

Culture method for isolation of *Helicobacter pylori* contamination of Endoscopic Units in Basra Hospitals

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ABSTRACT

Background: In this study, the first attempt in Basrah city to isolate and diagnose *H. pylori* from endoscopic units and workers by culture method.

Objective: to isolate and identify *H. pylori* by culture method and conventional tests from possibly contaminated sources and workers in endoscopy units in five hospitals.

Materials and methods: A total of 78 swabs were collected endoscopic units in five hospitals in Basrah city in Iraq namely: Altaalemi, Al-mawani, Al-Basrah general, Al-Shifaa and Al-Fayhaa during the period from September 2011 to June 2012.

Results: A total of 78 swabs, of which 12(15.38%) isolates of *H. pylori* were isolated by culture method and identified by biochemical tests. The highest frequency of *H. pylori* was in Altaalemi 7(29.16%) followed by Al-Shifaa 3(25%) and then Al-mawani 2(14.2%) and zero for the rest. The frequency of positive *H. pylori* in 13 samples from mouth of staff, was 3(23.1%). It was statistically significant ($P<0.05$) using SPSS program. Of the total 10 samples from forceps, 2(20%) showed positive tests for *H. pylori*, the frequency of positive *H. pylori* in 3 samples from sponge, was 2(66.7%), of the total 8 samples from flexible shaft, 2(25%) showed positive tests for *H. pylori*, 6 specimens from flexible cord transmitting light, air and water, 1(16.7%) showed positive tests for *H. pylori*, 5 specimens from Petridis for mouth pieces and 5 specimens from opening for biopsy channel, 1(20%) showed positive tests for *H. pylori*, Of the total 10, 6, 5, 5 and 2 samples from mouth, tables surface, beds, sinks and Pail sterilization respectively, the frequency of *H. pylori* was (0%).

Conclusion: *H. pylori* can be isolated by culture method from endoscopic units and workers and disinfection technique for manual cleaning and disinfection of the instrument may not remove the risk of *H. pylori* transmission.

استخدام طريقة الزرع لعزل جرثومة البوابة المعدية الملوثة لوحدة الناظور في مستشفيات محافظة البصرة

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^١قسم علوم الحياة / كلية العلوم / جامعة البصرة، ^٢فرع الطب الباطني / كلية الطب / جامعة البصرة / ^٣قسم علوم الحياة / كلية التربية / جامعة البصرة. المقدمة: تعد هذه الدراسة هي المحاولة الأولى في محافظة البصرة لعزل وتشخيص جرثومة *H. pylori* المسببة لقرحة المعدة من وحدة الناظور والعاملين بالوحدة بطريقة الزرع.

الهدف: عزل وتشخيص جرثومة *H.pylori* باستخدام طريقة الزرع والاختبارات التقليدية من العاملين والمصادر المحتمل تلوثها في وحدات الناظور لخمسة مستشفيات.

طريقة العمل: تم جمع ٧٨ مسحة من وحدات الناظور في خمسة مستشفيات في محافظة البصرة- العراق وكما يلي: م. التعليمي و م. الموائى و م. البصرة العام و م. الشفاء و م. الفيحاء خلال الفترة من شهر أيلول ٢٠١١ الى حزيران ٢٠١٢.

النتائج: من ٧٨ مسحة تم عزل ١٢ (١٥,٣٨%) عذلة من جرثومة *H.pylori* وشخصت باستخدام الاختبارات الكيموحيوية. أظهرت الدراسة إن أعلى نسبة عزل للجرثومة كانت في م. التعليمي (٢٩,١٦%) تلتها م. الشفاء (٢٥%) ثم م. الموائى (١٤,٢%) في حين لم تعزل في المستشفيات المتبقية. أظهرت نتائج العزل والتشخيص تردد جرثومة *H. pylori* بنسبة (٢٣,١%) من ١٣ عينة من العاملين بوحدة الناظور وبفارق معنوي ($P<0.05$) باستخدام البرنامج الإحصائي SPSS. وتم عزل جرثومة *H. pylori* بنسبة (٢٠%) من ١٠ عينات من الملاقط و (٦٦,٧%) من ٣ عينات من أسفنجة غسل الملاقط و (٢٥%) من ٨ عينات من العمود المرن و (١٦,٧%) من ٦ عينات من العمود

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الممرن الناقل للماء والهواء وبنسبة ١(٢٠%) من ٥ عينات من وعاء تنظيف آلة فتح الفم و ٥ عينات من فتحة إدخال سلك الملقط، ولم تعزل الجرثومة (صفر%) من ١٠ و ٦ و ٥ و ٥ عينات مأخوذة من آلة فتح الفم و سطوح المناضد والأسرة وأحواض الغسل و إناء التعقيم على التوالي. الاستنتاجات: عزل جرثومة *H. pylori* بطريقة الزرع من وحدات الناظور والعاملين فيها وان الطريقة البدوية لتنظيف وتعقيم الجهاز ربما لا تزال خطورة انتقال جرثومة *H. pylori*.

INTRODUCTION

There are reports of a high prevalence of *H. pylori* infections in institutions for people with intellectual disability and health care workers in these institutions because of their close contact.^[1] *H. pylori* inhabits the gastric mucosa, consequently, the endoscopes used to perform gastroscopies on patients affected become contaminated. Infection can be passed on to other patients via these endoscopes. *H. pylori* infection is more commonly found in gastroenterologists, endoscopy staff, intensive care nurses, groups of healthcare workers, and those caring for developmentally disabled individuals.^[2-4] The first recorded nosocomial infection with *H. pylori* was reported in 17 of 37 healthy subjects who took part in a study on acid concentrations in the stomach developed gastritis after an endoscopy.^[5] Study of Nurnberg, *et al.*,^[5] concluded that endoscopes are frequently contaminated with *H. pylori* immediately after gastro duodenal endoscopy in *H. pylori*-infected patients. Although, appropriate decontamination can certainly prevent transmission via this route.^[5,6] Iatrogenic transmission is possible, as *H. pylori* can survive manual cleaning and disinfection with 2% glutaraldehyde, particularly when there is ineffective cleaning before disinfection. De Schryver and Van Winckel,^[7] make survey for a literature search was performed using Pubmed (January 1990 to May 2001), various pathways of agent transmission, favoring person-to-person mode of transmission early in life. Faeco-oral, oral-oral and gastro-oral transmissions are proposed and may be of different relevance among various populations. Screening for colorectal cancer (CRC) by gastrointestinal endoscopy (flexible sigmoidoscopy or colonoscopy) has

been recommended for the average-risk population.^[8] The endoscopes have to be cleaned manually and/or by using automatic washing machines (best) especially designed for this purpose as the design and material of the endoscopes do not allow sterilization. Cleaning and disinfection of the devices has been a subject of concern, as transmission of infectious material cannot be entirely dismissed.^[9] *H. pylori* can be identified by various methods such as culture, urease breath test, and histological studies of biopsy specimens; however most of these methods are invasive and non-invasive include Serologic methods and PCR.

Aim: the objective of the present study was to isolate and identify *H. pylori* by culture method and conventional tests from possibly contaminated sources and workers in endoscopy units in five hospitals.

MATERIAL AND METHODS

Sample collection

A total of 78 swabs were collected from endoscopic units in five hospitals in Basrah city south Iraq namely: Altaalemi, Al-mawani, Al-Basrah general, Al-shifaa and Al-Fayhaa during the period from September 2011 to June 2012. These samples include 10 from forceps, 3 from sponge for washing, 8 from flexible shaft, 6 from flexible cord transmitting light, air and water, 10 from mouth piece, 5 from petridish for mouth pieces, 5 from opening for biopsy channel, 13 from mouth of staff, 6 from tables surface, 5 from beds, 5 from sink and 2 from sterilization pail. All swabs were cultured by streaking on chocolate and Modified Columbia Urea Agar (MCUA) medium^[10] and incubated in microaerophilic condition in anaerobic jar at 37 C° for 5-7 days.

The ways of disinfection in Al-Mawania, Al-Basrah and Al-Shifaa hospitals using medical gauze to clean forcipes once and then thrown it away except Al-sadr Altaalemi and Al-Fayhaa hospital using sponge instead of gauze .

Isolation and identification

The suspected purified colonies were chosen according to Gram staining and cultural characteristics and tested for the production of catalase, oxidase and urease.

Statistical analysis: Data were analyzed using SPSS program for window (version 10).

Culture result: This is the first attempt in Basrah city to isolate and diagnose *H. pylori* from endoscopic units and workers by culture method.

From a total of 78 swabs, of which 12(15.38%) isolates of *H. pylori* were isolated by culture method and identified by biochemical tests. From 24 samples from Altaalemi hospital, *H.pylori* was isolated in 7(29.16%), 14 samples from Al-mawani hospital, *H. pylori* was isolated in 2(14.2%), 12 samples from Al-Shifaa hospital, *H. pylori* was isolated in 3(25%), 13 and 15 samples from Al-Basrah and Al-Fayhaa hospital respectively, *H. pylori* was not isolated (0%) (table-1).

RESULTS

Table 1. Number and percentage of *H. pylori* isolates from twelve different sources in endoscopic units in five hospitals.

Sample source and number	Hospital									
	Al Altaalemi	Hp +	Al-Mawania	Hp +	Al-Basrah	Hp +	Al-Shifaa	Hp +	Al-Fayhaa	Hp +
forceps (10)	3	2	2	0	2	0	1	0	2	0
Sponge for washing (3)	2	2	0	0	0	0	0	0	1	0
Flexible shaft (8)	2	0	2	1	1	0	2	1	1	0
Flexible cord transmitting light ,air and water (6)	2	0	1	1	1	0	1	0	1	0
Mouth piece (10)	2	0	2	0	2	0	2	0	2	0
Petridis for mouth piece (5)	1	0	1	0	1	0	1	1	1	0
Opening for biopsy channel(5)	1	0	1	0	1	0	1	1	1	0
mouth of Staff (13)	6	3	2	0	2	0	1	0	2	0
Tables surface (6)	2	0	1	0	1	0	1	0	1	0
Bed (5)	1	0	1	0	1	0	1	0	1	0
Sink (5)	1	0	1	0	1	0	1	0	1	0
Pail sterilization (2)	1	0	0	0	0	0	0	0	1	0
Total =78 Hp+ =12(15.38%)	24	7 29.1 6%	14	2 14.2%	13	0 0%	12	3 25%	15	0 0%

Hp+: *H. pylori* positive

Al-Mawani, Al-Basrah general and Al-Shifaa hospitals not using sponge for cleaning.

The highest frequency of *H. pylori* was in Shifaa3(25%) and then Al-Mawani 2(14.2%) Altaalemi 7(29.16%) followed by Al- and zero for the rest. The colonies of *H. pylori*

were on MCUA medium small to middle in size, rounded and creamy in color, *H. pylori* on chocolate agar were translucent, rounded and tiny size. All *H. pylori* isolates were Gram negative, spiral, rods, or curved in shape. The biochemical tests performed to confirm the identity of *H.pylori* includ: Oxidase, catalase and urease which were positive in all.

Frequency of *H. pylori* according to the source of swab

Of the total 10 samples from forcipes, 2(20%) showed positive tests for *H. pylori*, but the difference was statistically not significant (P>0.05). The frequency of positive *H. pylori* in 3 samples from sponge for washing, was 2(66.7%) but it was statistically not significant (P>0.05). Of the total 8 samples from flexible shaft, 2(25%) showed positive tests for *H.*

pylori, but the difference was statistically not significant (P>0.05). 6 specimens from flexible cord transmitting light ,air and water, 1(16.7%) showed positive tests for *H. pylori*, but the difference was statistically not significant (P>0.05). 5 specimens from Petridis for mouth pieces and 5 specimens from opening for biopsy channel, 1(20%) showed positive tests for *H. pylori*, but the difference was statistically not significant (P>0.05). The frequency of positive *H. pylori* in 13 samples from staff, was 3(23.1%). It was statistically significant (P<0.05). Of the total 10, 6, 5, 5 and 2 samples from mouth piece, table surface, beds, sink and Pail sterilization respectively, the frequency of *H. pylori* was (0%) and the results show no significant (P>0.05) (table 2).

Table 2. frequency of *H.pylori* in twelve different source in endoscopic units.

Specimen Source	Hp +ve N (%)	Hp -ve N(%)	Mean	Std. Deviation	Variance	Chi-square	P-value
Forceps	2 (20%)	8(80%)	1.80	0.42	0.18	3.60	NS
Sponge for washing	2(66.7%)	1(33.3%)	1.33	0.58	0.33	0.33	NS
Flexible shaft	2(25%)	6(75%)	1.75	0.46	0.21	2.00	NS
Flexible cord transmitting light ,air and water	1(16.7%)	5(83%)	1.83	0.41	0.17	2.67	NS
Mouth piece	0(0.00%)	10(100%)	2.00	0.00	0.00	Cannot be performed	
Petridish for mouth pieces	1(20%)	4(80%)	1.80	1.80	0.20	1.80	NS
Opening for biopsy channel	1(20%)	4(80%)	1.80	0.45	0.47	1.80	NS
mouth of staff	3(23.1%)	10 (76.9%)	1.77	0.44	0.19	3.77	0.05
Tables surface	0(0.00%)	6(100%)	2.00	0.00	0.00	Cannot be performed	
Bed	0(0.00%)	5(100%)	2.00	0.00	0.00	Cannot be performed	
Sink	0(0.00%)	5(100%)	2.00	0.00	0.00	Cannot be performed	
sterilization Pail	0(0.00%)	2(100%)	2.00	0.00	0.00	Cannot be performed	

NS: no significant

DISCUSSION

Many studies have been performed about the contamination of endoscopic units by *H. pylori*, but no study has been done in Iraq specially in Basrah up to our knowledge, so the results of the present study will be compared with studies done in other countries. *H. pylori* is distributed worldwide and is found in developing countries in particular.^[11] Although there is much information about *H. pylori* infection, several aspects of the pathogenesis and epidemiology of this organism remains unclear.^[12] The transmission route of *H. pylori* infection has been the topic of several studies. Most infections are probably acquired in childhood, mainly via oral-oral or fecal-oral routes.^[13] Patients harboring *H. pylori* was reported by Al-Sulami, *et al*^[14] and it was also isolated from drinking water by culture method only^[15] and another study of Al-Sulami, *et al*^[16] showed *H. pylori* has been isolated and diagnosed from drinking water by culture method and a combination of biochemical and PCR test. In present study, *H. pylori* was isolated by culture method from different sources in endoscopic units and workers. Contamination of endoscopes may be due to inappropriate procedure of cleaning hygiene and sterilization or individual failure to adhere to the procedures. The frequency of *H. pylori* isolates was different from one hospital to another, the high percentage of *H. pylori* was 7(29.16%) in Altaalemi, 3(25%) in Al-shifaa, 2(14.2%) in Al-mawani, but there were no isolates from Al-Basrah and Al-Fayhaa hospital respectively, because of the use of more hygienic way of cleaning of the scope after use by disposable gauze instead of the same dish and sponge for all patients. The frequency of positive *H. pylori* in 3 samples from sponge in Al-sadr Altaalemi and Al-Fayhaa hospital, was 2(66.7%), reflecting the use of clean and forcipes sponge which represent an important source of pollution by *H. pylori*. Not taken samples of the sponge in Al-Mawania, Al-Basrah and Al-Shifaa hospitals

because they are using medical gauze to clean forcipes once and then thrown it away. The frequency of *H. pylori* was 0(0%) from mouth pieces, tables surface, bed, sink and Pail sterilization and the results showed no significant ($P>0.05$). Staff was an important source for contamination, the frequency of positive *H. pylori* in 13 samples from mouth of staff in Altaalemi hospital, was 3(23.1%), a statistically significant difference ($P<0.05$). Metanat, *et al.*,^[17] found the total frequency of *H. pylori* infection was 34% among healthcare workers in Zahedan, that is lower when compared to studies performed in other cities in Iran. Mastromarino, *et al.*,^[18] concluded that nurses had a significant higher prevalence of *H. pylori* infection ($P<0.01$). Of the total 10 samples from forceps, 2(20%) showed positive tests for *H. pylori*, the frequency of positive *H. pylori* in 3 samples from sponge for washing, was 2(66.7%), of the total 8 samples from flexible shaft, 2(25%) showed positive tests for *H. pylori*, 6 specimens from flexible cord transmitting light, air and water, 1(16.7%) showed positive tests for *H. pylori*, 5 specimens from Petridis of mouth and 5 specimens from opening for biopsy channel, 1(20%) showed positive tests for *H. pylori*. The frequency of positive *H. pylori* in 13 samples from mouth of staff, was 3(23.1%). Matysiak-Budnik^[19] showed an association between occupational exposure and an increased risk of infection. Williams,^[20] too, stated that there were increased occupational risks for endoscopy personnel. However, the evidence in this review appears contradictory, since the findings varied between no risk and a five times greater risk. De Schryver *et al.*,^[21] were able to show in their reviews increased risks for gastroenterologists and endoscopy personnel. Magalhaes Queiroz^[22] found controversial data on the occupational risk, but they considered only some of the studies also included here for gastroenterological personnel. Hildebrand, *et*

al.,^[23] concluded that gastroenterologists have a high risk of becoming infected with *Helicobacter pylori*, claiming the potential role of saliva or gastric juice droplets during endoscopy. Endoscopes are often cleaned and disinfected only manually in between the collection of gastric tissue specimens,^[24] on the other hand, gastric biopsy specimens may have been contaminated with *H. pylori* DNA remaining behind in the biopsy channels of the fiberoptic endoscopes. Indeed, it has been reported that patients can be infected with *H. pylori* through gastrointestinal equipment which is not properly disinfected^[25] *H. pylori* DNA has been detected by PCR in fluid flushed from endoscopes channels after cleaning and disinfection with 2% glutaraldehyde.^[26] Nurnberg, *et al.*,^[5] showed one of the 128 rinsing samples (0.8%) was found to be contaminated with *H. pylori* even after routine manual cleaning and disinfection-indicating that these cleaning and disinfection procedures may be insufficient to eradicate *H.pylori* from endoscopes completely.

Conclusions

1. *H. pylori* can be isolated by culture method from endoscopic units and workers .
2. Gastroenterological personnel are exposed to an increased risk of *H. pylori* infection.
3. Disinfection technique for manual cleaning and disinfection of the instrument may not remove the risk of *H. pylori* transmission and may be other bacteria and viruses.

Recommendations

- Use of the international way of cleaning of endoscopic and its accessories by washing machine after cleaning with medical gauze instead of sponge for washing forceps.
- Using mask and glasses to protect against *H. pylori* infection for this and other viral infection of medical personnel and prevent it transmission to the patients.

- For the future, use of disposable biopsy forceps.
- Use of disposable patients bed and covered sheets.
- Use of disposable mouth pieces.

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