THE DETECTION OF HELICOBACTER PYLORI IN COW MILK, CHEESE BY POUR PLATE AND POLYMERASE CHAIN REACTION METHODS

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Abstract: *Infectious occurred by Helicobacter Pylori is one of the most common food infectious in the world, however the origin of this bacteria is still in question. Transferring of this bacteria through raw milk to human is one of the proposed theory, therefore the present study was performed by aiming at determination of Helicobacter Pylori in tap water, cheese, cow milk and traditional ice cream in which overall 182 samples (ice cream samples=25, traditional cheese samples=47, raw cow milk samples=70, tap water samples=40) investigated. Results revealed that among 70 cow milk samples only one of them displayed Helicobacter Pylori in PCR method while in other samples (water, cheese and ice cream) were positive. Among all of the 182 samples, 16 of them including 2 samples from tap water (5%), 7 samples of cow milk, 6 samples of cheese and 1 sample of ice cream were positive in aforesaid bacteria by PCR method.*

Key words: *Water, row Cow Milk, Helicobacter Pylori, Ice Cream*

Introduction

Helicobacter Pylori is gram negative bacteria which colonize in gastric human mucosa. More than half of the world population is being infected by this bacteria, the infections are occurred 50 % in developed countries while 90 % in developing countries (Gomes et al, 2004; Das and Paul, 2007). Studies revealed that the most of cases has been infected

before the age of 10 (Klein et al, 1991; Parsons et al, 2001) and this bacteria is responsible for gastric ulcer. Despite of being commonly infected of human by these bacteria, the paths they can transfer to our body are still a controversial issue but the most common way is fecal-oral (Vale et al, 2010; Gomes et al, 2004).

Although this bacterium cannot grow in foodstuffs, they play a key role in transmitting of H. Pylori and dairy products are important among them due to their contribution in human's diet (Alborzi et al,2006; Basile et al,2006;Ghasemian Safaei et al,2011 and Johnson et al, 1997). The limited researches on foodstuffs and drink water have developed the theory of being as carrier of H. Pylori in Iran and other countries (Dore et al, 2011; Fujimura et al ,2002; Horiuchi et al,2001 and Quaglia et al,2008). Hence regarding its importance in human health, the target of this study is to investigate and determine of *H.Pylori* in water, milk in Shahrekord and Shiraz cities of Iran.

Material and method

Sampling

In this study 182 samples including ice cream samples=25, traditional cheese samples=47, raw cow milk samples=70, tap water samples=40 collected. Water samples in 1L bottles and other samples in 500cc bottles poured (sterilized bottles) and placed in the refrigerator.

Milk samples and prepared suspension from cheese and ice cream cultured by pour plate method on Brucella Agar culture media (MO74 HIMEDIA Co) enriched ,10 blood of calf embryo(RM-112 HIMEDIA Co) , 5 % defirinized sheep blood ,10 ml trimethoprim, 6 ml Cefixime and 6 ml VANCOMYCIN then cultured samples incubated in carbon dioxide incubator at 37˚C for 3-5 days. Suspicious colonies investigated by Gram, catalase, oxidase and Urease experiments (Quaglia et al,2008).

DNA extraction and *H pylori* tracing

Lage et al. (1995) presented the DNA extraction method. Genomic DNA Purification kit-Fermentas used to conduct DNA extraction base on manufacturer company instruction. The quality and quantity of extracted DNA evaluated by both electrophoresis on Agarose gel 1 % and spectrophotometer (λ=260 nm), then place in refrigerator before PCR experiment.

Applied primer was related to ureC gene and the sequences were:

"HP-F - 5'AAGCTTTTAGGGGTGTTAGGGGTTT-3'"

"HP-R: 5'-AAGCTTACTTTCTAACACTAACGC-3' "

This produces a product containing 294 basic pairs. To perform PCR, optimized concentration of reaction in 25 µl final volumes was determined 0.5 mM Magnesium Chloride, 1 µM of each primer, and 0.5 unit of taq DNA polymerase, 200µM of dNTP, then solution injected to the Thermo cycler model Eppendorf Co Germany and thermal processing set as: 94 ˚C for 1min, 56˚C for 1min, 72˚C for 1min and a final stage of 72˚C for 1min. Resultant evaluated by electrophoreses agarose gel(1.5%) containing ethydium bromide and observed with UV light.

Results and discussion

Current study aimed at investigation of contamination of tap water, raw cow milk, traditional ice cream and cheese. The results revealed that only 1 sample (0.55 %) among 182 samples infected with *H. Pylori* (figure 1). Results of PCR method showed that 16 samples (8.8%) among 182 samples contained the *H. Pylori* or its DNA (table1).

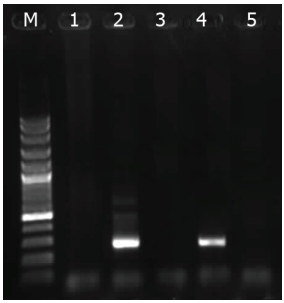

Figure1. M: 100 base pairs marker, 1st column: negative control (sterile distilled water), 2nd column positive column, 3rd and 5th columns: negative samples, 4th column: positive samples (contain *H. Pylori*).

جدول ۱ - وضعیت آلودگی آب‌های لوله کشی، شیر، پنیر و بستنی سنتی در شهرکرد و شیراز به *هلیکوباکترپیلوری* به روش PCR

شهر مورد مطالعه	نمونه‌ی مورد مطالعه	تعداد نمونه	تعداد نمونه‌های منفی (درصد)	تعداد نمونه‌های مثبت (درصد)
شهرکرد	آب لوله کشی شهری	۲۰	(۹۵/۰)۱۹	(۵/۰) ۱
	شیر خام گاو	۳۸	(۸۶/۸) ۳۳	(۱۳/۲)۵
	پنیر سنتی	۲۲	(۹۰/۱) ۲۰	(۹/۹) ۲
	بستنی سنتی	۱۴	(۹۲/۹) ۱۳	(۷/۱) ۱
شیراز	آب لوله کشی شهری	۲۰	(۹۵/۰)۱۹	(۵/۰) ۱
	شیر خام گاو	۳۲	(۹۳/۷) ۳۰	(۶/۳) ۲
	پنیر سنتی	۲۵	(۸۴/۰) ۲۱	(۱۶/۰) ۴
	بستنی سنتی	۱۱	(۱۰۰) ۱۱	(۰/۰) ۰
	تعداد کل نمونه‌ها	۱۸۲	(۹۱/۲)۱۶۶	(۸/۸)۱۶

Table1. Contamination conditions of tap water, milk, cheese, traditional ice cream to H. Pylori by PCR method in Sharekord and Shiraz city of Iran.

City	Studied samples	N.O. of samples	Negative samples	Positive samples (%)
Shahrekord	Tap water	20	19(95)	1(5%)
	Raw cow milk	38	33(86.8)	5(13.2)
	Cheese	22	20(90.1)	2(9.9)
	Traditional ice cream	14	13(92.9)	1(7.1)
Shiraz	Tap water	20	19(95)	1(5)
	Raw cow milk	32	30(93.7)	2(6.3)
	Cheese	25	21(84)	4(16)
	Traditional ice cream	11	11(100)	0(0)
	Total samples	182	166(91.2)	16(8.8)

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