **Methods:** A total of 150 serum samples were collected from students of the Higher Institute of Science and Technology in Msallata, with 100 L of each sterile serum transferred to a sample of *H. pylori* antigens kit (*H. pylori* Antigen Kit-Clinotech, USA). After 10 minutes, two distinct red lines in the group's control and test regions indicate a positive reaction. The isolated *H.pylori* from clinical blood was identified according to their morphological, cultural characteristics and consumption of broth manual some biochemical tests and confirmed by VITIK2 system. **Results:** A total of 63 (42.66%) of the 150 students tested positive for the virus. The prevalence of *H. pylori* infection was found to be related to age in a study of *H. pylori* seroprevalence. Infection rates were 45.5 percent among students aged 18-20, 85.7 percent among adults aged 31-40, 66.7 percent among those aged 41-50, and 28.6 percent among those aged 51 and up. Gender, age, and type of infection (symptomatic or asymptomatic seropositive infection) ($P<0.05$) all showed statistically significant differences (using Chi-square). The Biochemical characteristics of *H. pylori* was confirmed with excellent probability 99% after full Biochemical identification by VITIK2 system as well as the susceptibility information. **Conclusions:** This microorganism should be recognized as a possible cause of illness in children by community health personnel. Furthermore, the mode of transmission and possible methods of controlling the bacterial infection among students or in a community are public health concerns that need to be investigated further.

Keywords: *Helicobacter pylori*, infection, students, Libya

1. Introduction

Helicobacter pylori (*H. pylori*) is a major microorganism responsible for the development of a wide range of gastroduodenal diseases (Parsonnet *et al.,* 1991).

H. pylori is a gram-negative, microaerophilic bacterium with a helix that causes chronic low-level inflammation of the stomach lining and has been linked to the development of duodenal and gastric ulcers as well as stomach cancer. More than half of the world's population has *H. pylori* in their upper gastrointestinal tract, with more than 80% of those having asymptomatic infection. It is now the world's most common infection (Parsonnet *et al.,* 1991).

Infection with *H. pylori* is most commonly acquired during childhood and can last a lifetime if not treated (Torres *et al.,* 2000). According to seroepidemiologic studies, serum antibodies against *H. pylori* are present in 50% of adults in developed countries and nearly 90% of adults in developing countries (Chi *et al.,* 2009). Despite its high prevalence, little is known about the exact route of infection transmission in our society (Queiroz and Luzza, 2006).

As a result, knowing the prevalence of this infection is important. The objective of this research is to find out the prevalence of *H. pylori* infection among undergraduate students in Msalata Municipality, Libya.

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2. Materials and Methods

This study was conducted during the period from 2020-2021 at Bacteriology, labs, Department of Medical Laboratory Technology, high institute of science and Medical technology, Msalata, Libya.

I. Collection clinical specimens

Blood specimens were collected from 150 Higher diploma students, gender male and female and age (table, 1) at the Higher Institute of Science and Technology in Msallata Municipality from 8:00 a.m. to 12:00 p.m. in the laboratories section. They were asked to complete surveys in order to provide basic information such as their gender (male and female) and age (year).

Table 1: Risk factor of *H. pylori* infection higher diploma students regarding sex and age group.

Rick factor		Number of Higher diploma students (No = 150)	
Gender	**Male**	70 / 150	46.66 %
	Female	80 / 150	53.33 %
Age (year)	**18-20**	66 / 150	44.00 %
	21-23	84 / 150	56.00 %

II. Precaution clinical Blood

Safety precautions: Gloves and lab coat were wearied before beginning work. All biologic agents and chemicals were disposed in accordance with local environmental and safety regulations. These procedures can be found in the following **WHO (2004)**.

III. Collecting clinical specimens

The Higher diploma students were selected randomly from blood donors. Ten ml of venous blood samples were obtained by peripheral vein puncture under aseptic precaution from all students. Two ml of blood on sodium citrate to perform pro thrombin time. The blood specimens were refrigerated and arrived to the laboratory through one hour. The rest of blood was drawn in plain tube then put in water path at 37 C° for 30 minutes then centrifuged at 3000 rpm for 10 minutes then the resultant serum was divided in aliquots and stored at -20 C° for analysis.

IV. Serological assay

Using a sterile dispensable dropper, 3 drops of serum (approximately 100 mL) were transferred to the wells of the test kit each time (*H. pylori* Antigen Kit-Clinotech, USA). All sera that were not used were stored in a refrigerator. After 10 minutes, the test was conducted. The occurrence of two distinct red lines on the kit's control and test regions represented.

V. Isolation and enumeration of *H. pylori*

Aseptically (5g) of clinical blood sample was mixed with 95(ml) of sterile buffer peptone water and incubated in CO_2 incubator at 35°C for 24h (Al-Sulami *et al.,* 2008). One to ten (ml) mixture was transferred to nutrient broth and incubated in CO_2 incubator at 35°C for 72h. The enumerated cultures were re inoculated on MRS broth (FDA, 2002) selective medium and incubated in CO_2 incubator at 37°C for 24h. then put in refrigerator for further identification.

VI. Purification and identification of isolated *H. pylori.*

Bacterial colonies obtained from all previously mentioned media were chosen and picked up according to variation in culture characteristics and colony formation then purified by streak-plate method on Nutrient agar medium. Pure isolates were maintained on slants of the MRS broth medium at 4ºC for subsequent identification. Almost all microscopically examinations (Morphological characters: Shape, texture) and biochemical testing (Biochemical tests (Gram reaction, Motility test, Catalase test and Oxidase test) were carried out according to Bergey's manual, (2009); Collins & Lyne (2004) and Cheesbrough (2006).

VII. Haemolysis on blood agar medium

Blood haemolysis ability of the isolated bacterial colonies was tested using tryptic soy agar obtained from (Difco, USA), supplemented with 5% sterile human blood. Bacterial growth on blood agar medium showed the following features: a-haemolysis, β-haemolysis and no haemolysis.

Isolated *H. pylori* identification was confirmed by VITEK2 system carried out according to Shetty *&* Turner (1998) and Funke *et al.* (1998). The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substance. There are currently four reagent cards available for the identification of different organism as follows:

VIII. Culture Requirements

The parameters include acceptable culture media, culture age, incubation conditions and inoculums turbidity.

IX. Suspension preparation

A disposable bacterial needle used to transfer a single colony of a pure *H.pylori* culture and to suspended the *H.pylori* in 3.0 ml of sterile saline (aqueous 0.45% to 0.50%NaCl, pH4.5 to7.0) in a 12x75 mm clear plastic (polystyrene) test tube. The turbidity was adjusted according Table (2) and measured using a turbidity meter called the DensiChek.

Table 2: Suspension turbidities used for card inoculation

Product	McFarland Turbidity Range
GN	0.50-0.63
GP	0.50-0.63
YST	1.80-2.20
BCL	1.80-2.20

IX. Inoculation

Identification cards were inoculated with isolated *H. pylori* suspensions using an integrated vacuum apparatus. A test tube contain in g the microorganism suspension was placed into especial rack (cassette) and the identification card was placed in the neighbor in gloat while inserting the transfer tube into the corresponding suspension tube.

X. Antibiotic sensitivity test: (Vitek 2 system was used to antibiotic sensitivity test

Cefaclor, Cefotaxime, Cefoperazone, Cefepime, Clindamycin, Imipenem, Doxycycline, Levofloxacin, Ciprofloxacin, Amikacin, Sulphamethoxazole/Trimesoprim, Azithromycin, Ampicillin, Amoxicillin/Clavulinic acid, Pipracillin/Tazobactam and Nitrofurantion).

2.2. Statistical analysis

The Chi-squares (2) test was used $P< 0.05$ was considered significant.

3. Result

***3.1. H. pylori* detection**

H. pylori seropositivity was found in 63 of the 150 undergraduate students by Cast gram Rapid chromatographic Immunoassay (*H. pylori* Antigen Kit-Clinotech, USA). The positive samples were distinct pink colored band appeared on test regions, in addition to a pink line on the control line region, with a seroprevalence rate of 42.66 %. Males were infected at a rate of 58.57 % (36/70), while females were infected at a rate of 33.75 % (27/80). Males and females had significantly different levels of H. pylori infection (P 0.0286).

It was discovered that the prevalence of H. pylori infection among students was age-related. Students in grades 18-20 had the lowest H. pylori rate (23/63, 36.50 %), while students in grades 21-23 had the highest no significant rate (40/63, 49.63 %) 0.1175.

The males accounted for 42.9 % of the 108 seropositive students, while the females accounted for 23.1 %. Females accounted for 76.9 % of the 72 asymptomatic students studied, while males accounted for 57.1 %, 0.189.

Table 3: Risk factor of H. pylori infection among higher diploma students regarding sex and age group.

Parameter	Positive (n = 63)	Negative (n = 87)	P<0.05
Sex			
Male	36 (58.57%)	34(39.08 %)	0.0286
Female	27 (33.75%)	53 (60.91 %)	
Asymptomatic			
Male	9/36 (30.55%)	34	0.189
Female	13/27(48.14%)	53	
Age (yr)			
18-20	23(36-50%)	43 (49.42%)	0.1175
21-23	40 (63.49%)	44 (50.57%)	

3.2. Isolated *H. pylori* identification

In the present work, the isolated *H. pylori* from clinical blood was identified according to their morphological, cultural characteristics and consumption of broth manual some biochemical tests according to Bergey's manual, (2009) and confirmed by VITIK2 system. The morphological characters of *H. pylori* were straight rods to long spiral shape, motility, colonies were translucent to pale grayish and smooth texture. Biochemical characteristics were Gram negative, Beta hemolysis on blood agar, Facilitative anaerobic, positive, coagulase, catalase, oxidase and urease. The Biochemical characteristics of *H. pylori* was confirmed with excellent probability 98% after full Biochemical identification by VITIK2 system as well as the susceptibility information was provided as shown in table (4).

Microbiology Chart Report
BioMérieux Customer: 1005818356
Organism Quantity: 1 000 000 cfu/mL
Selected Organism: *Helicobacter pylori*

Table 4: Biochemical characteristics of *H. pylori* by VITEK 2

Identification Information	**Analysis Time: 5.80 hours**	**Status: Final**
Selected Organism	99% Probability	*Helicobacter pylor*
	Bionumber:	0003453103500250

Data of Biochemical tests for *Helicobacter pylori*												
APPA	-	ADO	-	PyrA	-	ARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	+	OFF	-
BGLU	-	dMAL	-	dMAN	+	dMNE	+	BXYL	-	BAlap	+
ProA	+	LIP	+	PLE	-	TyrA	+	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	+	MNT	+	5KG	-
lLATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	lHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	-	lMLTa	+	ELLM	-	lLATa	-		

Installed VITEK 2 ® Systems Version: 08.01

3.3. Antibiotic sensitivity of *Helicobacter pylori* isolate

According to VITEK 2, the antimicrobials sensitivity of *H. pylori* showed a range of susceptibilities ranging from sensitive to intermediate to resistant to the various antibiotics tested shown in table (5).

Table 5: Antibiotic sensitivity of *Helicobacter pylori* by VITEK 2.

Data of Antibiotic sensitivity for *Helicobacter pylori* and MIC					
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Ampicillin	>= 32	R	Imipenem	0.5	S
Amoxicillin / Clavulanic Acid	>= 32	R	Amikacin	>= 64	R
Ticarcillin	>= 128	R	Gentamicin	>= 16	R
Piperacillin/Tazobactam	64	I	Tobramycin	>= 16	R
Cefalotin	>= 64	R	Nalidixic Acid	>= 32	R
Cefoxitin	>= 64	R	Ciprofloxacin	>= 4	R
Cefotaxime	>= 64	R	Ofloxacin	>= 8	R
Ceftazidime	>= 64	R	Nitrofurantoin	256	R
Ertapenem	>= 8	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

Installed VITEK 2 ® Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
Therapeutic Interpretation Guideline: NATURAL RESISTANCE
AES Parameter Set Name: Global CLSI-based+
Natural Resistance AES Parameter Last Modified: Oct 24, 2017 14:16 CDT

4. Discussion

H. pylori is one of the most common bacterial infections in humans, and it affects children in developing countries at a young age. Endoscopy and serology are two traditional diagnostic tools that can be too invasive for children, but the (Robert *et al.,* 1993).

The results of this study showed that the seroprevalence of *H. pylori* was 42.66% among undergraduate students at the Higher Institute of Science and Technology Msallata, which is consistent with many studies in some developing countries (Jafri *et al.,* 2010). This seroprevalence rate, however, was lower than that reported in (Chi *et al.,* 2009; Ishaleku and Hope 2010).

This pathogenic bacteria infection is acquired during childhood, and its prevalence rises with age, with the patient's infection lasting for the majority, if not all, of his or her life (Jafri *et al.,* 2010). The fact that males have more symptomatic *H. pylori* infections than females is usually related to other risk factors such as stress and smoking, which aggravate *H. pylori* infections, according to (Zhang *et al.,* 2010).

Females have a higher prevalence of asymptomatic *H. pylori* infection than males, possibly because females engage in more social activities than males, according to (Queiroz and Luzza, 2006).

As a result, community health workers should be aware of this microbe as a possible source of illness in children. They can help by promoting good hygiene and educating families about how to prevent children from contracting the bacteria. Furthermore, the mode of transmission and potential means of controlling the bacterial infection among students or a community are public health concerns that need to be investigated further.

Our results demonstrated the antimicrobial susceptibility of *H. pylori* with a range of sensitivities ranging from sensitive to moderate to resistance to different antibiotics tested according to several similar studies (Wheeldon *et al.,* 2004) and (Ziemniak, 2006).

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