

Short Communication

Real Time PCR assay for detection of *Helicobacter pylori* infection and clarithromycin susceptibility in biopsy specimens in southern Denmark

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Keywords

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- Real-Time PCR
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- rapid urease test
- clarithromycin resistant

Abstract

In this study, we have investigated the current situation of *Helicobacter pylori* infection with a real-time PCR assay, our routine culture method, and a rapid urease test in Southern Denmark. 52 patients who came to gastric endoscopy examination on the two hospitals in Southern Denmark were taken to the study. All the biopsy samples were cultured and further examined for antibiotic resistance in our routine methods. A commercial Real-Time PCR assay was employed to detect *H. pylori* DNA from the biopsy samples. 15 of the biopsies from one of the hospitals were selected for investigation using a rapid urease test. Only positive samples were sent for *H. pylori* cultivation/PCR.

Of 52 patients who underwent gastric endoscopy and taken biopsy, 10 were shown culture positive of *H. pylori* and 20 were shown PCR positive. All culture positive samples were also PCR positive. Of 15 urease breath test positive patients, 7 were shown culture positive of *H. pylori* and 14 were shown PCR positive. Of 10 culture positive samples, 2 were shown clarithromycin resistant. These findings show that the Real-Time PCR assay is more sensitive and accurate to detect *H. pylori* than our routine culture method and clarithromycin resistant *H. pylori* has increased in Denmark in last 10 years.

INTRODUCTION

Helicobacter pylori is a ubiquitous pathogen infecting the population in many countries including Scandinavia and *H. pylori* antibiotic resistance is increasing worldwide. The bacterium colonizes the stomach of humans leading to a lifelong chronic gastritis, peptic ulcer disease, and gastric cancer [1-3]. Accurate diagnosis of *H. pylori* infection is a crucial part in the effective management of many gastro duodenal diseases. Various diagnostic methods are developed to detect *H. pylori* infection [4-6]. Among them, the most common used methods in the microbiological laboratory are *H. pylori* culture and rapid urease test. However, more and more laboratories have used Real-Time PCR for detecting *H. pylori* infection in recent years. Each method has its own advantages, disadvantages, and limitations.

The choice of one method or another could be depended on availability and accessibility of diagnostic tests, level of laboratories, clinical conditions of patients, and likelihood ratio of positive and negative tests on different clinical circumstances.

In this study, we have compared a real-time PCR assay with our routine culture method, and in some cases including a rapid urease test, for detection of *H. pylori* infection in biopsy specimens in Southern Denmark. Since antibiotic resistance to *H. pylori* has been increased in last decade and last Danish study on *H. pylori* antimicrobial resistance was more than years ago, the current situation of antimicrobial resistance to *H. pylori* infection has also been investigated.

MATERIALS AND METHODS

Biopsies from 52 patients, who came to gastric endoscopy

examination in the period from March – September 2012 on two locations in Southern Denmark, were included in the study. Mean age of the 52 patients (21M/31F) were 56 years (range 20-82).

The biopsies were rolled on the surface of 5% blood-agar containing three different antibiotics, vancomycin, polymyxin B and trimetoprim, and placed in a tube in the freezer (-80 °C) for later PCR-investigation. Agar plates were incubated 4 days in a microaerophilic atmosphere (6% O₂), 37 °C. Characteristic grey, translucent, urease positive colonies were identified as *H. pylori* and examined using E test for susceptibility to amoxicillin (1 µg/ml), clarithromycin (0.25 µg/ml), tetracycline (4 µg/ml) and metronidazole (8 µg/ml). Numbers in parenthesis are the limits for full sensitivity.

15 of the biopsies from one of the departments were selected for investigation using a rapid urease test (Pronto Dry, Medicoline Aps, Køge, Denmark). Only positive samples were sent for cultivation/PCR.

H. pylori DNA were extracted from biopsy tissues by using a QIAGEN DNeasy® Blood & Tissue Kit (QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany). A novel commercially available Real-Time PCR assay (*Helicobacter pylori* Real-TM, Sacace Biotechnologies, Como, Italy) was employed to this study. This kit is a test for the detection of *H. pylori* DNA in the biopsy samples of stomach mucous membrane, sputum, faces and other biological materials, which contains positive control and internal control. Each reaction tube contains 15 µl of reaction mix and 10 µl of bacterial DNA extraction. Real-Time PCR analysis was performed on a Stratagene MX3005P.

RESULTS AND DISCUSSION

The results in table 1 shows that of 52 patients who underwent gastric endoscopy and taken biopsy, 10 were shown culture positive of *H. pylori* and 20 were shown PCR positive. All culture positive samples were also PCR positive. These results show that specificity of our routine *H. pylori* culture method versus the Real-Time PCR method was 100%, while the sensitivity was 50%. According literatures, culturing of *H. pylori* from gastric biopsy specimen is a highly specific but less sensitive method, even the

experienced laboratories recover the bacterium from only 50% to 70% of actually infected biopsies [7-8]. In general, culturing has almost 100% specificity, but the sensitivity of culture shows significant variation, which is mainly due to it is not easy to culture *H. pylori* from gastric biopsy. PCR provides excellent sensitivity and specificity, greater than 95%, as compared with other conventional tests and has more accurate results of detecting *H. pylori* antibiotic [5].

Table 2 shows that of 15 urease breath test positive patients, 7 of them were shown culture positive of *H. pylori* and 14 were shown PCR positive. These results show that sensitivity of our routine *H. pylori* culture method versus the Urea breath test was also 50 %, but the sensitivity of the Urea breath test versus the Real-Time PCR was 93%. The rapid urease test is more sensitive than routine culture method. This method is based on the production of large amounts of urease enzyme by *H. pylori*, which splits the urea test reagent to form ammonia, enabling its detection by a rapid indirect test. Commercial rapid urease tests typically have specificities above 95%-100% and sensitivity range from 85%-95% [9].

Minimum inhibitory concentration (MIC) of Amoxicillin, Clarithromycin, Metronidazole and Tetracyclin of the ten culture *H. pylori* positive samples were tested. Table 3 shows that two of the ten isolates were clarithromycin resistant and all ten isolates were sensitive to metronidazole, amoxicillin, and tetracyclin. Last Danish study on *H. pylori* antimicrobial resistance was more than years ago, which showed that resistance to clarithromycin was 7% and to metronidazole was 28% [3]. Our data shows that resistance to clarithromycin is 20%. *H. pylori* antibiotic resistance is varied geographically, for example, resistance to clarithromycin in Japan is 86.4% [10] and in Sweden is 1.5% [11]. However, the *H. pylori* antibiotic resistance to clarithromycin has been increased worldwide recent years [12].

In conclusion, the data from our study show that the Real-Time PCR assay and urease assay are more sensitive and accurate to detect *H. pylori* than the routine culture method and there is an clear indication that clarithromycin resistant *H. pylori* is increased in Denmark.

Table 1: Comparing Sacace Real-Time PCR assay and routine culture method on detecting *H. pylori* in 52 gastric biopsy samples.

	PCR Positive	PCR Negative	Total
Culture Positive	10	0	10
Culture negative	10	32	42
Total	20	32	52

Sensitivity: Culture/Real-Time PCR = 50%
 Specificity: Culture/Real-Time PCR = 100%

Table 2: Comparing Sacace Real-Time PCR assay and routine culture method on detecting *H. pylori* in 15 urease test positive gastric biopsy samples.

	PCR Positive	PCR Negative	Total
Culture Positive	7	0	7
Culture negative	7	1	8
Total	14	1	15

Sensitivity: Culture/Urease test = 50%
 Sensitivity: Real-Time PCR /Urease test = 93%

Table 3: Minimum inhibitory concentration (MIC) of Amoxicillin, Clarithromycin, Metronidazole and Tetracyclin of 10 isolates of *H. pylori* by E-test.

No.	Gender	Age (year)	Amoxicillin	Clarithromycin	Metronidazole	Tetracyclin
1	F	71	S	S	S	S
2	M	58	S	S	S	S
3	M	53	S	S	S	S
4	M	56	S	S	S	S
5	F	78	S	S	S	S
6	F	53	S	S	S	S
7	F	57	S	R (32)	S	S
8	M	34	S	S	S	S
9	M	64	S	S	S	S
10	F	56	S	R (>256)	S	S

S: susceptible. Amoxicillin: MIC < 1 µg/ml; Clarithromycin: MIC < 0.25 µg/ml; Metronidazole: MIC < 8 µg/ml; Tetracyclin: MIC < 4 µg/ml.
 R: resistant.
 F: female; M: male.

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REFERENCES

- Sokic-Milutinovic A, Alempijevic T, Milosavljevic T. Role of Helicobacter pylori infection in gastric carcinogenesis: Current knowledge and future directions. *World J Gastroenterol.* 2015; 21: 11654-11672.
- De Francesco V, Giorgio F, Hassan C, Manes G, Vannella L, Panella C, et al. Worldwide *H. pylori* antibiotic resistance: a systematic review. *J Gastrointest Liver Dis.* 2010; 19: 409-414.
- Petersen AM, Gjøde P, Vinge OD, Jensen S, Krogfelt KA. Helicobacter pylori antimicrobial resistance and risk factors in Denmark 1998-2004: no need for concern? *Helicobacter.* 2006; 11: 210-211.
- Lopes AI, Vale FF, Oleastro M. Helicobacter pylori infection - recent developments in diagnosis. *World J Gastroenterol.* 2014; 20: 9299-9313.
- Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SS, et al. Diagnosis of Helicobacter pylori infection: Current options and developments. *World J Gastroenterol.* 2015; 21: 11221-11235.
- Uotani T, Graham DY. Diagnosis of Helicobacter pylori using the rapid urease test. See comment in PubMed Commons below *Ann Transl Med.* 2015; 3: 9.
- Grove DI, Koutsouridis G, Cummins AG. Comparison of culture, histopathology and urease testing for the diagnosis of Helicobacter pylori gastritis and susceptibility to amoxicillin, clarithromycin, metronidazole and tetracycline. *Pathology.* 1998; 30: 183-187.
- Loffeld RJ, Stobberingh E, Flendrig JA, Arends JW. Helicobacter pylori in gastric biopsy specimens. Comparison of culture, modified giemsa stain, and immunohistochemistry. A retrospective study. *J Pathol.* 1991; 165: 69-73.
- Tseng CA, Wang WM, Wu DC. Comparison of the clinical feasibility of three rapid urease tests in the diagnosis of Helicobacter pylori infection. *Dig Dis Sci.* 2005; 50: 449-452.
- Murakami K, Furuta T, Ando T, Nakajima T, Inui Y, Oshima T, Tomita T. Multi-center randomized controlled study to establish the standard third-line regimen for Helicobacter pylori eradication in Japan. *J Gastroenterol.* 2013; 48: 1128-1135.
- Storskrubb T, Aro P, Ronkainen J, Wreiber K, Nyhlin H, Bolling-Sternevald E, et al. Antimicrobial susceptibility of Helicobacter pylori strains in a random adult Swedish population. *Helicobacter.* 2006; 11: 224-230.
- Ghotaslou R, Leylabadlo HE, Asl YM. Prevalence of antibiotic resistance in Helicobacter pylori: A recent literature review. See comment in PubMed Commons below *World J Methodol.* 2015; 5: 164-174.

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