



## Isolation of *Helicobacter Pylori* from Raw Milk and Study on Its Survival in Fermented Milk Products

Lamiaa M. Talaat AL Shrief and Salwa S. Thabet\*

Food Hygiene Department, Animal Health Research Institute, Agriculture Research Center (ARC), Egypt.

\*Corresponding Authors: Salwa S. Thabet, E-Mail: [salwas.t.73@gmail.com](mailto:salwas.t.73@gmail.com)

### ABSTRACT

This research aimed to know if raw milk is a possible source of *Helicobacter pylori* infection to human and assessing the inhibitory effect of fermented milk products on *H. pylori* growth. Sixty samples of cows' milk and raw marketable milk (30 samples each) were tested to detect *H. pylori* and were collected from farms and supermarkets in Assiut City, Egypt. The pathogen could be isolated from 13.33 and 6.66 % of cows' milk and marketable milk, respectively, by using the conventional culture method. Confirmation of the isolated strains using the PCR technique revealed that, 50% of the isolated strains were positive for the 16S rRNA gene. The strong antibiogram of *Lactobacillus acidophilus* was evident, where the count of the tested two strains of *H. pylori* (S1 and S2) was sharply decreased to 2.6 and 2.17 log cfu/ g yoghurt and 2.47 and 3.04 log cfu/ml rayeb at the time of fermentation and could not be isolated at the first day. In case of *Bifidobacterium bifidum*, the count of S1 and S2 on the first day was 2.3 and 2.77 log cfu/ g yoghurt and 2.95 and 2.7 log cfu/ ml rayeb. Regarding the viability, *H. pylori* strains have remained viable for two days in yoghurt that was not supplemented with probiotics and could survive for seven days in control milk samples. There was no significant difference between the growth patterns of S1 and S2 in all treatments. Finally, fermented milk products containing probiotics were more effective in the survival of *H. pylori* than fermented milk products with no probiotics with a significant difference (P-value < 0.05).

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*Helicobacter pylori* is a microaerophilic spiral-shaped, Gram-negative bacterium that colonizes the gastric mucosa of more than 50% of the worldwide population. It is considered the major cause of peptic ulcer disease, duodenal ulcer, type B gastritis, gastric adenocarcinoma, lymphoma of mucosa-associated lymphoid tissue and gastric B-cell and human is the main reservoir of *H. pylori* infection (Crowe, 2019; Parikh and Ahlawat, 2021).

The prevalence of *H. pylori* infection in developed countries is around 34.7%, while in developing countries is 50.8% (Zamani et al., 2018). The infection of this bacteria is acquired very early in life and is often persistent lifelong without inducing symptoms if not properly treated (Kalali et al., 2015).

The role of foods especially milk and milk products in the transmission of *H. pylori* is still unknown (Dixit et al., 2012), but the presence of *H. pylori* in the stomach of domestic animals without

inducing any disease and its recovery from milk, meat and the stomach of ruminants suggests that, these animals and milk may act as reservoirs for *H. pylori* infection (Sambashivaiah et al., 2011). In addition, milk and milk products are suitable for the growth and survival of *H. pylori* and consequently, its transmission to humans (Vale and Vitor, 2010).

Treatment of *H. pylori* is difficult due to its colonization of the epithelial cells that line the antrum of the stomach and the access of antibiotics in this place is limited (Goodwin and Armstrong, 1990). In addition to the inactivation of antimicrobial agents in the stomach, as well as, antibiotic therapy is accompanied by the growth of antibiotic resistance species and unwanted side effects (Gotteland and Gruchet, 2003; Megraud and Lehours, 2007). For these reasons, the development of alternative methods to prevent the colonization of *H. pylori* and searching for new better therapies have become an essential demand, so much attention has focused on probiotics in recent years.

Probiotics are defined as nonpathogenic life microorganisms that have a beneficial effect on the host (FAO/ WHO, 2001). Lactic acid-producing bacteria and Bifidobacterium are the most commonly used probiotics (Sullivan and Nord, 2005). The antagonism of these groups of bacteria against pathogens is based on acids and inhibitory substances. These substances are oxygen peroxide, derivatives of oxygen metabolism, reuterin, aromatic compounds and bacteriocins (Georgalaki et al., 2013; Madureira et al., 2011). The resistance of lactobacilli to acid assists its persistence in the stomach; hence, they are used for *H. pylori* treatment and prophylaxis (Kaur, 2020).

Therefore, the main objective of this work was to detect the possibility of *H. pylori* transmission from milk and milk products and to determine its survival in fermented milk products.

## MATERIALS AND METHODS

### 1. Detection of *H. pylori*:

#### 1.1. Collection of samples:

A total of sixty random raw milk samples were examined for isolation of *H. pylori* including thirty samples of individual lactating cows were collected from the farms in Assiut City and thirty samples of raw milk were purchased from dairy shops. The dairy shops milk samples were tested for heat treatment using the Storch's test (Lampert, 1975). 100 ml of milk from each sample in a sterile glass container was transported to the laboratory with a minimum delay in an icebox.

#### 1.2. Isolation of *H. pylori* (Stevenson et al., 2000):

One ml from each milk sample was inoculated in a test tube containing 9 ml brain heart infusion broth supplemented with *H. pylori* selective supplement (Oxoid, UK) and 4% iron supplemented calf serum and incubated at 37°C for 24- 48 h under microaerophilic condition (5% O<sub>2</sub>, 15% CO<sub>2</sub> and 80 % Nitrogen) using gas generation kits (Oxoid, UK). One loopful from the inoculated tubes was streaked on plates of Columbia agar containing 5% defibrinated sheep blood and *H. pylori* selective supplement and incubated at 37°C under microaerophilic conditions for 3- 5 days. The suspected colonies were identified based on their morphological characteristics and biochemical tests (Fox et al., 2000).

### 2. Molecular identification of the isolated strains (Chong et al., 1996):

The previously identified six positives and four suspected strains using biochemical tests were sent to the Reference Laboratory for Veterinary Quality Control on Poultry Production in Animal Health

Research Institute, Dokki, Giza, Egypt for molecular confirmation by PCR technique.

### 2.. DNA extraction:

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

### 2.2. PCR amplification:

Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystems 2720 thermal cycler. Primers supplied from Metabion (Germany) are listed in Table (1).

Table 1: Primers used for identification of 16S rRNA of *H. pylori*

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)		Final extension
				Secondary denaturation	Annealing	
16S rRNA	CTGGAGAGA	109	94°C for 5 min.	94°C for 30 sec.	60°C for 30 sec.	72°C for 7 min.
	CTAAGCCCTCC					
	GCTGATTGTGC					

### 2.3. Analysis of the PCR Products:

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Appllichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis,

15 µl of the products were loaded in each gel slot. Gelpilot 100 bp (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and all data were analyzed through computer software.

### 3. Survival of *H. pylori* in fermented milk products :

#### 3.1. Culture preparation:

##### 3.1.1. Indicator organism:

Two strains of *H. pylori* (S1 and S2) used in the experiment were the previously isolated and identified strains. They were propagated in brain heart infusion broth at 37°C for two days and tenfold serial dilution was made to detect the density of the organism/ ml broth.

##### 3.1.2. Strains of probiotics:

*Lactobacillus. acidophilus* 20079 and *Bifidobacterium. bifidum* ATCC 15696 strains were the used reference strains in the experiment. *L. acidophilus* strain was cultured on De Man, Rogosa and Sharpe broth (MRS broth), while *B. bifidum* strain was grown on MRS broth supplemented with cysteine and was incubated at 37°C for 48 h. anaerobically, then, tenfold serial dilution was made to detect their count/ ml of the inoculated broth.

#### 3.2. Experimental technique:

##### 3.2.1. Preparation of yoghurt (Abo- Donia, 2008):

One liter of milk was purchased from Assiut City supermarkets and displaced to the laboratory. Milk was boiled for a few minutes and cooled to 45°C and 2% yoghurt culture (yoghurt sold in supermarket and was examined for *H. pylori* free by plating on Columbia agar plates) was added and mixed well. One portion was taken as control negative before inoculation with *H. pylori* strains and the remaining portion was divided into three groups of two bottles; the first bottle was inoculated with S1 and the second with S2 of *H. pylori* in a count of  $5 \times 10^6$  cfu/ ml (6.69 logs cfu/ ml). The first group was inoculated with *L. acidophilus* and the second with *B. bifidum* to obtain a count of  $5 \times 10^9$  cfu/ ml (9.69 cfu/ ml). The third group was not inoculated with probiotics. Finally, all bottles were kept at 40 °C until fermentation (3- 4 h.), then kept in the refrigerator at  $4 \pm 2$  °C.

##### 3.2.2. Preparation of rayeb (Abo- Donia, 2008):

One liter of fresh milk was divided into three groups of two bottles one bottle was inoculated with S1 and the second with strain S2 of *H. pylori* at a count of  $5 \times 10^6$  cfu/ ml (6.69 log cfu/ ml). The first group was inoculated with *L. acidophilus*. The second with *B. bifidum* to obtain a count of  $5 \times 10^9$  cfu/ ml (9.69 cfu/ ml) and the third group was not inoculated with

probiotics and then the content of each bottle was put in shallow or deep earthenware pots and was kept in warm dark place till the cream was raised. Milk was coagulated and this curd is named rayeb, then kept in the refrigerator. In addition, 100 ml of fresh milk was made rayeb and was not inoculated with *H. pylori* as a control negative.

##### 3.2.3. Preparation of control positive:

500 ml milk was boiled for 15 min and cooled to 37°C, then was put in two bottles. One bottle was inoculated with S1 and the second with S2 of *H. pylori* in a count of  $5 \times 10^6$  cfu/ ml (6.69 log cfu/ ml) as control positive.

##### 3.2.4. Enumeration of *H. pylori* in the inoculated products:

Tenfold serial dilution of the inoculated groups, control positive and control negative of yoghurt and rayeb were carried out using 0.1 % peptone water. The cells number of *H. pylori* was determined by plating on Columbia blood agar plates. Its count was detected at the time of fermentation of the products and on the first, second, third and seventh days.

#### 4. Statistical analysis:

The Statistical analysis of the data was performed using Bartlett's test for homogeneity and Prism 5 by Turkey's Multiple Comparison Test to detect the significant difference between different treatments.

## RESULTS

As recorded in Table 2, *H. pylori* could be isolated from 4 out of the examined 30 cows' milk samples in a percentage of 13.33. On the other hand, the incidence of *H. pylori* in the tested marketable milk samples was 6.66%, where it could be isolated from 2 out of the examined samples.

Table 2: Incidence of *H. pylori* in the examined samples based on biochemical identification:

Samples	No. of examined	Positive samples	
		No	%
Cows'	30	4	13.33
Marketable	30	2	6.6%

As observed in Figure1 and Table 3, confirmation of the isolated strains from Cows' milk and Marketable milk by using PCR revealed that two and one out of the identified strains were confirmed positive for *H. pylori* with a percentage of 50%. Consequently, the incidence of *H. pylori* in the tested samples was 6.66 and 3.33%, respectively.

Table 3: Results of molecular conformation of the isolated *H. pylori* strains by using PCR technique:

Samples	Prevalence of <i>H. pylori</i> in the isolated		Incidence of <i>H. pylori</i> in the examined	
	No.	%	No.	%
Cows' milk	2/ 4	50	2/30	6.66
Marketable	1/ 2	50	1/30	3.33

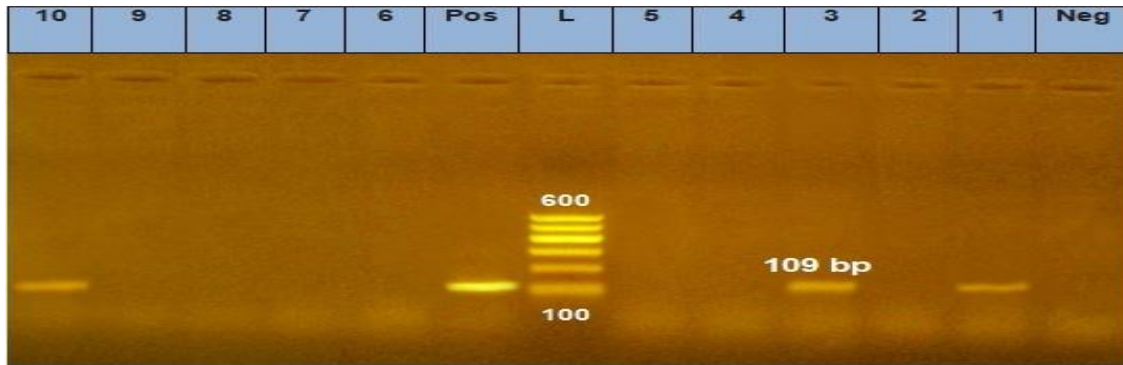


Fig. 1: Molecular identification of the isolated *H. pylori* strains by using PCR.

The data illustrated in Table 4 represented the effect of yoghurt on the survival of *H. pylori*, where the organism could be isolated from yoghurt supplemented with *L. acidophilus* in a count of 2.6 and 2.17 log cfu/ g for S1 and S2, respectively, at zero time and could not be detected at the first day. While in case of *B. bifidum*, the initial count of *H. pylori* was 4.3 and 4.01 log cfu/ g and could be isolated on the first day in a count of 2.3 and 2.77 logs cfu/ g. On the contrary, *H. pylori* could survive in yoghurt with no probiotics for two days with a count of 4.77 and 4.3 logs cfu/ g at the time of fermentation, 4.6 and 3.95 log cfu/ g on 1<sup>st</sup> day, 2.0 and 2.7 at 2<sup>nd</sup> day and could not be isolated at the 3<sup>rd</sup> day.

Table 4: Survival of *H. pylori* in yoghurt supplemented with *L. acidophilus* and *B. bifidum*:

Day	<i>L. acidophilus</i>				<i>B. bifidum</i>				No probiotics			
	S1		S2		S1		S2		S1		S2	
	count	pH	count	pH	Count	pH	count	pH	count	pH	count	pH
Zero time	2.6	4.3	2.17	4.4	4.3	4.5	4.01	4.4	4.77	4.8	4.3	4.9
1 <sup>st</sup> day	ND	ND	ND	ND	2.3	4.3	2.77	4.2	4.6	4.6	3.95	4.6
2 <sup>nd</sup> day	ND	ND	ND	ND	ND	ND	ND	ND	2.0	4.5	2.7	4.5
3 <sup>rd</sup> day	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Zero time = time of fermentation, ND = not detected, S1 = strain 1 of *H. pylori* and S2 = strain 2 of *H. pylori*

According to the reported data in Table 5, the strains of *H. pylori* could survive in rayeb supplemented with *L. acidophilus* during fermentation of the product with a count of 2.47 and 3.04 log cfu/ml and failed to be detected on the first day. In case of *B. bifidum*, *H. pylori* strains could resist to the first day and their initial count was 4.6 and 4.5 log cfu/ ml and on the 1<sup>st</sup> day was 2.95 and 2.7 log cfu/ ml. On the other hand, the pathogen could withstand for two days in rayeb with no probiotics in a count of 5.95 and 5.32 at time zero and the microbial

population of S1 and S2 was 4.6 and 3.69 log cfu/ ml on the 1<sup>st</sup> day, 3.04 and 3.95 log cfu/ ml at the 2<sup>nd</sup> day, then, they could not be detected at the 3<sup>rd</sup> day.

Table 5: Survival of *H. pylori* in Rayeb supplemented with *L. acidophilus* and *B. bifidum*:

Day	L. acidophilus				B. bifidum				No probiotics			
	S1		S2		S1		S2		S1		S2	
	Count	pH	count	pH	Count	pH	count	pH	count	pH	Count	pH
Zero time	2.47	5.9	3.04	5.7	4.60	5.8	4.50	6.0	5.95	6.0	5.32	6.1
1 <sup>st</sup> day	ND	ND	ND	ND	2.95	5.4	2.70	5.6	3.6	5.8	3.69	5.9
2 <sup>nd</sup> day	ND	ND	ND	ND	ND	ND	ND	ND	3.04	5.1	3.95	5.3
3 <sup>rd</sup> day	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Zero time = time of fermentation, ND = not detected, S1 = strain 1 of *H. pylori* and S2 = strain 2 of *H. pylori*

Regarding Control samples, strains of *H. pylori* could survive in control milk samples for seven days with a low decreasing percentage and their count was 6.6 and 6.3 log cfu/ ml at zero time. While, the bacterial densities of S1 were 5.8, 5.2, 4.7 and 4.3 log cfu/ ml at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 7<sup>th</sup> days and S2 was found in a population of 5.27, 4.95, 4.69 4.2, respectively ( Table 6).

Table 6: Results of control samples.

Day	Control +ve				Control -ve	
	S1		S2		Yoghurt	Rayeb
	Count	pH	Count	pH		
Zero time	6.6	6.7	6.3	6.8	ND	ND
1 <sup>st</sup> day	5.8	6.7	5.27	6.7	ND	ND
2 <sup>nd</sup> day	5.2	6.7	4.95	6.7	ND	ND
3 <sup>rd</sup> day	4.7	6.6.	4.69	6.7	ND	ND
7 <sup>th</sup> day	4.3	6.6	4.2	6.6	ND	ND

Zero time = time of fermentation, ND = not detected, S1 = strain 1 of *H. pylori* and S2 = strain 2 of *H. pylori*

## DISCUSSION

Although the internal contamination of milk with *H. pylori* is not likely to be found, its presence in the environment leads to external milk contamination from the surrounding. In addition to milk properties that provide *H. pylori* the opportunity for transition to human (Talaei *et al.*, 2015).

Comparing the summarized results in Table 1 and 2 with the recorded findings by other authors revealed that, the highest incidence was reported by Elhariri *et al.*, (2017), who found that, 30.0% of the tested cows' milk were seropositive for *H. pylori* by

using different ELISA kits. Mousavi *et al.*, (2014) and Yahaghi *et al.*, (2014) could isolate the organism from cows' milk in a nearly similar percentage to the present study (16.0 and 16.66%) and similar results for PCR detection of *H. pylori* were recorded by Ranjbar *et al.*, (2018), who could detect *H. pylori* in 7.5% of the examined bovine milk samples. On the other hand, lower results were recorded by Talimakhani and Mashak (2017), who could isolate *H. pylori* from 4% of the examined bovine milk samples. Guessoum *et al.*, (2018) found that, 6.0 and 13.0% of the examined samples were positive for the organism by using ELISA assays and PCR technique and failed to isolate

*H. pylori* by the classical culture method. Also, **Thabet (2018)** could isolate *H. pylori* from 2.5% of the evaluated cows' milk by culture method and confirmation of the isolated strains by PCR. On the contrary, **Azevedo et al., (2007)** and **Tabatabaei (2012)** could not isolate *H. pylori* by culture method. Concerning marketable milk, **Sayed et al., (2010)** could isolate *H. pylori* in percentage similar to the result of this work (6%) by culture method.

Nonetheless, the incidence of *H. pylori* in the present study was low in relation to the highest results recorded by other authors who used PCR and ELISA for detection of *H. pylori*, where this organism is fastidious and complex growth media are required for its growth, in addition, PCR assay is more sensitive and accurate (**Abdel-Latif et al., 2016**). So, recently developed methods for detecting *H. pylori* must be applied in the research directly on the examined samples. Detection of *H. pylori* in dairy shops milk and its presence in the tested cows' milk in this literature supports the theory that, milk and milk products play a role in the transmission of *H. pylori* to humans.

In recent years, the strong antagonistic effect of probiotics against pathogenic bacteria is a major concern concerning the processing of dairy products and the potential risk of *H. pylori* infection as a result of consumption of contaminated milk and milk products was promoted us to study the patterns of *H. pylori* growth in fermented milk products, which are considered the most suitable vehicles of probiotics to the consumer.

The results achieved and recorded in Tables 4 and 5 revealed that, the inoculated strains of *H. pylori* can survive during fermentation of yoghurt and rayeb supplemented with *L. acidophilus* with a high decline in the count of S1 and S2 and could not be detected at the first day of the experiment. Regarding the effect of *B. bifidum*, the reduction rate in the count of S1 and S2 is lower than that recorded for *L. acidophilus* besides the survival of the tested strains for one day. In case of the products not supplemented with probiotics, the decreasing percentage in the count of S1 and S2 was lower than that reported in yoghurt and rayeb supplemented with probiotics. In addition, the evaluated strains remained viable to the second day with a high count.

There is little and scant literature studying the survival of *H. pylori* in yoghurt. At the same time, **Bohmeler et al., (1996)** recorded the survival of the organism for three h at 37° C. Contrarily, **Sadek et al., (2011)** recorded that, *H. pylori* could be detected on the first day in yoghurt supplemented with *L. acidophilus*, while, in case of supplementation with *B. bifidum*, *H.*

*pylori* could not be recovered at the first day and could survive for 10 days in yoghurt with no probiotics.

The behavioral patterns of *H. pylori* in milk (control positive) are characterized by a low decreasing percentage in its population along with the experiment as recorded in Table 6. Where the evaluated strains could withstand for seven days and the rate of decline in its population is low in relation to the recorded data in case of fermented milk. The survival of *H. pylori* in milk samples was in agreement with the demonstrated results of many publishers; **Elhariri et al., (2018)**, **Poms and Tatini (2001)** and **Quaglia et al., (2007)** recorded the survival of *H. pylori* in pasteurized milk for 10, 5 and 9 days, respectively.

The statistical analysis of the recorded data revealed that, there was a homogeneity between the results of all treatments in yoghurt and the results of control samples with a P-value of 0.482 as well in rayeb and control samples with a P-value of 0.306. There was no significant difference between the survival of S1 and S2 of *H. pylori* in control samples and all treatments and between the effect of *L. acidophilus* and *B. bifidum* ( $P > 0.05$ ). However, there was a very high significant difference between the growth patterns of this pathogen in control samples and fermented milk products with probiotics ( $P < 0.001$ ). There was a highly significant difference between the growth of *H. pylori* strains in control samples and fermented milk products with no probiotics ( $P < 0.01$ ). While there was a significant difference between *H. pylori* growth in acidophilus fermented products and fermented products with no probiotics with a P-value  $< 0.05$ .

Although yoghurt's pH is lower than that reported for rayeb, the behavior of *H. pylori* in yoghurt and rayeb was nearly similar, whereas, there are many mechanisms for the antagonism of probiotics other than pH behind the inhibition of the pathogen growth.

In addition to the dissolved oxygen and oxygen permeation in the packages could affect the viability of *B. bifidum* spp. *H. pylori* strains were recovered from yoghurt and rayeb supplemented with *B. bifidum* on the first day contrary to the addition of *L. acidophilus*, where the acidity of the products affects the viability of *B. bifidum*. On the other hand, *L. acidophilus* can survive at low pH, whereas, it could survive well at pH 3.0 or above. The members of the genus *Bifidobacterium* are sensitive to O<sub>2</sub> (**Sonomoto, 2011**).

Although, the inhibitory effect of yoghurt and rayeb not supplemented with probiotics on the viability of *H. pylori* as a result of low pH, while in vivo, *H.*



*pylori* have the ability to survive low pH by the breakdown of urea found in foods to carbon dioxide and ammonia to neutralize the area around it, while in fermented milk products urea is exhausted at the time of ripening (Moble, 2001).

*B. bifidum*, unlike *Lactobacillus*, has an antagonistic effect on *H. pylori* but in addition to its antagonism, it improves the pathological lesions of the gastric mucosa and shifts the stages of peptic ulcer to a mild degree and decreases urease activity of *H. pylori*. In addition, *B. bifidum* declines the count of *E. coli* in the intestine and consequently decreases the molecular hydrogen in the stomach that is used as a source of energy for *H. pylori* growth (Olson and Maier, 2002; Wang *et al.*, 2004).

It is obvious from the demonstrated data that probiotics have an inhibitory effect on *H. pylori* growth. The variations between the antagonistic effect of *L. acidophilus* and *B. bifidum* were clearly evident and *L. acidophilus* was more effective than *B. bifidum*. Moreover, the viability and activity of probiotics are the main consideration during the processing of fermented milk because they must survive during the shelf life to confer health benefits to humans and be found in an adequate count that can suppress the growth of pathogenic bacteria. So, over-acidification can be limited by good manufacturing practices and using a culture of reduced over-acidification behavior (Kneifel *et al.*, 1993).

Fermented milk products containing probiotics especially *Lactobacilli* and *Bifidobacterium*, have beneficial effects on humans as a consequence of their obvious antagonism against *H. pylori* as reported in this literature. Therefore, we have attempted to develop a fermented dairy product containing an adequate amount of probiotics that strongly affect the in vitro viability of *H. pylori* to render fermented milk product *H. pylori* free and to be used for the treatment of *H. pylori*; on the other hand, the viability of probiotics must be taken in consideration.

## CONCLUSION

The findings of this study declared that milk and milk products have an impact role in the transmission of *H. pylori* infection to humans and the antagonistic activity of fermented milk products against this organism, especially through probiotics, is clearly evident. In addition, complementary and extended studies in the future to elucidate the factors affecting the growth of *H. pylori* in fermented milk products must be carried out to establish a precise data of the probiotics densities to be used, supplementation of fermented milk products with more than one probiotic

and consequently, using these products as an alternative method for treatment of this pathogen.

## Declaration of Conflicting Interests:

The authors revealed that there is no potential conflicts of interest.

## REFERENCES

- ABDEL-LATIF, EMAN, F., MAROUF, S. A., and HAMZA, DALIA, A., 2016. Molecular and serological detection of *H. pylori* in cow's milk and its impact on human health. *I. J. B. P. A. S.*, 5: 60- 66.
- ABO- DONIA, S. A. 2008. Origin, history and manufacture process of Egyptian dairy products: An overview. *Alexandria J. Food Sci. Technol.*, 5: 51- 62.
- AZEVEDO, N. F., ALMEDIA, C., CERQUEIRA, L., DIAS, S., KEEVIL, C.W., and VIERIA, M.J., 2007. Coccoid form of *H. pylori* as a morphological manifestation of cell adaptation to the environment. *Appl. Environ. Microbiol.*, 73: 3423- 3427. <http://dx.doi.org/10.1128/AEM.00047-07>
- BOHMELEER, G., GERWERT, I., SCUPIN, E., and SINELL, H. J., 1996. Epidemiology of *H. pylori* in human : Studies on survival capacity of *H. pylori* in food . *Dtsch. Tieraztl. Wschenschr.*, 102: 438- 443. PMID: 9035978
- CHONG, S.K., LOU, Q., FITZGERALD, J. F., and LEE, C. H., 1996. Evaluation of 16S rRNA gene PCR with primers Hp1 and Hp2 for detection of *H. pylori*. *J. Clin. Microbiol.*, 34: 2728– 2730. <https://dx.doi.org/10.1128%2Fjcm.36.2.603-603.1998>
- CROWE, S. E., 2019. Helicobacter pylori infection. *New England J Med.*, 380(12):1158–1165.
- DIXIT, M. D., BHAT, K. G., and DAYAL, A., 2012. Role of *H. pylori* specific heat shock protein -60 antibodies in the etiology of coronary artery disease. *Microbiol., Res.* 3.
- ELHARIRI, M., ELHELW, REHAB, HAMZA, DALIA and EL- MAHALAWY, HEBA, S., 2017. Serological evidence and risk factors for *H. pylori* infection in animals and humans. *J. Infe. Dev. Ctries.*, 11: 414- 419.
- ELHARIRI, M., HAMZA, DALIA, ELHELW, REHAB and HAMZA, EMAN, 2018. Occurrence of cagA+ vacA s1a m1 i1 *Helicobacter pylori* in farm animals in Egypt and ability to survive in experimentally contaminated UHT milk. *Scientific Report*, 8(1).
- EL- SHAROUD, W. M. 2009. Prevalence and survival of *Campylobacter* in Egyptian dairy product. *Food Research International*, 42: 622- 626. DOI:10.1016/j.foodres.2009.0.009.
- FAO/ WHO, 2001. Health and Nutritional Properties of Probiotics in Food Including Powder Milk and Live LAB. Food and Drug Administration/ World Health Organization. Expert Consultation Report.
- FOX, J. G., CHEIN, C. C., DEWHIRST, F. E., PASTER, B. J., SHEIN, Z., MELITO, P. L., WOODWARD, D. L., and REDGERS, F. G., 2000. *H. Canadensis* sp. Nov. isolated from humans with diarrhea as an example of an emerging pathogen. *J. Clin. Microbiol.*, 38: 2546- 2549. <https://doi.org/10.1128/jcm.38.7.2546-2549.2000>

- GEORGALAKI, MARINA, K., PAPANIMITIYOU, K., ANASTASIOU, RANIA, POT, B., VAN DRIESSE, G., DEVREESE, B., and TSAKLIDOU, E., 2013.** Macedonicin, the second food-grade lantibiotic produced by streptococcus macedonicus ACA-DC 198. *Food Microbiol.*, 33: 124-130. <http://dx.doi.org/10.1016/j.fm.2012.09.008>
- GOODWIN, C. S., and ARMSTRONG, J. A., 1990.** Microbiological aspects of *H. pylori* (Campylobacter pylori). *European journal of clinical microbiology, infectious disease* .Official Publication of European of Clinical Microbiology, 91-13.
- GOTTELAND, M., and CRUCHET, I. A., 2003.** Suppressive effect of frequent ingestion of *Lactobacillus Johnson ii Lal* on *H. pylori* Colonization in asymptomatic volunteers. *J. Antimicrob. Chemother.*, 51: 1317- 1319.
- GUESSOUM, MERYEM, GUECHI, Z., and ADNANE, M., 2018.** First –time serological and molecular detection of *H. pylori* in milk from Algerian local-breed cows. *Veterinary world*, 1326- 1330. <https://doi.org/10.14202/vetworld.2018.1326-1330>
- KALALI, B., FORMICHELLA, L., and GERHARD, M., 2015.** Diagnosis of *H. pylori*: changes towards the future. *Diseases* 3(3):122–135. <https://dx.doi.org/10.3390%2Fdiseases3030122>
- KAUR, L., GORDON, M., BAINES, P. A., IHEOZOR-EJIOFOR, Z., SINOPOULOU, V., and AKOBENG A. K., 2020.** Probiotics for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev.* 2020, 3:CD005573. [10.1002/14651858.CD005573.pub3](https://doi.org/10.1002/14651858.CD005573.pub3)
- KNEIFEL, W., JAROS, D., and ERHARD F., 1993.** Microflora and acidification properties of yoghurt and yoghurt- related products fermented with commercially starter cultures. *Int. J. Food Microbiol.*, 18: 179- 189.
- MADUREIRA, A. R., PINTADO, M. E., GAMES, A. M., and MALCATA, F. X., 2011.** Incorporation of probiotic bacteria in whey cheese decreasing the risk of microbial contamination. *J. Food Prot.*, 47: 1194- 1199.
- LAMPERT, L. M., 1975.** *Modern Dairy Products*. 3<sup>rd</sup> ed. Chemical Pub. Co., Inc., New York.
- MEGRAUD, F., and LEHOURS, P., 2007.** *H. pylori* detection and antimicrobial susceptibility testing. *Clin. Microbiol. Rev.*, 20: 280- 322. <https://doi.org/10.1128/cmr.00033-06>
- MOBLEY, H. L. T., 2001.** Mobley H. L. T and Mendz, G. L. and Hazell, S. I. (eds) *H. pylori: Physiology and Genetics*. U. S. National library of medicine.
- MOUSAVI, M., SAFARPOOR DEHKORDI, F., and RAHIMI, F., 2014.** Virulence factors and antibiotic resistance of *H. pylori* isolated from raw milk and unpasteurized dairy products in Iran. *J. Venomous Anim. Toxins including Trop. Dis.*, 20: 1-7.
- OLSON, J. W., and MAIER, R. J., 2002.** Molecular hydrogen as an energy Source for *H. pylori*. *Science*, 29: 1788 – 1790.
- PARIKH, N. S., and AHLAWAT, R., 2021.** *Helicobacter pylori*. In StatPearls [Internet]; StatPearls Publishing: Treasure Island, FL, USA, 2021; Available online: <https://www.ncbi.nlm.nih.gov/books/NBK534233/> (accessed on 25 February 2021).
- POMS, R. E., and TATINI, S. R., 2001.** Survival of *H. pylori* in ready to eat foods at 4°C. *Int. J. Food Microbiol.*, 63 :281- 6. DOI: 10.1016/s0168-1605(00)00441-4.
- QUAGLIA, N. C., DAMBROSIO, A., NORMANO, G., PARISI, A., FIRINS, A., LORUSSO, V., and CELANO, G. V., 2007.** Survival of *H. pylori* in artificially contaminated ultrahigh temperature and pasteurized milk . *J. Food Microbiol.*, 24: 296- 300. doi: 10.1016/j.fm.2006.04.008.
- RANJBAR, R., FARSANI, F. Y., and DEHKORDI, F. S., 2018.** Phenotypic analysis of antibiotic resistance and genotypic study of the *vacA*, *cagA*, *iceA*, *oipA* and *babA* genotypes of the *H. pylori* strains isolated from raw milk. *Antimicrobial Resistance and Infection Control*, 7:115 <https://doi.org/10.1186/s13756-018-0409-y>
- SADEK, ZEINAB, I., ABOU SHADY, HALA, M., YOUSSEF, SADEK, ZEINAB, I. AND ABOU SHADY, HALA, M., YOUSSEF, and HEBAT ALLAH, I., 2011.** Role of probiotic strains in the management of *H. pylori*. *Advances in Food Science*, 33: 237- 245.
- SAMBASHIVAIAH S, BILICHODMATH, S., and NANJIAIAH, N., 2011.** *H. pylori* in periodontal pockets of chronic periodontitis patients with and without type II diabetes mellitus : a randomized controlled trial. *Microbiol. Res.* 2. <https://doi.org/10.4081/mr.2011.e12>
- SAYED, AMAL, S. M., HUSSEIN, ASMAA, A., HASSAN, MONA, A., EL-DIN, SALWA, S., AHMED, EMAN, M. M., and NOMAN, HANAN, A., 2010.** A study on *H. pylori* and *H. helimanni* in milk, soft cheese and the health hazard on children in Assiut Governorate . *Assiut Vet. Med. J.*, 56: 14- 25.
- SONOMOTO, K. & A. YOKOTO, 2011.** Lactic acid bacteria and *Bifidobacterium*: Caister Academic Press. ISBN 928.
- STEVENSON, T. H., CASTILLO, A., LUCIA, L. M., and ACUFF, G. R., 2000.** Growth of *H. pylori* in various liquid and plating media. *Appl. Microbiol.*, 30: 192- 196. doi: 10.1046/j.1472-765x.2000.00699.x.
- SULLIVAN, A., and NORD, C. E., 2005.** Probiotics and gastrointestinal diseases. *J. Intern. Med.*, 257: 78-92.
- TABATABAEI, M. 2012.** Application of molecular and cultural methods for identification of *Helicobacter spp.* In *Different Animal Sources*. *Glob. Vet.*, 8: 292- 297.
- TALAEI, R., SOUOD, N., MOMTAZ, H., and DABIRI, H., 2015.** Milk of life stock as a possible transmission route of *H. pylori* infection. *Gastroenterol. Hepatol. Bed. Bench.*, 8(1): 530-536.
- TALIMKHANI, A., and MASHAK, Z., 2017.** Prevalence and genotyping of *H. pylori* isolated from meat, milk and Vegetable in Iran. *Jundishapur Journal of Microbiology*, 10 (11): e14240. <https://dx.doi.org/%2010.5812/jjm.14240>
- THABET, ASMAA, S., 2018.** Occurrence of *H. pylori* in Cattle, buffaloes and patients with gastritis and peptic ulcer . M. S. Ss. Thesis, Fac. Vet. Med., Assiut University.
- VALE, F. F., and VITOR, J. M. 2010.** Transmission pathway of *H. pylori*: Dose food plays a role in rural and urban areas. *Int. J. food Microbiol.*, 138:1-12. doi: 10.1016/j.ijfoodmicro.2010.01.016. Epub 2010 Jan 18.



- WANG, K. Y., LI, C. S., LIU, D. S., PERNG, Y. C., SU, D. C., WU, C. M., JAN, C. H., HUANG, T. N., and WANG, W. M. 2004. Effects of ingesting *Lactobacillus* and *Bifidodacterium* Containing yoghurt in subjects with Colonized *H. pylori*. *Am. J. Clin. Nutr.*, 80: 737 – 741. <http://dx.doi.org/10.1093/ajcn/80.3.737>
- YAGHI, E., KHAMESIPOU, E., MASHAYEKHI, FATEMEH, M., DEHKORDI, F. S., SAKHAEI, M. H., MASOUAHDIMANESH, KHAMENEIE, M., and MARYAM, K., 2014. *H. pylori* in vegetables and salads: genotyping and antimicrobial resistance properties. *Biomed. Res. Int.*, 757941. <https://doi.org/10.1155/2014/757941>
- ZAMANI, M., EBRAHIMTABAR, F., ZAMANI, V., MILLER, W., ALIZADEH-NAVAEI, R., SHOKRI-SHIRVANI, J., and DERAKHSHAN, M., 2018. Systematic review with meta-analysis: the worldwide prevalence of *H. pylori* infection. *Aliment Pharmacol Ther* 47(7):868–876.

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