

Isolation and Identification of *Helicobacter* spp. From Gastric Biopsies and Autopsies in Human and Animal Gastric Patients

Hamed Wafy<sup>1\*</sup>, Shymaa Abdelmalek<sup>2</sup> and Alaa Eldin H. Mostafa<sup>1</sup>

(1) Department of Microbiology, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

(2) Microbiology department, Faculty of Veterinary Medicine, Cairo University.

\*Corresponding author: [wafy\\_hamed@yahoo.com](mailto:wafy_hamed@yahoo.com)

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ABSTRACT

*Helicobacter* infection is a major public health issue worldwide, about 4.4 billion individuals worldwide were confirmed to be infected. *Helicobacter* is a human pathogen that is transmitted from human to human and has a zoonotic relationship. *Helicobacter* gastritis, peptic ulcer, and perforation may lead to cancer. This study aims to try to isolate the *Helicobacters* from gastric biopsies and autopsies in human patients and animals (dogs and cats).

**Methodology:** 105 gastric biopsies were collected from dyspeptic patients and 5 gastric autopsies were collected from dogs and cats. The samples were cultivated and characterized by microbiological and molecular tools (PCR).

**Results:** The results showed few harvested isolates (n=22) after characterization the confirmed *Helicobacter* isolates were 10 only from humans and animals.

**Conclusion:** The conclusion is the microbiological method is so costive, time-consuming, and less sensitive. The presence of accurate and rapid tests is so crucial for specific and sensitive detection of *Helicobacters*.

**Keywords:** Gastric biopsies, *Helicobacter*, Isolation, PCR,

INTRODUCTION

The discovery of *Helicobacter pylori* in 1982 ushered in a paradigm shift in the knowledge and treatment of gastroduodenal diseases. (Megraud and Lehours, 2007).

*Campylobacter pylori* was the old name for *Helicobacter pylori* (*H.pylori*) (Al-Sulami et al., 2010). Experiments with volunteers and self-ingestion revealed that these bacteria may colonize the human stomach and irritate the mucosa (Morris et al., 1991).

*H. pylori* colonization in the stomach can cause upper gastrointestinal disorders like chronic gastritis, peptic ulcer disease, gastric mucosa-related lymphoid tissue lymphoma, and gastric cancer, according to early results. The degree of inflammation is likely to be the cause of *H. pylori*-related illnesses. (Abdalsadeg et al. 2012).

Although the majority of people do not acquire symptoms as a result of *H. pylori* colonization, between 10% and 15% of people may

experience symptoms, and the clinical outcome of the infection will be determined by complex interactions between host and bacterial characteristics. (Cogo et al., 2011).

*H. pylori* is a gram-negative bacteria that looks like a curved rod or a short spiral. It is thought to be one of man's most prevalent pathogenic illnesses, with prevalence rates in developed countries ranging from 30 to 60%, depending on age and socioeconomic position. (Vinette et al., 2002). The route of transmission and other elements of *H. pylori* infection epidemiology are still unknown. (Tiwari et al., 2005). A variety of strategies have been used to identify virulence factors that aid *H. pylori* in colonizing the host and contributing to disease progression. One of these factors is the urease enzyme, which *H. pylori* requires to live in the low-pH stomach lumen on its route to the more neutral gastric mucosa. (Montecucco and Rappuoli, 2001). For colonization, *H. pylori* also needs motility and chemotaxis genes, presumably so that it can find and travel to its chosen infection site and stay there. (Ottemann and Lowenthal, 2002).

*H. pylori* infection diagnosis is sometimes difficult (Tiwari et al., 2005; Abdalsadeg et al., 2012). Invasive procedures (endoscopy with biopsies for histology, culture, and a fast urease test) can be used (Vaira and Vakil, 2001). Culturing *H. pylori* from gastric biopsies is difficult. Microscopy and the fast urease test can be highly specific if done correctly, however, they are based on biopsy material and hence, like culture, are theoretically susceptible to sampling mistakes (Yoshida et al., 1998). Because invasive methods are costly, less invasive methods such as serological blood testing and the urea breath test have grown in popularity (Zagari et al., 1999; Bazzoli et al., 1997).

*H. pylori* is the gastric pathogen that colonizes the human stomach and causes a spectrum of diseases such as chronic active gastritis, gastric ulceration, Mucosal associated Lymphoid Tissue (MALT) lymphoma, and gastric cancer among others (Warren, 1983; Marshall and Warren, 1984; Nomura et al., 1991; Kidd and Modlin, 1998; Brown, 2000; Go, 2002).

The organism has been regarded as microaerophilic, as its optimal growth occurs in the presence of 5–15% oxygen (Marshall and

Warren, 1984; Langenberg et al., 1984; Goodwin and Armstrong, 1990).

*Helicobacter* infection is a major public health problem. In 2015, a systematic review shows that about 4.4 billion individuals worldwide were confirmed to be *Helicobacter* positive (Hooi et al. 2017).

This study aimed to isolate and identify *Helicobacter* from gastric biopsies and autopsies in human patients and pet animals (dogs and cats).

## MATERIALS AND METHODS

This study was approved by the Research Ethics Committee process number (HAM00116) Informed consent was obtained from all patients.

This research was conducted following the principles of Egypt's Declaration of Independence and was authorized by Medicine Cairo University's Institutional Animal Care and Use Committee Ref; results will be available soon.

### Samples:

105 total biopsy samples were collected from dyspeptic patients recruited to different hospitals. Eighty samples were from El-Maadi Military Hospital and 25 samples were from The Military Production, Helwan, Cairo from November 2020 to December 2021. The gastric biopsies were collected in sterile saline. The mean of patients aged 45 yrs. (30-50 yrs). The samples were collected in sterile saline and transported immediately to the Microbiology Laboratory, Faculty of Veterinary Medicine, Cairo University.

Animal samples: 5 samples were collected from Gastric autopsies from Mongrel dogs at the Surgery Department, Faculty of Veterinary Medicine, Cairo University. The samples were collected in sterile cups with sterile saline and transmitted to the Microbiology department, Faculty of Veterinary Medicine, Cairo University (Abdelmalek, et al., 2021)

### Isolation of *H. pylori*:

Sample processing: The gastric biopsies and autopsies were homogenized by an electric automated homogenizer till all tissue disappeared within the fluid. (Rabiea, et al., 2021).

### Isolation on selective medium:

The homogenized samples were spread onto Columbia blood agar base (HiMedia) supplemented with 7% horse blood, Campylobacter enrichment supplement, and Helicobacter selective supplement. Incubation at 37°C for 7-10 days in microaerophilic conditions (5-15% O<sub>2</sub> and 10% CO<sub>2</sub>) in an anaerobic jar using microaerophilic sachets (HiMedia) (Mégraud and Lehours, 2007).

#### **Morphological and biochemical:**

After the incubation period, the colonial morphology was observed. The morphological characterization of the *Helicobacter* bacilli was examined by Gram's stain.

The biochemical identification of the obtained isolates was performed by the Catalase test using 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), Oxidase test using oxidase discs (HiMedia), Urease test using Urea hydrolysis media (Oxoid), and Nitrate reduction test using the nitrate medium with its indicator (indicator A and indicator B).

#### **Molecular identification:**

DNA was extracted by a Genomic DNA extraction kit (Cat. No. 51604, Qiagen, Germany). DNA content and purity were determined spectrophotometrically by measuring the A<sub>260</sub>/A<sub>280</sub> optical density ratio. PCR: The PCR mixture included 1x master mix (amaROnePCR™, Cat.No. SM213-0250, GeneDireX, Inc.), 0.25 μmol forward, and reverse primers (ConsH), Forward primer: 5' TCG CTA AGA GAT CAG CCT ATG TCC T 3', Reverse primer: 5' ATT CCA CCT ACC TCT CCC ACA CT 3'. To produce 435bp (Abdelmalek et al. 2021), 10 ng DNA, and up to 20 μl nuclease-free water. The amplification cycle for ConsH was as follows: initial denaturation at 94°C/3 minutes, 30 cycles of the following temperatures: 94°C/30 sec, annealing at 60°C/1 minutes, and cyclic extension at 72°C/2 minutes, final extension at 72°C/5 minutes. Gel electrophoresis of the PCR products was performed in 1.5% agarose (Vivantis, cat.no: pco701) against a 100 bp DNA ladder (Vivantis, cat.no: NL1405) (Abdelmalek et al. 2021).

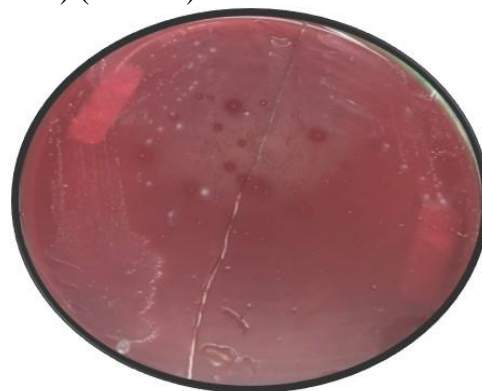
## **RESULTS**

Our results revealed 20 obtained isolates from 105 human samples (19%), 3 isolates were obtained from 5 animal samples (60%). The colonial morphology of the obtained isolates

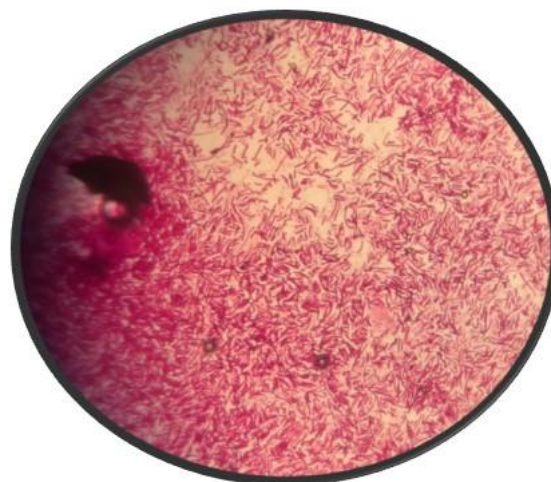
are non-hemolytic small pinpointed creamy white colonies (Figure 1). The morphological features of all 23 obtained isolates were Gram's negative bacilli by Gram's stain (100%) (Figure 2) (Table 1).

The biochemical identification was explained in table 1 and illustrated in Figure 3, the catalase test was positive to all obtained isolates (100%). The oxidase test was positive in 18 human isolates (94%) and 2 animal isolates only (66.6%), the urease test was positive in 14 human isolates (70%) and 2 animal isolates (66.6%). The nitrate reduction test was positive in 15 human isolates and 2 animal isolates (75% and 66.6%, respectively).

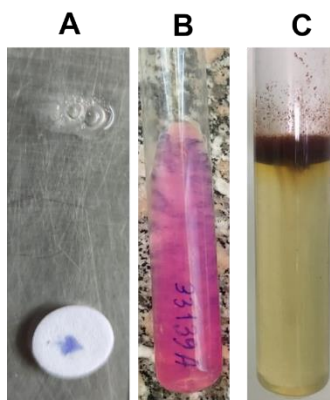
The molecular identification by 16srRNA PCR revealed 8 positive *Helicobacter* isolates from 20 human obtained isolates (40%). Only two animal isolates were positive to PCR (66.6%) (Figure 4) (Table 1).



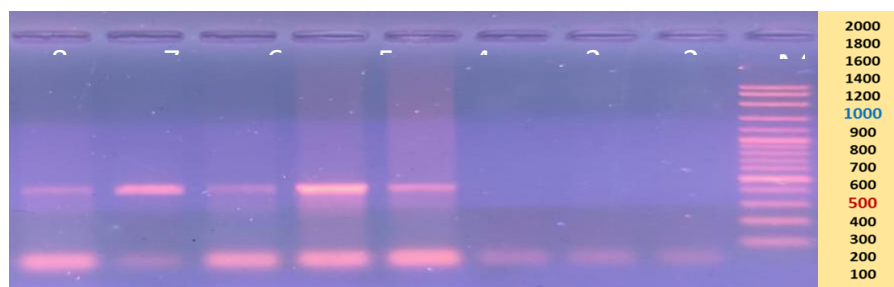
**Figure (1):** Grayish white small colonies of *Helicobacter* on Columbia blood agar medium with supplements.



**Figure (2):** Gram's negative curved bacilli of the isolates (*Helicobacter*) by Gram's stain under oil immersion lenses.



**Figure (3):** Biochemical identification of the isolates Helicobacters: A; catalase test in upper and positive oxidase lower. B; Urease test positive. C; positive nitrate reduction test.



**Figure (4):** 16srRNA PCR of the isolates using ConsH primers: M; 100bp DNA ladder. 1-3; negative samples. 4-8, positive 16srRNA PCR of 435 bp.

**Table (1):** Result demonstration of the local isolates from study samples, isolation, identification by biochemical and PCR:

	Sample no. (n)	Isolation	Gram' stain	Biochemical tests (+ve)				16srRNA PCR
				Catalase (+ve)	Oxidase	Urease	Nitrate reduction	
<b>Human samples</b>	105	20 (19%)	Gram's negative bacilli	20 (100%)	18 (94%)	14(70%)	15(75%)	8(40%)
<b>Animal samples</b>	5	3 (60%)	(100%)	3 (100%)	2 (66.6%)	2(66.6%)	2(66.6%)	2(66.6%)

## DISCUSSION

In the recent study, the aim was trials for isolation of Helicobacters from gastric biopsies and autopsies in dyspeptic patients and dead pet animals. Identification of the obtained isolates by different microbiological methods (culture, morphological, biochemical, and molecular by PCR).

The results were obtained that only 23 isolates were harvested from the total samples used in the study as explained in table 1. Only 23 isolates from 105 human biopsies and 5 gastric autopsies. 20 isolates from human samples and only 3 isolates from animal samples. These

results mean that Helicobacter is highly fastidious and difficult to be isolate (Euzeby, 2013). The colonial morphology showed in figure1, small greyish-white colonies on Columbia blood agar medium with campylobacter enrichment supplement and Helicobacter selective supplement (Owen, 1998). The morphology of the isolated bacteria demonstrated by Gram's stain and examined under an oil emersion lens showed Gram's negative bacilli (Figure 2) ( Dewhirst, et al., 2005). The biochemical identification by a set of biochemical, Catalase, Oxidase, urease, and nitrate reduction. The results showed in table 1

and Figure 3 in both types of samples. The Genus *Helicobacter* should be Oxidase positive. Catalase test positive in all *Helicobacters* except *H.canis*. Nitrate reduction and urease production are variable among species. The urease positive *Helicobacters* as *H.pylori*, *H.suis*, and *H.bilis*. the urease negative, as *H.pullorum*, *H.canis*, and *H. cinaedi*. Nitrate reduction positive *helicobacters* as *H. cinaedi*, *H.pullorum*, *H.bilis*. The nitrate reduction is negative as *H.pylori*, *H.suis*, and *H.canis*.

16srRNA specific primers (ConsH) is newly designed in 2021 by Abdelmalek et al., which showed great specificity. This primer was used in 16srRNA gene detection and isolates identification and gave positive bands at 435 bp in 10 confirmed *Helicobacter* isolates from 23 total local isolates. That reported that the molecular diagnosis by PCR is the most accurate and confirmatory test. (Abdelmalek et al., 2021).

From the previous results, we concluded that the *Helicobacters* are highly fastidious and difficult to isolate from the clinical samples. The media are not considered selective due to the spread of antibiotic-resistant bacteria such as *E.coli*. The morphological examination is not characteristic for *Helicobacter* only but there are variants of Gram's negative bacilli, which need further identification. The biochemical identification is not unique to *Helicobacters* only and many bacteria give the same biochemical profiling. The molecular identification by PCR especially the housekeeping gene (16srRNA) using the newly designed primers (ConsH) which has high specificity and sensitivity (Abdelmalek et al., 2021). The PCR is considered the gold standard test due to the specific catch of the specific gene. The results were obtained revealed that only 8 positive human isolates to PCR (40%), but only 2 positive animal isolates were positive to PCR (66.6%). The previous results between humans and animals due to the type of collected samples, the biopsy samples are small in size and the harvested bacilli are small numbers, but the autopsy samples are larger and harvest more bacilli (Hooi et al., 2017). There are several methods now available for the detection of the presence of *Helicobacter*, each one has its advantages, disadvantages, and limitations. the selection of

the method is according to if endoscopy is necessary or not (Garza-González et al., 2014).

## CONCLUSION

The *Helicobacter* spp. isolation is so difficult, although bacteriological isolation is the gold standard test. Molecular identification is the most accurate and specific tool. The microbiological isolation and characterization are so costive, time-consuming, and less sensitive.

## AUTHOR CONTRIBUTIONS:

W.H. Concept design, methodology, fund, grafting.

Sh.A. Methodology, manuscript drafting, and revision.

A.M. Supervision, analysis, drafting, and editing.

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