Original isolation of *Campylobacter pyloridis* from human gastric mucosa

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Abstract

During April and May 1982, in the Department of Microbiology at Royal Perth Hospital, Western Australia, campylobacter-like organisms were cultured from eleven gastric mucosa biopsy specimens. Most isolates did not appear before 3 days incubation, and grew at 37° C but not at 42° C. They grew best in a humidified atmosphere in a carbon dioxide incubator containing 8% carbon dioxide, and died rapidly in air. In contrast to current concepts of *Campylobacter*, the organisms had multiple polar flagella, but it is now known that other *Campylobacter* species can also exhibit this feature. Analyses in England and Australia have shown that two isolates have a DNA base composition of 36–37 mol % guanosine plus cytosine. This is in the *Campylobacter* range. We propose the name *Campylobacter pyloridis* sp. nov. and the strain Royal Perth Hospital 13487 has been designated as the type strain and has been deposited in the National Collection of Type Cultures in London (NCTC 11637). In our study of one hundred gastroscopy specimens, the campylobacter-like organisms were seen histologically in the mucosa of 95% of forty patients with active gastritis, in 87% of thirty one patients with peptic ulcers, and in only 6% of thirty one apparently healthy mucosal specimens.

Introduction

Many years ago histopathologists noted the presence of 'spiral' bacteria in gastrectomy specimens (Doenges, 1938; Freedburg and Barron, 1940). From biopsy material of the gastric antrum, Steer and Colin-Jones (1975) reported that they cultured *Pseudomonas aeruginosa*, but their illustration showed curved bacteria. At Royal Perth Hospital since 1979 campylobacter-like bacteria have been noted (by JRW) in biopsy material from patients with gastritis. The bacteria were closely associated with the surface of the gastric mucosa, beneath the surface mucus layer. A prospective study of such material including culture was undertaken during 1982, and Warren and Marshall (1983) have made a preliminary report of their histological findings and the 'unidentified curved bacilli'. At the Second International Workshop on *Campylobacter* Infections in September, 1983, Marshall and Warren (1983) presented more data and the bacteria were considered to be campylobacter-like organisms (CLO); the name *Campylobacter pyloridis* for these mucosally-associated gastric bacteria was suggested (Skirrow, 1983). The present communication records the microbiological details of the first isolation of this organism which is apparently new to human microbiology.

Materials and methods

Between March and May 1982, patients scheduled for routine elective gastroscopy at the Royal Perth Hospital Gastroenterology Unit were invited to join this study, and in one hundred patients who consented, the gastric antrum was biopsied. The study group consisted of sixty three males and thirty seven females, aged 20–88 years (mean 55 years); eighteen had a gastric ulcer, nine a duodenal ulcer, four had ulcers in both places, fifty three had endoscopic gastritis or duodenitis, and sixteen had no clinically observed lesions.

For our study three specimens of intact gastric antral mucosa were taken at a distance from any focal lesion. One specimen was examined histologically, and the other two placed in chilled anaerobic broth transport medium. However, it has since been found satisfactory to send specimens for culture in a sterile jar in a drop of saline.

The specimens were processed microbiologically within 1 h of collection in the following ways. One specimen was used to make a Gram-stained smear. Under sterile conditions the other specimen was minced and inoculated into serum broth and onto blood agar, and chocolate agar without antibiotics, and on Skirrow's medium containing vancomycin, polymyxin and trimethoprim (Skirrow, 1977). The plates were incubated at 37°C, in a mixture of air (25%) and anaerobic gas mixture (75%); the composition of the anaerobic gas mixture was hydrogen (10%), carbon dioxide (10%) and nitrogen (80%).

The first thirty four specimens were incubated for only 48 h and CLO were not obtained. By accident the next specimen was cultured for 6 days during the Easter holiday, and on 14 April a growth of CLO was observed on the chocolate agar. Fortunately the plates were not covered with a heavy growth of Enterobacteriaceae which frequently occurred with the prolonged moist culture. All plates from subsequent specimens were incubated for at least 4 days, and it was found that isolates did not appear after this time. Other parameters for culture were discovered later and are reported under the Results.

Organisms other than CLO were identified, but were not obtained in any regular pattern. Histologically, Gram-negative rods and Gram-positive cocci were often seen, but they were scattered at random on the luminal surface of the mucus layer, unlike the CLO which were small, curved rods, constant in type, which were on the epithelial surface deep in the mucus. Histological sections were stained with the Warthin-Starry silver stain which revealed the CLO most sensitively.

Results

The histological results will be presented elsewhere. In summary CLO correlated most closely with active gastritis, whether or not the patient had a peptic ulcer. In forty specimens from patients with active gastritis, of whom 50% had a peptic ulcer, CLO were seen in the gastric mucosa in thirty eight (95%). CLO were seen in eighteen (62%) of twenty nine patients with chronic gastritis and in two (6%) of thirty one patients without gastritis.

Only ninety six biopsies were examined microbiologically. CLO were seen in the Gram stains of six of the first thirty four specimens, but were not obtained on culture. Of the next sixty two specimens CLO were seen in twenty eight and cultured from eleven (18%) of these. Failure to culture CLO may have been due to the heavy growth of Enterobacteriaceae, such as *Proteus rettgeri*, which often covered the plates after 3-4 days incubation. Of the last sixty two specimens five had large numbers of CLO on Gram stain, and CLO were cultured in every case; these were all patients with active chronic gastritis. In twenty two of this group of specimens few CLO were seen on Gram stain, but six cultures were obtained. The CLO were S-shaped or curved Gram-negative rods, 3 μm by 0.5 μm.

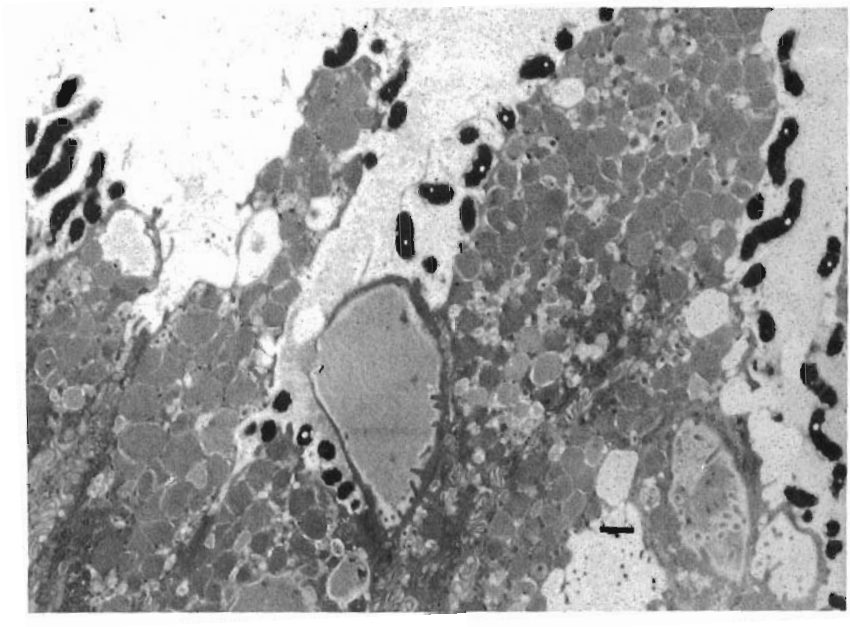

Figure 1 Thin section survey micrograph showing campylobacter-like organisms, including many curved profiles, on the surface of gastric mucous epithelial cells. Bar = 1 μm. x 4,800.

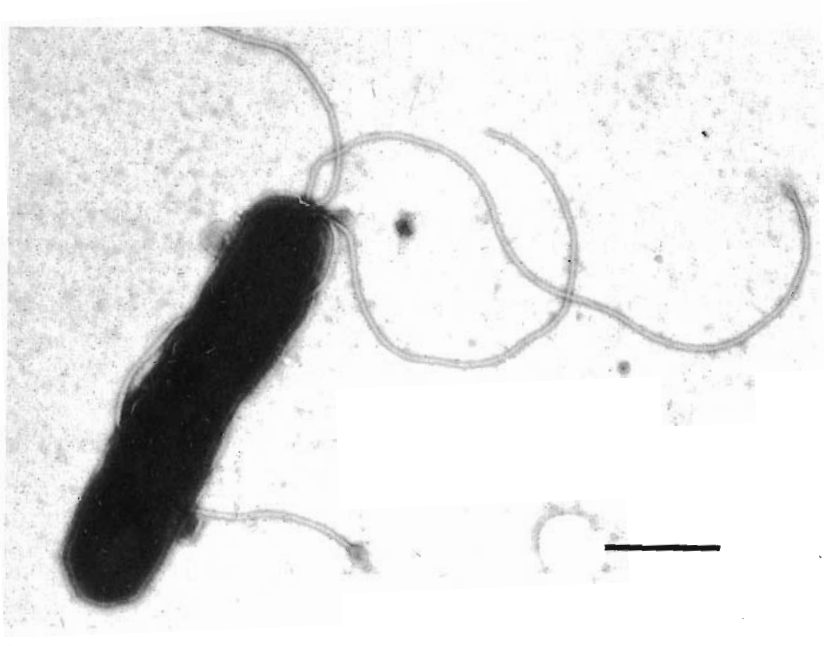

Figure 2 Bacterial cell from hanging-drop preparation. Negative stain electron micrograph (2% PTA, pH 6.8; bar = 1 μm). Multiple flagella arise from one pole; note the terminal bulb. x 16,000.

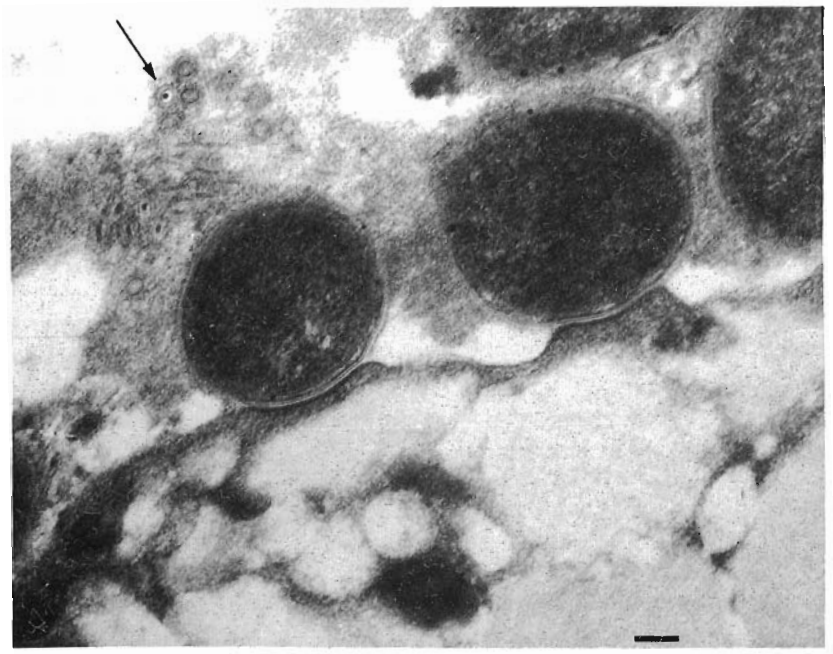

Figure 3 Sectioned organisms at higher magnification, with profiles of transected sheathed flagella (arrow). Bar = 0.1 μm. x 56,250.

In tissue sections and Gram stained smears from tissues the bacteria usually appeared smaller and more curved (Figure 1) than cultured CLO which were irregular and larger than *C. jejuni*. In electron micrographs the organisms had smooth coats, usually with four sheathed flagella arising from one end of the cell (Figures 2 and 3). Growth was evident after 2 or 3 days as 1 mm, translucent colonies. Fresh, moist plates were more successful for isolation than dried plates. The organisms died rapidly when left in air. In old cultures coccoid bodies appeared.

We have now discovered that cultures can be routinely maintained on 7% blood agar plates incubated at 37°C in a humidified atmosphere of air and 8% carbon dioxide, such as is obtained in a carbon dioxide incubator, with water in the bottom shelf. These conditions have also been shown to be adequate for the isolation of the organisms (McGechie, personal communication).

Characterization tests were performed (by HR); a chocolate agar slope with brain-heart infusion broth at the bottom allowed hanging drop preparations to be made, and all isolates were motile. *C. pyloridis* were oxidase and catalase positive and produced hydrogen sulphide, detected by lead acetate paper, but they failed to metabolise glucose, did not produce indole nor hydrolyse urea, and they did not reduce nitrite nor nitrate. They did not oxidise gluconate and did not grow in the presence of 1% glycine or 1% bile salts or 3.5% sodium chloride.

The organisms appeared to be sensitive to erythromycin, tetracycline, kanamycin, gentamicin, cephamandole, cefoxitin, rifampicin, and colistin. One of the twelve isolates was resistant to penicillin, four isolates were resistant to metronidazole and all strains were resistant to naladixic acid and trimethoprim and sulphaphurazole.

Two isolates of *C. pyloridis* have been received by the National Collection of Type Cultures in London, and we designate Royal Perth Hospital Isolate 13487 to be the type strain, with the NCTC number 11637. We propose the name *Campylobacter pyloridis* sp. nov. (Gr. noun *pylorus* gatekeeper; gen. sing. *pyloridis*). For growth *C. pyloridis* requires 5–20% oxygen plus 5–20% carbon dioxide. Maximum humidity and haematin are required for optimum growth on solid media, and the optimum temperature is 37°C. Growth also occurs at 30°C, but not at 25°C or 42°C. The rapid, darting motility organisms spin around their long axes and can be seen in hanging-drop preparations. DNA analysis of the type strain has shown that the DNA base composition is 37.1 mol % guanosine plus cytosine (Owen, personal communication). Another isolate RSB 6 (= NCTC 11639) was sent to the University of Queensland, and has shown a DNA base composition of 35.8 ± 0.5 mol% G + C (Sly, personal communication).

Discussion

An original difficulty in deciding the genus of our isolates was that *Campylobacter* have been defined as having one polar flagellum (Pead, 1979), and so the possession of four polar flagella indicated that our organism might be *Spirillum* species (Warren and Marshall, 1983). Ritchie *et al.* (1966) described multiflagellate forms of *C. fetus* although without sheaths. these forms were thought to be aberrant.

Other *Campylobacter* species have been found recently to have a proportion of their population with up to five polar flagella (Skirrow *et al.*, 1983). Specific antiserum against the campylobacter group antigen has shown fluorescence with *C. pyloridis* (Price *et al.*, 1984). As the DNA base composition is 36–37 mol% G + C, *C. pyloridis* is probably a *Campylobacter* species. However, sheathed flagella have never been demonstrated in any other species of *Campylobacter* and the genus may need to be redefined to include such organisms.

Recently, we have found that *C. pyloridis* do not need haematin for growth although the organisms grow more slowly in its absence; on 10% horse-serum agar colonies are visible after about 4 days incubation. We are pleased that during 1983 *C. pyloridis* have been obtained in culture outside Australia, from a biopsy (by BJM) in Worcester, England (Lancet, 1983), and by Price *et al.* (1984).

Since *C. pyloridis* were seen in the gastric mucosa of all patients with duodenal ulcer, and 80% of patients with gastric ulcer, therapy that is antibacterial may be more successful than other therapy in achieving healing of peptic ulcers. *C. pyloridis* are sensitive to bismuth, and ulcer treatment with tri-potassium di-citrato bismuthate complex (De-Nol, Gist-Brocades), is associated with a lower relapse rate than cimetidine. Germ-free rats are not susceptible to the production of experimental gastric ulcers, and normal rats are protected from such ulcers by antibiotics (Sato *et al.*, 1983).

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