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Studies on *Helicobacter* from Human Stomachs in Taiz City, Republic of Yemen

Abstract:Forty-one bacterial isolates were obtained from gastric juice and gastric biopsy specimens from persons 30-70 years old. The samples were taken from patients who chewed qat (the fresh leaves of the plant *Catha edulis*) and patients who didn't chew qat. The specimens were collected at different hospitals in Taiz city, Republic of Yemen. These bacterial isolates were identified as four species: *Helicobacter pylori*, *Helicobacter bizzozeronii*, *Campylobacter jejuni* and *Campylobacter sputorum*. *H. pylori* was recorded as occurring in a higher percentage of qat chewers (42%) than in non chewers (25%), in gastric biopsies (37%) than in gastric juice specimens (20%) and in those aged from 50-70 years (35%) than in those aged from 30-50 years (33%). Antibacterial activity of Christ's thorn honey and garlic, onion, and cabbage extracts against 14 isolates of *H. pylori* revealed that the most promising effect was achieved with Christ's thorn honey followed by garlic, onion and cabbage extracts. The antibacterial effect of garlic and onion at concentration 15% increased by mixing it with honey in a ratio 1:1.

Keywords: *Helicobacter*, *Campylobacter*, qat, Yemen

Introduction

The discovery of *Helicobacter pylori* in Western Australia in 1983 (Marshall and Warren, 1983) not only introduced a whole new group of bacteria to science, but also revolutionized our concept of gastroduodenal pathology in particular peptic ulcer diseases. The historical associations between bacteria and peptic ulcer disease are described by Rathbone and Heatley (1992). *H. pylori* has been associated with peptic ulcer diseases and cancers of the human gastrointestinal tract (Versalovic and Fox, 1999). Chronic *Helicobacter pylori* are reduced with *Allium* vegetable intake (Gara, et al. 2000).

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دراسات على بكتيريا الهليكوبكتير المعزولة من المعدة
لمرضى في مدينة تعز، الجمهورية اليمنية

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المستخلص: تم في هذه الدراسة عزل واحد وأربعون سلالة بكتيرية من عينات نسيجية، وأخرى من العصير المعدي لأشخاص تتراوح أعمارهم من 30 - 70 عاماً، يمزج بعضهم الأوراق الخضريه لنبات القات (كاسا ايدولوس) يومياً والبعض الآخر لا يمارس هذه العادة. صنفت السلالات البكتيرية المعزولة إلى أربعة أنواع هي هليكوبكتير بيلورى، هليكوبكتير بيزوزيروناى، كمبيلوبكتير جيجوناي، وكمبيلوبكتير سبيوتورم. سجلت الهليكوبكتير بيلورى نسب عالية في الأشخاص ماضغي القات وفي العينات النسيجية وفي الأعمار من 50 - 70 سنة. تم دراسة تأثير عسل نحل العلب اليمني وتأثير المستخلص المائي لنبات الثوم، البصل، الكرنب على أربعة عشر سلالة للهليكوبكتير بيلورى، حيث أعطى العسل أعلى تأثير يليه تأثير مستخلص نبات الثوم، البصل والكرنب على التوالي، بينما إرتفع تأثير مستخلص الثوم والبصل مع إضافة العسل إليهم بنسبة 1:1.

كلمات مدخلية: هليكوبكتير، كمبيلوبكتير، بكتيريا، القات، اليمن.

The Yemeni habit of chewing the fresh leaves of the plant *Catha edulis* produces mild cerebral stimulation. In addition, the immune response to *H. pylori* colonization decreases in Yemeni patients who regularly chew qat (El-Guneid, et al. 1991).

The objectives of the present study were to (i) isolate the common bacteria from the upper alimentary tract from patients suffering from stomach or stomach and duodenal ulcers; (ii) study the effect of some natural plant extracts and Christ's thorn honey on different isolates of *H. pylori*.

Materials and Methods

Twelve gastric juice and eight gastric biopsy specimens were taken from human patients with either stomach ulcer or stomach and duodenal ulcer by endoscopy. Specimens were cultured fresh and not delayed in transport for more than two hours. During the culturing process the specimens were

Characteristics	Bacterial isolates															
	402	501	502	503	601	602	701	702	703	801	802	803	901	902	1001	1002
Growth in the air	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Microaerophilic at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 42°C	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-
Curved rod-shape	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram stain reaction	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease production	+	-	-	-	+	-	-	+	-	-	+	-	+	-	+	+
Catalase production	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+	+
Nitrate reduction	-	+	-	+	-	-	+	+	+	-	-	+	-	+	+	-
Oxidase production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H ₂ S production	-	+	-	+	-	-	+	-	+	-	-	+	-	+	-	-
Growth in presence of 1% glycine	-	+	+	+	-	+	+	+	+	+	-	+	-	+	+	-
Susceptibility to :-																
Nalidixic acid	R	R	S	R	R	S	R	R	R	S	R	R	R	R	R	R
Ciprofloxacin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Cephardine	I	R	R	R	R	I	R	R	R	R	R	R	R	R	I	R

Characteristics	Bacterial isolates					
	1301	1401	1501	1502	1601	1602
Growth in the air		-	-		-	-
Microaerophilic at 37°C	+	+	+		+	+
Growth at 42°C	-	-	+	-	-	-
Curved rod-shape	+	+	+	+	+	+
Gram stain reaction	-ve	-ve	-ve	-ve	-ve	-ve
Motility	+	+	+	+	+	+
Urease production	+	+	-	+	+	-
Catalase production	+	+	+	+	+	-
Nitrate reduction	-	-	-	-	-	+
Oxidase production	+	+	+	+	+	+
H ₂ S production	+	-	-	-	-	+
Growth in presence of 1% glycine	-	-	+	-	-	+
Susceptibility to :-						
Nalidixic acid	R	R	S	R	R	R
Ciprofloxacin	S	S	S	S	S	S
Cephardine	R	R	R	R	I	R

+ = growth - = no growth -ve = negative S = susceptible R = resistant I = intermediate

H. pylori was originally named *Campylobacter pyloridis*; however, its name was changed to *C. pylori* and later transferred by Goodwin and Armstrong (1989) to *Helicobacter* as the type species of the genus.

H. bizzozeronii has been cultured from dogs by Hanninen and Happonen (1997) and it is morphologically indistinguishable from *H. heilmannii*, which colonize the human stomach and are associated with gastritis. The two organisms may be the same (Nachamkin and Skirrow, 1998).

A new subspecies of *C. jejuni* has been isolated from human clinical specimens including gastric epithelium biopsies (Kasper and Dickgiesser, 1985). *C. sputorum* was also recovered from the oral cavity and gingival cervices of humans. The organism was not recognized as an agent of human disease, although a few clinical isolates have been reported (Koneman, *et al.* 1997).

The incidence of *H. pylori* in the gastric biopsy specimens was higher than that in gastric juice specimens. *H. pylori* were recorded more in patients aged from 50–70 years than patients aged from 30–50 years (Table 2). In developed countries prevalence of *H. pylori* increases with age. (Nachamkin and Skirrow, 1998). Occurrence of *H. pylori* in qat chewers was higher than in non-chewers with the high percentage of *H. pylori* in qat chewers being 42% while it was 25% in non-chewers. El-Guneid, *et al.* (1991) recorded that one of the striking findings of his study was the presence of *H. pylori* in the lower end of the esophagus in all 12 cases that reported the intake of qat. The daily intake of qat was associated with a higher prevalence of duodenal ulcer.

Table 2: Percentage of *Helicobacter pylori* in relation to number of bacterial strains.

Sources of bacterial strains	Total No.	No of <i>H. pylori</i>	% of <i>H. pylori</i>
Gastric juices	25	5	20%
Gastric biopsies	16	6	37%
Qat chewing	21	9	42%
Non Qat chewing	20	5	25%
Persons 30-50 years	18	6	33%
Persons 50-70 years	23	8	35%

Antibacterial activity of the onion, garlic and cabbage extracts against the 14 strains of *H. pylori* was observed. 30% concentration of onion and garlic revealed higher activity than 15% concentration (Table 2). The antibacterial effect of

garlic was relatively higher than that of onion while cabbage recorded low antibacterial potentiality compared with garlic and onion (Table 3).

Table 3: Antibacterial activities of garlic, onion and cabbage extracts against 14 strains of *Helicobacter pylori*.

Isolates of <i>H. pylori</i>	Inhibition zone (mm)				
	Onion 15%	Onion 30%	Garlic 15%	Garlic 30%	Cabbage 30%
101	10.5	22.0	13.0	24.0	14.5
203	12.0	22.5	11.0	22.5	14.5
301	11.5	23.0	11.4	22.5	13.5
402	11.0	24.5	11.4	22.5	13.5
601	11.5	23.0	11.5	23.0	12.5
802	10.5	21.5	12.3	24.0	13.0
901	11.5	22.0	13.4	26.0	14.5
902	12.0	23.0	13.6	16.5	13.5
1002	12.0	23.5	14.0	26.0	12.5
1201	11.0	22.5	12.0	24.0	12.5
1301	11.0	22.0	12.5	23.5	13.5
1401	10.5	22.5	11.0	22.5	14.5
1502	11.0	23.0	10.6	22.0	14.5
1601	10.5	22.5	11.3	22.5	12.5

Inhibition zone diameters mm; original diameter = 5mm)

Potentiality of medicinal plants against tested organisms refers to the nature of the active constituent or constituents of the crude extraction obtained from medicinal plant (Higazey, *et al.* 1997, Moreira, *et al.* 1997, Khilare and Gangawane, 1997 and Rastogi, *et al.* 1998). Several studies have reported that garlic extract possesses potent activity against *H. pylori* (Cellini, *et al.* 1996, Sivam, *et al.* 1997, Ohta, *et al.* 1999, Wet, *et al.* 1999, Gara, *et al.* 2000).

Anti-*H. pylori* activity of Christ's thorn honey, a mixture of garlic extract with honey and a mixture of onion extract with honey was observed (Table 3). The Christ's thorn honey showed excellent anti-*H. pylori* effect followed by the effect of honey-garlic mixture and effect of honey-onion mixture respectively (Table 3). The potential effect of garlic and onion against the 14 strains of *H. pylori* increased by mixing with Christ's thorn honey. Osato, *et al.* (1999) recorded that honey from New Zealand and Saudi Arabia at concentrations approximating 20% (v/v) inhibits the growth of *H. pylori in vitro*. Molan (1996) reported that 5% manuka honey solution halted the growth of strains of *H. pylori* isolated from samples of stomach ulcers. One antibacterial substance in the honey is

hydrogen peroxide, but it was found in tests on 26 honeys that two from vipers' bugloss and manuka had antibacterial properties even when hydrogen peroxide had been removed (Somal, *et al.* 1994).

As demonstrated by the excellent anti *H. pylori* effect of Christ's thorn honey and garlic extract that was achieved in this research, it can be recommended that the patients suffering from stomach ulcer or stomach and duodenal ulcer can eat Christ's thorn honey and garlic extract to reduce the ulcers.

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