Isolation of a "*Helicobacter heilmanii*"-like Organism from the Human Stomach

"*Helicobacter heilmanii*" ("*Gastrospirillum hominis*") is a spiral-shaped microorganism seen in the gastric mucosa of approximately 0.5% of patients with gastritis (1–4). The classification of this uncultured organism as a *Helicobacter* sp. is based on sequencing of the 16S rRNA gene after amplification by polymerase chain reaction directly from infected gastric biopsies (5). Culturing of "*Helicobacter heilmanii*" has been unsuccessful, whereas spiral organisms have been cultured from animals; *Helicobacter felis*, for example, has been cultured from cats (6). Serological examination of patients with "*Helicobacter heilmanii*" infection has demonstrated that this microorganism is more closely related to *Helicobacter felis* than to *Helicobacter pylori* (7). We describe culture of an organism resembling "*Helicobacter heilmanii*" from a human gastric biopsy.

A 23-year-old male patient had an upper gastrointestinal endoscopy because of continuing dyspepsia. Eight biopsies (4 for histology and 4 for microbiology) were obtained according to the Sidney classification of gastritis. Biopsies for histological examination were formalin fixed and stained with hematoxylin-eosin. Biopsies for microbiology were transported in serum broth (Statens Seruminstitut, Copenhagen) and sown on nonselective 7% lysed horse blood agar plates (Statens Seruminstitut) within four hours after endoscopy. The plates were grown in an anaerobic chamber with 5% O_2, 10% CO_2, and 85% N_2, but without H_2, in a 95% moist atmosphere at 36°C for up to seven days.

In histological sections spiral-shaped organisms resembling "*Helicobacter heilmanii*" ("*Gastrospirillum hominis*") developed in foveolae in biopsies from antral mucosa but not from corpus mucosa. *Helicobacter pylori*-like organisms could not be seen. Culture revealed uniform, small, translucent colonies distinctly resembling *Helicobacter pylori* after five days on the primary plates. In phase contrast microscopy two forms of the organisms could be identified: 10 to 15% of the organisms were long spiral-shaped rods and the remaining majority were short ox-bow rods resembling *Helicobacter pylori*. Both were gram-negative rods. Subcultures of the organisms revealed *Helicobacter pylori*-like colonies after two to three days.

Pure culture of long, spiral-shaped rods was obtained after subculture. The organisms were oxidase, catalase, and urease positive, as are other *Helicobacter* species. They did not produce acid from sugars and did not hydrolyse hippurate. Electron microscopy revealed a bundle of flagella at one of the cultured organisms and a lack of the periplasmic fibrils that are seen in most spiral organisms isolated from animals (Figure 1). The organisms were susceptible to amoxicillin, metronidazole, tetracycline, and erythromycin, and were eradicated by triple combination therapy with amoxicillin, metronidazole, and omeprazole.

This previously uncultured organism resembled in many respects *Helicobacter pylori*. Only microscopy of the colonies distinguished them from *Helicobacter pylori* in contrast to *Helicobacter felis* or "*Helicobacter rappini*," which grows in a tiny, swarming layer. In addition, the biochemical reactions of the organism and its susceptibility to antibiotics resembled those of *Helicobacter pylori*. Without sufficient microscopic investigation of colonies, this microorganism could easily be overlooked. By electron microscopy, the cultured microorganism differs from other cultured helicobacters and gastric spiral bacteria. In histological sections it is indistinguishable from "*Helicobacter heilmanii*" and may be a culture of this organism. Further characterisation of the organism is in progress.

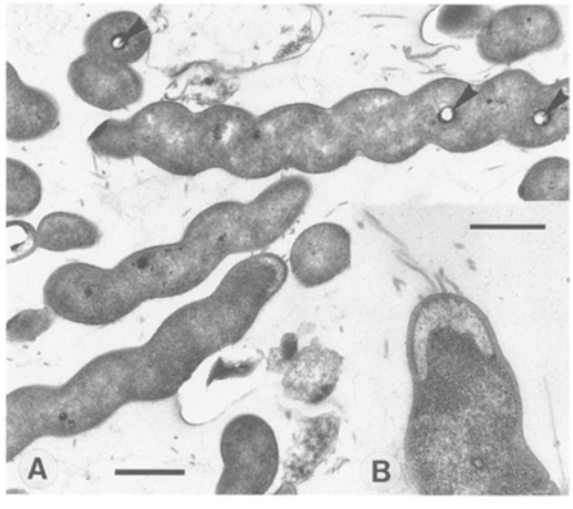

Figure 1: Representative electron micrographs of a two-day-old culture of spiral-shaped organisms. (A) The inclusions (arrowheads) were found in a small percentage of the organisms. Original magnification, 14,600 x Bar = 1 μm. (B) Polar end from another spiral-shaped organism illustrates the flagellated end, with the electron-lucent zone beneath. Original magnification, 31,300 x Bar = 0.5 μm.

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Novobiocin-Salmonella-Shigella Agar for Isolation of *Salmonella* Spp.

One of the objectives of using selective culture media for the isolation of *Salmonella* spp. is the inhibition of saprophyte flora. Bile salts and brilliant green are two examples of the substances used in the traditional culture media for this purpose. The addition of novobiocin to selective media for the isolation of *Salmonella* spp. is also an established technique. For example, Hoben et al. (1) in 1973 used a novobiocin-Hektoen enteric (HE) agar combination. In 1986 Devenish et al. (2) described a culture medium, novobiocin-brilliant green-glucose (NBG) agar, whose inhibitory power was due not only to the presence of brilliant green but also to the novobiocin, which inhibited the growth of *Proteus* spp. Later, Poisson (3) formulated the medium novobiocin-brilliant green-glycerol-lactose (NBGL) agar, which was similar to the previously mentioned medium.

We compared salmonella-shigella (SS) agar (Difco, USA) containing novobiocin (10 mg/l) (SS-NOV; Sigma, USA) with SS agar without novobiocin. One hundred nineteen stool samples (salmonella free) were inoculated with 119 strains of *Salmonella* (1 per sample) from our collection and belonging to the following species: *Salmonella enteritidis* (n = 87), *Salmonella typhimurium* (n = 17), *Salmonella virchow* (n = 7), *Salmonella derby* (n = 3), *Salmonella brenedey* (n = 2), *Salmonella infantis* (n = 2), and *Salmonella anatum* (n = 1). In 1994 species distribution in our laboratory was as follows: *Salmonella enteritidis* (51.9%), *Salmonella typhimurium* (25.1%), *Salmonella virchow* (12.2%), and others (10.8%).

The samples were prepared by adding 10^4 cfu/g to 20 g of loose stools and then mixing gently for several minutes. This mixture was diluted by adding an appropriate volume of 0.9% NaCl, and one drop of this suspension was used to inoculate each media. After incubation at 37°C for 24 h, the plates were screened by means of the C_8-esterase test (MUCAP test, Biolife, Italy) (4, 5), noting the number of H_2S-positive and MUCAP-positive colonies per plate. At least three of these colonies were subcultured on Kliger agar and then identified with the API 20 E System (bioMérieux, France) if the reactions in the tube were compatible with those for *Salmonella*. Serological confirmation of the identification of isolates was performed using commercially prepared salmonella 0 antisera (Difco). Sensitivities were compared by the discrepant paired chi-square test.

Table 1: Comparison between salmonella-shigella (SS) agar and salmonella-shigella agar with novobiocin (SS-NOV) for detection of *Salmonella* in 119 inoculated stool samples.

	SS agar	SS-NOV
Sensitivity	84.1%	96.5%[a]
No. of colonies (mean)	17	30
Standard deviation	13	21
Range	0–100	0–110
Median	10	20
(P_{25}-P_{75})[b]	2–25	10–40

[a] $p < 0.001$.
[b] Interqartile range.