

# Patterns of colonisation of *Campylobacter pylori* in the oesophagus, stomach and duodenum

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**SUMMARY** Thirty five subjects underwent upper gastrointestinal endoscopy and multiple biopsy (30 patients, five normal subjects). A total of 11 biopsies per subject from four sites (oesophagus (three), gastric body (two), antrum (three), duodenum (three)) were examined for inflammation and the presence of *Campylobacter pylori* and using standard methods of culture and by light (LM) and electron microscopy (EM). The organism was cultured from oesophageal biopsies in eight of 30 (27%) patients but could not be identified at this site by LM or EM. There was evidence of oesophageal inflammation in 20 patients which was associated with the local finding of *C pylori* in five (25%) including two of seven (29%) with Barrett's mucosa. Antral *C pylori* was present in 22 of 23 (96%) patients with chronic active gastritis. The organism was found in the antrum and oesophagus in four of 22 patients (18%), in the antrum and duodenum in four of 22 patients (18%) and in all three sites in a further two of 22 patients (9%). Antral *C pylori* was found in five of six patients with peptic ulceration. *C pylori* was cultured from the duodenum in six patients with confirmation by LN and EM in three, but only on areas of gastric metaplasia. The organism was not found in the normal group. This study indicates that *C pylori* may be irregularly isolated from the oesophagus and duodenum in patients with antral *C pylori* and chronic active gastritis. The role of *C pylori* in the oesophagus is most likely that of a commensal or contaminant.

Since the isolation of *C pylori* in 1982<sup>1</sup> there has been renewed interest in a possible bacterial cause for upper gastrointestinal disease. It is now generally accepted that the presence of this organism in the gastric antrum is frequently associated with histological gastritis and peptic ulceration.<sup>2</sup> Current evidence supports *C pylori* as being a cause of gastritis but its role in the pathogenesis of ulcers is less clear.<sup>3</sup> As diseases of the upper gastrointestinal tract such as duodenal ulceration (DU) and gastro-oesophageal reflux (GOR) frequently coexist<sup>4</sup> it may be that in some cases the unknown link could be *C pylori*. This has not been formally investigated as most trials have tended to concentrate on the

organism at one site<sup>5-10</sup> or in a specific condition.<sup>11,12</sup> Similarly the role of *C pylori* in the oesophagus is unclear. It was originally thought not to colonise this site<sup>13</sup> but this has recently been questioned.<sup>14,15</sup> The aim of this study was to prospectively investigate patterns of isolation of *C pylori* in the oesophagus, stomach, and duodenum in a mixed group of patients.

## Methods

### PATIENTS

Forty one consecutive dyspeptic subjects referred for oesophagogastroduodenoscopy (OGD) gave informed consent to take part in this study. Thirty five successfully underwent endoscopy, biopsy, and laboratory assessment and form the study group (Table 1). The subjects were subsequently divided for comparison into a patient group (n=30) and

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Table 1 Endoscopic findings of the study population

Normal group (On endoscopy and histology)	n
Endoscopic appearance of patient group	30
Oesophagitis	6
Peptic ulcer plus oesophagitis	2 (DU=1, GU=1)
Barrett's oesophagus	6
Barrett's plus adenocarcinoma	1
Hiatus hernia	1
Duodenal ulcer	2
Gastric ulcer	2
Carcinoma of stomach	1
Normal	9

a normal group (n=five) based on presence or absence of any histological inflammation or endoscopic abnormality. The mean age in the patient group (15 men) was 55 years (range 22–74) and in the normal group (four men) was 53 years (range 35–72). This study received ethical committee approval.

#### ENDOSCOPY AND BIOPSY

Endoscopy was performed under sedation (iv Diazemuls) and topical throat spray (Xylocaine) using the Olympus XQ 10 endoscope. From each subject a total of 11 biopsies were taken from four sites: oesophagus (three), body of stomach (two), antrum (three), first part of duodenum (three). Biopsies were taken on 'first pass' starting with the oesophagus. Separate biopsy forceps were used for each site. Oesophageal biopsies were taken 5 cm above the gastro-oesophageal junction and in the case of Barrett's oesophagus to include an area below the squamocolumnar junction. Antral biopsies were taken 5 cm proximal and duodenal biopsies 1 cm distal to the pylorus. Samples were immediately transported to the laboratory in a suitable medium and bacteriological plating was done within one hour. Any apparent discomfort for the subject led to the procedure being abandoned.

The endoscopes and forceps were sterilised between subjects using glutaraldehyde and then washed with tap water. Checks for bacterial contamination after cleaning were negative.<sup>16</sup> All specimens in this study were submitted 'blind' for analysis.

#### MICROBIOLOGICAL INVESTIGATION

Crushed biopsies from the oesophagus, antrum and duodenum were inoculated onto two plates containing brain-heart infusion agar plus 7% horse blood and 1% Isovitalex (BBL Microbiology Systems). Plate 1 contained vancomycin, trimethoprim and polymyxin B (Skirrow's oxid medium) and plate 2 amphotericin (2 mg/l), nalidixic acid (20 mg/l) and vancomycin (6 mg/l). Culture was carried out under

microaerobic conditions (Campypak-BBL Microbiology Systems). The plates were inspected on the third, fifth, and seventh day. Identification of *C pylori* was by colony morphology, Gram staining, and testing for the presence of the enzymes oxidase and urease. The amount of growth was estimated visually using a scale of + to +++++. Any amount of growth was viewed as a positive result.

#### HISTOLOGICAL INVESTIGATION

Specimens from the oesophagus, body of stomach, antrum and duodenum were fixed in 10% neutral buffered formalin and 3 µm sections cut. Staining was with haematoxylin and eosin, periodic acid schiff/alcian blue and a modified Giemsa stain.<sup>17</sup> Biopsies were examined by LM for the presence of the organism. Active inflammation was recorded as mild, moderate or severe depending on the number of neutrophil polymorphs present in the lamina propria and epithelial surface (+ to +++). Barrett's oesophagus was diagnosed by endoscopy and histological confirmation of the presence of columnar epithelium.

#### ELECTRON MICROSCOPY

Fresh biopsies from all four sites were fixed in 3% glutaraldehyde in a 0.15 M cacodylate buffer for 24 hours and then post fixed in 1% osmium tetroxide for one hour. The tissue was embedded in epoxy resin and semi-thin sections cut and stained with 0.5% toluidine blue. Selected specimens had ultra-thin sections cut and stained by 2% uranylacetate and lead citrate. Sections of interest, in particular from the oesophagus and duodenum, were examined using the Corinth 500 electron microscope to confirm the results of LM.

#### SOURCE OF *C PYLORI* IN THE OESOPHAGUS

In an attempt to discover whether oesophageal *C pylori* originates from a reservoir in the stomach or from the oropharynx, six of eight subjects with a positive oesophageal culture reattended four to six weeks later for a buccal smear. The inside of the mouth, cheek and gums were swabbed and cultured in a similar manner to the biopsy samples. Results were compared with those obtained from 30 asymptomatic laboratory staff.

#### Results

The endoscopic appearance of all subjects is shown in Table 1.

#### *C PYLORI* IN THE GASTRIC ANTRUM AND BODY

Twenty three patients had histological evidence of chronic active gastritis in the antrum which was

Table 2 Results of endoscopy, culture and histological examination in patients found to have *C pylori* at sites other than the stomach

Patient	Endoscopic appearance	Histology ( <i>C pylori</i> : CP →+++ No. of organisms Inflammation: inflm →+++)				Culture (Estimate of CP growth +→+++++) Oes/Antr/Duo		
		Oesophagus CP/inflm	Body Cp/inflm	Antrum CP/inflm	Duodenum CP/inflm	Oes	Antr	Duo
A	Normal	-	-	-	+	++	-	-
B	Oesophagitis	-	+	-	-	+++	-	-
C	Barrett's	-	-	-	++	+	+	-
D	Oesophagitis	-	-	+	+	+	++	-
E	GU	-	+	-	-	++	+	++++
F	Barrett's	-	+	-	-	++	+	+++
G	DU	-	-	-	-	++	+	++++
H	DU	-	-	-	-	-	+	+
I	H hernia	-	+	+	-	+	+	++
J	Oesophagitis	-	+	+++	+	+++	++	-
K	Oes and DU	-	-	+	+	+	++	++++
L	Normal	-	-	-	+	+	-	+

associated with the presence of *C pylori* in 22 (96%). The organism was seen by LM in 21 of 22 (95%), cultured in 20 of 22 (91%), with concordance occurring between both examinations in 19 (86%). The antrum was the source of the heaviest growths of the organism. In the body of the stomach *C pylori* was identified by light microscopy in 16 patients being associated with local histological inflammation in 12 (75%). The presence of the organism in the body of the stomach was always associated with antral *C pylori*.

#### ASSOCIATION OF ANTRAL *C PYLORI* WITH THE ORGANISM AT OTHER SITES

*C pylori* was isolated both from the antrum and oesophagus in four of 22 patients (18%), from the antrum and duodenum in four of 22 patients (18%) and from all three sites (antrum, oesophagus, duodenum) in a further two of 22 patients (9%) (Table 2). Whenever duodenal *C pylori* was found it was always associated with antral *C pylori*. Conversely, two patients with oesophageal *C pylori* had no evidence of the organism at any other site. *C pylori* was not found in the histologically and endoscopically normal group.

#### *C PYLORI* IN THE OESOPHAGUS

*C pylori* was cultured from the oesophagus in eight of 30 patients (27%) but could not be seen at this site by LM or EM (Table 2). In six of eight patients oesophageal *C pylori* was associated with the presence of the organism in the antrum. In the remaining two patients the oesophagus was the only site of isolation. There was no correlation between oesophageal *C pylori* and the organism in the body of the

stomach. A total of 20 patients had endoscopic or histological evidence of oesophageal inflammation including seven with Barrett's mucosa. *C pylori* was cultured from five of 20 (25%) but was not isolated from the remaining 15 (75%). The organism was found in two of seven patients with Barrett's oesophagus (29%). Examination of the culture plates of oesophageal biopsies showed a scanty growth (+) in five of eight and a heavy growth in three of eight (++ - +++). A heavy oesophageal growth corresponded with a similar growth from the antrum in one of three patients.

#### *C PYLORI* IN THE DUODENUM

The organism was cultured from the duodenum in six patients with a mixture of diagnoses (Table 2). In three patients this could be confirmed by LM and EM but only in areas of gastric metaplasia. A further four patients with gastric metaplasia did not have the organism. Whenever the organism was cultured from the duodenum it was always associated with antral gastritis and *C pylori*. The positive duodenal cultures tended to be less heavy than those from the antrum.

#### *C PYLORI* AND PEPTIC ULCERATION

*C pylori* and histological chronic active gastritis was present in the antrum of five of six patients with peptic ulceration (three DU, two GU) but was absent at all sites in one patient with a GU. Three of these patients also had oesophageal *C pylori* (two DU, one GU) and two had duodenal *C pylori*.

#### BUCCAL SWABS

The organism was not detected on follow up examination in the oropharynx of six patients with a

positive oesophageal culture including the two patients where this was the only site of isolation. Similarly, the organism was not found in 30 asymptomatic laboratory staff.

### Discussion

Despite extensive research the cause of peptic ulceration is still not known.<sup>18</sup> The current debate has been altered by the isolation of *C pylori* and the finding, confirmed in this study, that its presence is frequently associated with gastritis and peptic ulceration.<sup>2</sup> There is now good evidence to suggest that this organism may be a cause of gastritis but its role in the genesis of ulceration is controversial.<sup>3</sup> This study has attempted to address two areas of limited knowledge: the patterns of colonisation and disease in the upper gastrointestinal tract and the role of the organism in the oesophagus.

We have shown that *C pylori* may be isolated irregularly from the oesophagus in a diverse group of patients (27%) but could not be found at any site in histologically normal controls. The organism was only infrequently associated with endoscopic and histological oesophagitis (25%). In Barrett's oesophagus where, because of mucosal similarities to the stomach,<sup>19</sup> one might have expected an increased prevalence, the organism could only be cultured in two of seven (29%) cases. These results tend to suggest that *C pylori* is of limited importance in this organ and that its role is either that of a commensal or more likely a contaminant. This hypothesis is further supported by the fact that the organism could not be identified at this site by LM or EM and the cultures obtained tended to be much less heavy.

If *C pylori* is not pathogenic in the oesophagus, where does it come from? The method and checks used in this study suggest that contamination between patients or different sites in the same patient is unlikely. Other possible sources of the organism are the mouth or stomach. In this study we were unable to culture the organism from the oropharynx of patients or controls and have so far failed to isolate *C pylori* from any food or drink. It has been shown that *C pylori* may be isolated from gastric juice in infected subjects.<sup>20</sup> As six of eight of our patients with oesophageal *C pylori* also had the organism present in the antrum it may be postulated that they were transmitted by means of GOR of contaminated gastric juice. This theory is supported by recent work on bismuth treatment which shows that *C pylori* may be present in the oesophagus and in the antrum in 40% of a small group of patients with severe oesophagitis and proven GOR.<sup>14</sup>

In two patients *C pylori* was isolated only from the oesophagus. Contrary to the previous discussion this

might tend to support an independent and possibly pathogenic role for the organism at this site and requires further review (Table 2). In patient A, a 45 year old white man with frequent GOR symptoms but normal endoscopy, there was evidence of antral and body inflammation but his oesophagus was histologically normal. The most likely explanation would seem to be that the organism had been missed in the specimens taken. The results in patient B, a 57 year old man, are the only ones in this study to show the presence of the organism in association with oesophagitis alone. He previously underwent a vagotomy and pyloroplasty for duodenal ulceration which had now healed. It is possible that the bile seen in his stomach at endoscopy may be important as it has been shown that antral *C pylori* progressively diminish with time after gastric drainage procedures possibly due to excessive bile reflux.<sup>21</sup>

Previous reports indicate that *C pylori* is only seen in the duodenum in areas of gastric metaplasia.<sup>7, 22-24</sup> Our results do not support this as only three of six patients with a positive duodenal culture had associated gastric metaplasia. The most likely reason for this is that our biopsies simply missed the areas of metaplasia in these three patients. The alternative explanation that the organism came from the antrum through a contaminated endoscope or infected gastric juice would call into question the possible link between *C pylori* and gastric metaplasia in the genesis of duodenal ulceration.<sup>6</sup>

In addition to showing that oesophageal or duodenal *C pylori* is usually associated with the presence of the organism in the stomach, this study suggests that the organism may on occasions be present in all three sites examined. In two of three patients with duodenal ulceration *C pylori* was found in the oesophagus, stomach, and duodenum. It is possible that excessive and widespread distribution of this organism is a feature of duodenal ulceration.

The frequent coexistence of antral *C pylori*, gastritis and peptic ulceration is now generally accepted. Within the limits of this relatively small study on a wide group of patients we have shown that patterns of colonisation of the oesophagus and duodenum can exist in association with antral *C pylori*. The pathogenic role of this organism, however, is still controversial. We suggest that oesophageal *C pylori* is a commensal or a contaminant probably originating from the stomach. Its role in the duodenum is less clear but may be similar to that in the oesophagus. Further work is required on larger groups of patients and controls to confirm these results.

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