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Detection of *Helicobacter pylori* DNA in Palestinian water samples

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Dedication

To
My Dear Mother, Father, Brothers and Sisters
For their Patience, Encouragement and Support
With Love and Respect

Declaration:

I certify that this thesis submitted for the degree of Master is the result of my own research, except where otherwise acknowledged, and that this thesis or any part of the same has not been submitted for a higher degree to any other university or institution.

Signed ... ... ليندا عطية ....

Linda Abed EL-haleem Atta Atteyeh

Date: 17/01/07

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Abstract

About half of the world's population is estimated to be infected with *Helicobacter pylori* (*H. pylori*), a gastric bacterium that contributes to the development of peptic ulcer disease and gastric cancer. *H. pylori* is more prevalent in low-income areas of the world and social and economic development decreases the prevalence as reflected in comparisons both within and between countries. The infection is typically acquired in early childhood and once established commonly persists throughout life unless treated. The route of transmission is uncertain, but most of the studies suggest person to person transmission this suggested route could be through gastro-oral, oral-oral and fecal-oral routes are likely possibilities. Hence, gastroenteritis may facilitate dissemination of the infection. The community and environment may play additional roles for *H. pylori* transmission in some (low-income) settings. Furthermore, host and bacterial factors may modify the probabilities of acquisition and persistence of the infection. Studies employing microbiological and molecular biological techniques have demonstrated that *H. pylori* has the ability to survive when introduced into water and that *H. pylori* is present in water and other environmental samples all over the world. Epidemiological studies have shown that water source and exposures related to water supply, including factors related to sewage disposal and exposure to animals, are risk factors for infection. Humans and animals shed the bacteria in their faeces and the mechanisms for entry into water, and for survival, ingestion and infection are dependent upon a range of environmental influences. The understanding of *H. pylori* occurrence and transmission is of practical importance if future study deems prevention of the infection desirable in some high-prevalence populations one of the most common human gastrointestinal pathogens.

Molecular biological techniques, for example PCR were employed in many studies to detect the presence of pathogens' DNA in drinking water and other water resources. These

sensitive techniques were utilized at a very narrow scale in research conducted in Palestine. In this study, PCR techniques were employed to detect the presence of *H. pylori* DNA in water samples collected from different geographical areas of Palestine. Primers specific to the 16S rRNA gene and the UreA gene of *H. pylori* were used to amplify microbial DNA extracted from water samples collected from municipal water, groundwater and dug wells. Sample collection was done prior to application of any kind of disinfection procedure to these water samples.

Forty-six water samples were collected from three different regions: seventeen samples from the Jericho district, sixteen samples from the Tulkarem district and thirteen samples from Wadi Al Arroub. *H. pylori* was detected in four (9%) out of the forty-six tested samples. One of the positive samples was collected from ground water wells in the Jericho district and the other three were collected from groundwater in Wadi Al Arroub.

In addition to the major goal of detecting *H. pylori*, this study was expanded to test the presence of other waterborne pathogens; for this purpose, PCR was used to amplify fragments of the wecE gene specific to Enterobacteriaceae and the blaTEM specific for β-lactamase producing Enterobacteriaceae. Twenty-four (52%) out of the forty-six water samples were tested positive for Enterobacteriaceae, while six (13%) of the samples—all from Tulkarem district—were tested positive for β-lactamase producing Enterobacteriaceae. Screening for the presence of 16S rRNA of *Legionella pneumophila* among the forty-six collected water samples returned positive results in two samples (4%) collected from Wadi Al-Arroub. In conclusion, most of the samples was contaminates with one pathogen at least. This indicates the poor microbiologic quality for the water used for human consumption, and irrigation And this needs to be handled with high priority from the Palestinian health ministry to maintain controlled alternative water resources.

ملخص

بكتيريا *Helicobater pylori*هي نوع من البكتيريا الممرضة الأكثر انتشارا وهي مرتبطة بالعديد من الأمراض المتعلقة بالمعدة والأمعاء عند الإنسان، ليس هنالك طريقة معرفة ومتفق عليها لانتقال هذه البكتيريا عند الإنسان. هنالك العديد من الطرق المقترحة مثل الانتقال من شخص الى شخص عن طريق الفم والتي تعتبر أكثر الطرق المقترحة قبولا بين العلماء. بالإضافة إلى الانتقال طريق من شخص الى شخص فإن هناك بعض التقارير الأولية افترضت احتمالية الماء كمصدر أو طريقة لانتقال هذه البكتيريا. و قد اعتمدت هذه التقارير على بعض الدراسات التي أظهرت وجود المادة الوراثية لبكتيريا *H. pylori* في مياه البلدية وفي مصادر أخرى من مياه الشرب.

إن العديد من الدول المتقدمة أخذت باستخدام التقنيات الجزيئية المعتمدة على تفاعل السلسلة المتبلمرة PCR في فحص ودراسة وجود الميكروبات في مياه الشرب. هذه التقنيات الفعالة لم يتم استخدامها في المعاهد الفلسطينية إلا على نطاق ضيق. في هذه الدراسة قمنا باستخدام تقنية تفاعل السلسلة المتبلمرة PCR من اجل فحص وجود المادة الوراثية لبكتيريا *H. pylori* في عينات من الماء جمعت من ثلاث مناطق جغرافية مختلفة من فلسطين. استخدمت بادئات تفاعل (Primers) خاصة بكل من جين 16S rRNA و جين UreA وذلك لمضاعفة قطعة معينة من هذه الجينات من عينات من ال DNA تم استخلاصها من مياه البلدية و المياه الجوفية بالإضافة إلى مياه الآبار المحفورة قبل أن تتعرض هذه العينات لأي نوع من المعالجة بهدف تعقيمها.

جمعت عينات الماء من محافظات أريحا و طولكرم ومن وادي العروب جنوب بيت لحم حيث كان عددها 46 عينة موزعة على النحو الآتي: 17 عينة من محافظة أريحا، 16 عينة من محافظة طولكرم و 13 عينة من وادي العروب. أربع من هذه العينات (9%) أظهرت نتائج ايجابية لوجود *H. pylori*، واحدة من هذه العينات كانت من منطقة طولكرم أما العينات الثلاث المتبقية فكانت من وادي العروب في حين لم يتم العثور على *H. pylori* في أي من العينات السبعة عشر التي جمعت من محافظة أريحا.

بالإضافة إلى الهدف الأساسي من هذه الدراسة والمتمثل في فحص وجود *H. pylori*، تم توسيع هذه الدراسة لتشمل فحص وجود أنواع أخرى من الممرضات الناشئة عن الماء: Enterobacteriaceae و *Legionella pneumophila*. من اجل فحص وجود هذه الممرضات في عينات المياه التي جمعت (46 عينة)، تم استخدام تفاعل السلسلة المتبلمرة PCR لمضاعفة قطع من wecE gene و من جين bla_{TEM} β-lactamase. أربعة وعشرون (52%) من العينات ال46

أظهرت نتائج ايجابية لوجود Enterbacteriaceae بالاعتماد على مضاعفة wecE gene. ستة (9%) من هذه العينات – جميعها من محافظة طولكرم – أظهرت نتائج ايجابية لوجود β-lactamase producing Enterobacteriaceae بالاعتماد على مضاعفة جين. β-lactamase bla_{TEM}. عندما تم فحص وجود *Legionella pneumophila* في عينات المياه المدروسة تم التحقق من وجودها في عينتين (4%) كانت قد جمعت من واد العروب. (78%) من مجموع العينات المدروسة كانت ملوثة ببكتيريا *H. pylor*i وأنواع أخرى من الممرضات الناشئة عن الماء. و هذا أمر مقلق يجب أخذه بعين الاعتبار من قبل السلطات المختصة.

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