

Isolation of *Streptococcus Mobilis* from a Japanese Couple with *Helicobacter Pylori*-Negative Chronic Gastritis, Intestinal Metaplasia, and Reactive Gastropathy

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Abstract

Reactive gastropathy associated with the infection of gastric microbiota has received limited attention compared to *Helicobacter pylori*. We have reported the association between the three types of classification for reactive gastropathy and unidentified coccoid bacteria. This study aimed to culture and isolate gastric microorganisms from *H. pylori*-negative Japanese couples with dyspeptic symptoms. They were diagnosed with Type III reactive gastropathy, characterized by preneoplastic gastric mucosal changes such as intestinal metaplasia.

Two novel bacterial types, *Streptococcus mobilis* IV and a species closely related to *Streptococcus oralis* subsp. *tigurinus* were isolated from gastric biopsy specimens. After administering a sequential combination of antibiotics over six weeks, the couple became asymptomatic with no adverse effects. Given the intracellular and intranuclear invasive characteristics of the bacteria *S. mobilis*, along with its production of DNase, hydrogen sulfide (H₂S), and CHO toxin, it is important to conduct careful and regular surveillance for Type III reactive gastropathy in populations with a high incidence of gastric cancer. This research significantly enhances our understanding and management of reactive gastropathy.

Keywords: : Intestinal Metaplasia, Reactive Gastropathy, *Streptococcus*.

Abbreviations

BHI: Brain Heart Infusion.

CHO: Chinese Hamster Ovary.

EGD: Esophagogastroduodenoscopy.

GEJ: Gastroesophageal Junction.

SMLB: *Streptococcus Mobilis*-like Bacteria.

WSS: Warthin-Starry silver.

Introduction

Reactive gastropathy, also known as chemical gastropathy, bile reflux gastritis, and type C gastritis, is the second most common type after *H. pylori* gastritis [1, 2]. The definitive diagnosis is made through the histological examination of a gastric biopsy, as endoscopic findings are often nonspecific. The histological features of reactive gastritis include foveolar hyperplasia with tortuosity of elongated crypts, mucosal and lamina propria edema, capillary congestion and vasodilatation in lamina propria,

smooth muscle hyperplasia in lamina propria, and sparse chronic inflammatory cell infiltration [1, 2]. Reactive gastropathy is associated with various factors such as bile, pancreatic secretions, alcohol, nonsteroidal anti-inflammatory drugs (NSAIDs), and other chemicals and medications [1, 2].

Although microorganisms in the gastrointestinal tract produce various chemicals, enzymes, and toxins, the role of gastric microbiota in causing reactive gastropathy, aside from *H. pylori*, has not been extensively studied. We previously reported an association among three classification types of reactive gastropathy with unidentified coccoid bacteria; however, we could not successfully isolate or molecularly identify the organism [3]. Reactive gastropathy has been described as “the constellation of endoscopic and histological changes resulting from chemical injury to the gastric mucosa” [2, 4]. A thorough evaluation of histological and endoscopic findings are essential to diagnose reactive gastropathy accurately.

Over the last three decades, we have reported the involvement of a novel bacterium, *Streptococcus mobilis* (previously proposed name: *Okadaella gastrococcus*, ATCC BAA-2258), in various esophagogastroduodenal pathologies. *S. mobilis* is an intracellular and intranuclear invasive, acid-tolerant, Gram-stain-variable bacterium found in both *H. pylori*-positive and *H. pylori*-negative gastropathies. Its involvement in the gastric cancer cascade has been suggested based on its intranuclear presence and various biochemical products, including CHO cytotoxin, DNase, and H₂S, which may also contribute to reactive or chemical gastropathy. This study aimed to isolate gastric microorganisms from patients with reactive gastropathy and treat the dyspeptic symptoms of a Japanese couple.

Methods

A Japanese couple, a 48-year-old husband and a 45-year-old wife, presented with dyspeptic symptoms and consented to undergo an esophagogastroduodenoscopy (EGD). During the procedure, gastric biopsy specimens were collected from the pre-pyloric antrum and the mid and proximal antrum for histological and microbiological examinations and from the gastroesophageal junction (GEJ). The gastric biopsy specimens from the stomach were placed in a 3 mL chocolate agar slope (bioMérieux) with 0.5 mL of BHI broth (Remel) and a 3 mL chocolate agar slope containing 0.5 mL of sterile normal saline. Immediately after the completion of EGD, the biopsy specimens on the agar slope with BHI broth and the agar slope with normal saline were cultured on a chocolate agar plate (bioMérieux) using a sterile disposable needle to compress and streak the specimen on the agar surface. After the procedure, the biopsy specimens were returned to their containers. The gastric aspirate collected into the sterile reservoir of the endoscopic unit was transferred into a sterile container and then cultured on the chocolate agar plate. The chocolate agar slope containing 0.5 mL of sterile normal saline or BHI broth and the chocolate agar plate without biopsy specimens served as their respective controls. These specimens were incubated for 3–14 days under microaerophilic and anaerobic conditions at 37°C.

The medium of biopsy specimens, with BHI broth or normal saline, was examined daily using a Gram stain, cultured on a chocolate agar plate with a disposable sterile probe, and incubated under anaerobic conditions at 37 °C. Isolated bacteria from the biopsy specimens were subcultured under anaerobic conditions for 72 hours on 5% horse blood agar (bioMérieux) at 37°C. CampyGen™ (Oxoid) and AnaeroGen™ (Oxoid) were used for microaerophilic and anaerobic conditions, respectively. This study employed the Gram stain method, utilizing crystal violet and safranin. Biochemical tests, including Rapid ID 32 STREP (bioMérieux), RapID STR (Remel), RapID ANA II (Remel), and antibiotic sensitivity tests using disc diffusion and E-tests, were performed on isolated Gram stain-variable coccoid bacteria from the couple and Gram-positive bacteria from the husband.

The antibiotics used for diffusion disc tests included azithromycin, chloramphenicol, colistin, gentamicin, doxycycline, erythromycin, kanamycin, metronidazole, suxamethonium, sodium polyanetholsulfonate (SPS), tetracycline, and vancomycin. For

the E-tests, the antibiotics used were amoxicillin, amikacin, azithromycin, clarithromycin, cefotaxime, gatifloxacin, penicillin, rifampicin, and tetracycline.

According to their protocols, the couple's isolates underwent 16S rRNA gene sequencing analysis and phylogenetic tree reconstruction at the TechnoSuruga Laboratory in Japan. H&E, Warthin-Starry silver (WSS) stains, and *H. pylori* immunohistochemistry were performed on biopsy specimens fixed in 10% formalin at Sullivan and Nicolades Private Pathology for histological examination. *H. pylori*-positive specimens were used as controls for the WSS stain and immunohistochemistry methods. Based on their sensitivity responses to the isolated bacteria, the patients were treated with omeprazole and antibiotics, including amoxicillin, doxycycline, clarithromycin, and rifampicin.

Results

Endoscopic Appearance and Histology

The histology examination of the specimens from the gastroesophageal junction (GEJ) identified an inflamed squamocolumnar junction with intestinal metaplasia in the cardia, suggestive of Barrett's esophagus in the wife, and without intestinal metaplasia in the husband. The WSS stain revealed intracellular and extracellular *S. mobilis*-like bacteria (SMLB) in the squamocolumnar junction. Figure 4a illustrates the endoscopic appearance of the wife's pre-pyloric antrum. The histologic examination identified reactive gastritis with intestinal metaplasia against a background of chronic gastritis (Fig. 4b). Multiple extracellular and intracellular SMLB were observed in the intestinal metaplasia (Fig. 4c, 4d).

Under WSS stain, SMLB were also noted in the vessels (Fig. 4c), the lamina propria, and interstitial space (Fig. 4d). Figure 5a shows the endoscopic appearance of the husband's pre-pyloric antrum. The histological examination revealed reactive gastritis with focal intestinal metaplasia against mild chronic gastritis (Fig. 5b). SMLB were detected in the mucous and mucosa (Fig. 5c) under WSS stain. Dark-stained coccoid microorganisms, slightly larger than SMLB, were found in the gastric pits of the specimens (Fig. 5c). The WSS stain demonstrated not only *H. pylori* but also, incidentally, intracellular SMLB in the controls, as shown in Figure 5d. *H. pylori* immunohistochemistry was negative in both cases. Bile reflux was not observed in either case during the endoscopic examination.

Microbiology

Facultative anaerobic and lactic acid-producing coccoid bacteria were isolated as single cells, pairs, or chains. Isolates from the husband and wife exhibited identical Gram-stain-negative reactions (Fig. 6b) and similar biochemical and antibiotic sensitivity results. The phenotypic characteristics of the isolates resembled those of *S. mobilis*: facultative anaerobic, mesophilic, alpha-hemolytic, catalase-negative, oxidase-negative, urease-negative, H₂S-positive, and Gram-stain-variable coccoid bacteria.

The tests used in this investigation were unable to identify the isolated bacteria. Additionally, it was unfortunately not possible to continue subculturing the isolate from her husband due to unknown reasons. The available tests also did not identify the Gram-positive coccoid bacteria isolated from the husband (Fig.

6c). Subsequently, two isolates, one from the wife (Gram-negative coccoid bacteria) and the other from the husband (Gram-positive bacteria) were sent to the TechnoSuruga laboratory in Japan for molecular identification. 16S rRNA gene sequencing analysis of the bacteria isolated from the wife identified it as a new species of *S. mobilis* (Fig. 7). Furthermore, the analysis revealed that the unidentified Gram-positive bacteria isolated from the husband represented a novel species of *Streptococcus*, closely related to *Streptococcus oralis* subspecies *tigurinus* (Fig. 7).

Treatments

The couple sought treatment for persistent dyspepsia and histological findings of intestinal metaplasia, a preneoplastic change (Type III reactive gastropathy). Based on the sensitivity results of the isolated bacteria, they underwent a sequential combination of antibiotics and a proton pump inhibitor over six weeks.

The wife received amoxicillin 500mg t.d.s., doxycycline b.i.d., and omeprazole b.i.d. for two weeks, followed by amoxicillin 500mg t.d.s., rifampicin 600mg daily, and omeprazole b.i.d. for four weeks. The husband, who had a dual infection with *S. mobilis* and *S. oralis*-like bacteria, was treated with amoxicillin 500mg t.d.s., clarithromycin 500mg b.i.d., and omeprazole b.i.d. for two weeks, followed by amoxicillin 500mg t.d.s., rifampicin 600mg daily, and omeprazole b.i.d. for four weeks.

Their dyspeptic symptoms completely resolved. After a two-week course of either amoxicillin, doxycycline, and omeprazole or amoxicillin, clarithromycin, and omeprazole, followed by a subsequent four-week sequential course of amoxicillin, rifampicin, and omeprazole, without adverse effects.

Discussion

Reactive gastropathy can be classified into Types I, II, and III [3]. Type I reactive gastropathy is illustrated in Figure 1. The endoscopic appearance may be normal (Fig. 1a) or show mild to moderate gastritis without erosion, haematin, or petechial hemorrhage. The histological diagnosis indicates mild reactive gastritis (Fig. 1b). WSS stain reveals numerous coccoid bacteria in the interstitial space, hyperplastic fibroblasts, and smooth muscle fibers (Fig. 1c).

Type II reactive gastropathy is illustrated in Figure 2. The endoscopic appearance features erosions (Fig. 2b, 2c), haematin, and petechial hemorrhage (Fig. 2a) in the background of gastritis. The histological diagnosis indicates moderate reactive gastritis (Fig. 2d). WSS stain reveals numerous coccoid bacteria in the interstitial space, associated with smooth muscle fibers and present in the gastric mucosal cells (Fig. 2e). The bacteria can be observed in the muscle fibers under higher magnification.

Type III reactive gastritis is illustrated in Figure 3. The endoscopic appearance reveals erosions, haematin, and petechial hemorrhage (Figs. 3a, 3b) in a background of gastritis. Histological examination confirmed intestinal metaplasia (Fig. 3c) in severe reactive gastritis (Fig. 3d). Narrowband imaging can di-

agnose intestinal metaplasia but not reactive gastritis. Numerous coccoid bacteria are observed on the mucosal cells and intestinal metaplasia using WSS stain (Fig. 3e).

In previous investigations, intracellular SMLB were consistently found in *H. pylori*-positive specimens under WSS staining, as shown in Figure 5d. Based on our studies over the past 30 years, SMLB infection consistently coexists with *H. pylori* infection. However, *H. pylori* infection does not occur in the context of SMLB-associated gastric mucosal pathologies. Biopsy samples obtained from the couple revealed mild gastroesophagitis without macroscopic evidence of bile reflux. In the wife's case, intestinal metaplasia in the cardia could not exclude Barrett's esophagus. These findings supported the report of a tendency for an increased incidence of reactive gastropathy among patients with inflammation of GEJ [5].

Although the researchers concluded that bile reflux induced inflammation, our findings obtained through special staining methods and electron microscopy suggested a possible association between SMLB infection, gastroesophagitis, and Barrett's esophagus, which has been presented at international meetings (unpublished). The couple was free from NSAIDs, smoking, and hepatorenal dysfunction. The wife did not drink alcohol but intermittently took the antihistamine fexofenadine. Conversely, the husband consumed 375 mL of beer and 400 mL of wine daily. Consequently, drug and alcohol use as potential causes of reactive gastropathy cannot be entirely excluded.

In the couple's cases, histological examination revealed areas of focal intestinal metaplasia in their antral biopsy specimens. Mild reactive gastritis was also observed alongside mild chronic gastritis. Therefore, the classification is Type III reactive gastropathy for both cases. SMLB were identified in the couple's gastric mucosa, and a novel *S. mobilis* was isolated. Unfortunately, the isolate of *S. mobilis* from the husband was lost during subculture for an unknown reason. However, the phenotypic features of the isolates from the couple had been studied prior to the loss of the husband's isolate. The results of the Gram stain, biochemical tests, and sensitivity tests were identical. The phenotypic features of the isolated *S. mobilis* from the couple suggested intrafamily transmission. However, this requires investigation and confirmation through molecular studies.

To verify whether the bacteria found in the biopsy specimens during the histological examination are *S. mobilis*, a monoclonal antibody immunohistochemistry or FISH (fluorescence in situ hybridization) test would provide the answer. Considering the improvement in dyspeptic symptoms following sequential treatments, this evidence further supports the association between Type III reactive gastropathy and *S. mobilis* infection. In addition to our previous efforts to isolate gastric microorganisms, at least two bacteria, *S. mobilis* IV and a new species closely related to *S. oralis* subsp. *tigurinus* could be associated with reactive gastropathy.

Therefore, further investigations are required to clarify the relationship between gastric microbiota and reactive gastropathy. This may represent a rare case of reactive gastropathy with intestinal metaplasia in the context of chronic gastritis. Type III re-

active gastropathy should be monitored regularly, as it can lead to gastric cancer over time due to preneoplastic mucosal changes,

particularly in populations with a higher incidence of gastric cancer, such as the Japanese.

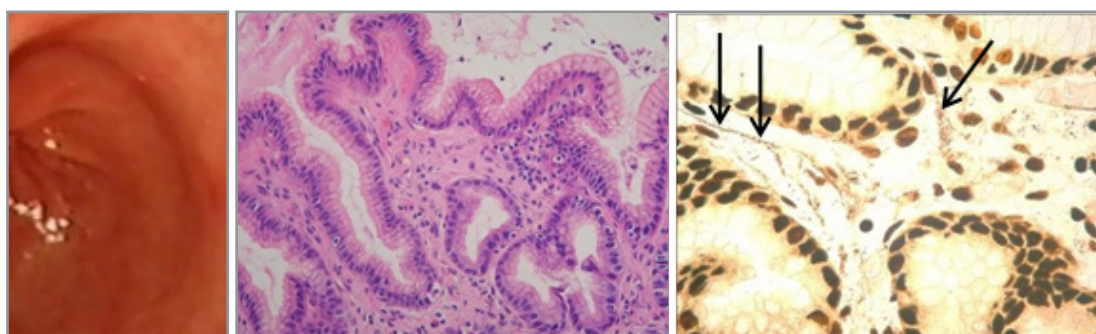


Figure 1: Type I Reactive Gastropathy.

Endoscopic View of The Antrum

- The gastric mucosa shows no erosion, petechial hemorrhage, or hematin. Histological examination with H&E staining reveals mild reactive gastritis
- Multiple SMLB (arrows) are observed in the interstitial space under WSS
- Staining
- X: magnification of the objective lens.

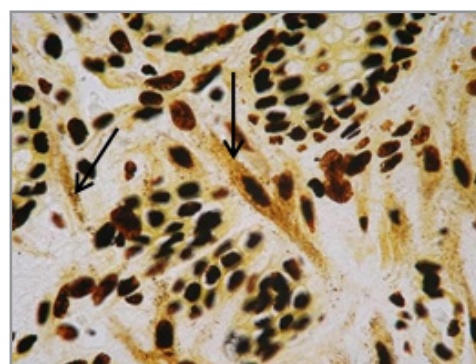
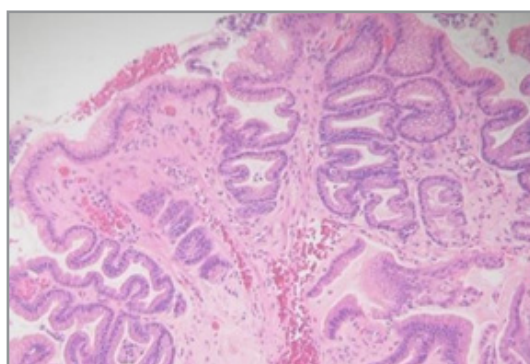


Figure 2: Type II Reactive Gastropathy.

The endoscopic view of the antrum reveals multiple petechial hemorrhages and hematin in The gastric mucosa

- (a), as well as numerous elevated erosive lesions
- (b, c). There is no evidence of macroscopic intestinal metaplasia. Histological examination with H&E staining reveals moderate reactive gastritis
- (b). Multiple SMLB (arrows) in the interstitial space are associated with smooth muscle fibers under WSS staining
- (c). SMLB can be observed in the gastric pit, peri-vascular area, and intravascular site (not shown). X: magnification of the objective lens.

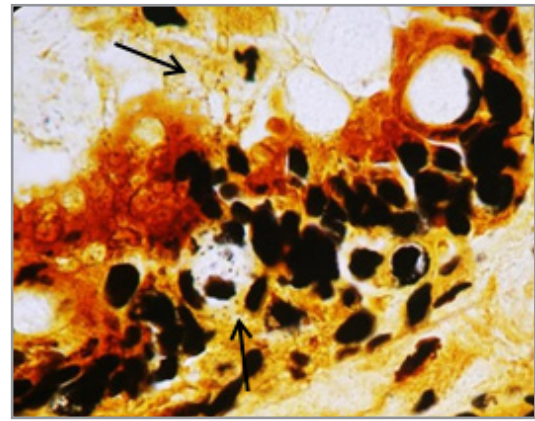
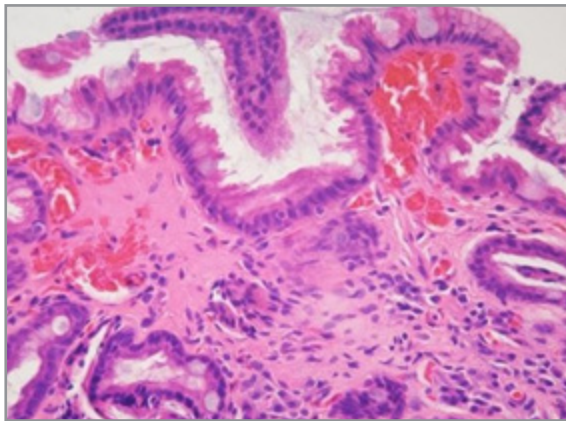
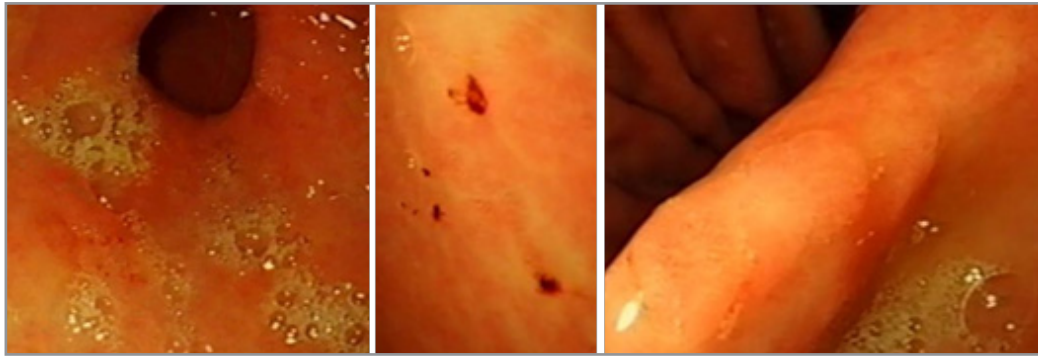


Figure 3: Type III Reactive Gastropathy.

The endoscopic view of the antrum reveals multiple erosive lesions

- The gastric mucosa shows several petechial hemorrhages and hematin
- An area of intestinal metaplasia is observed at the angular notch of the lesser curvature

- Histological examination with H&E stain demonstrates intestinal metaplasia in the background of severe reactive gastritis
- Multiple SMLB (arrows) are identified within the mucosal cells, lamina propria, and intestinal metaplasia under WSS stain (e). X: magnification of the objective lens.

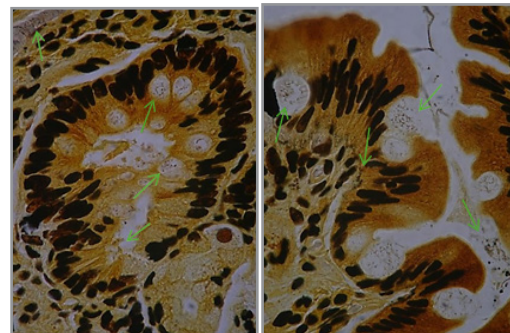
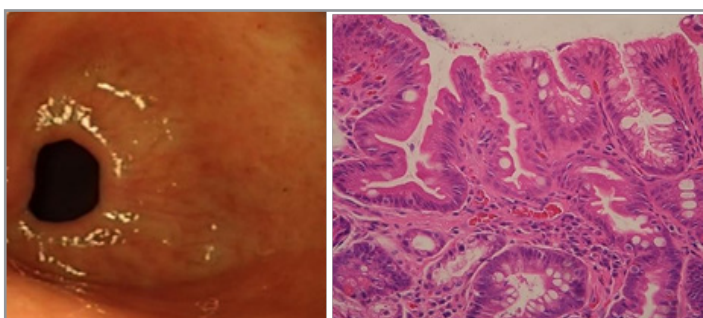


Figure 4: The Case of The Wife.

The endoscopic appearance of the pre-pyloric antrum is unremarkable

- The gastric mucosa has no erosion, petechial hemorrhage, or hematin. There is no macroscopic intestinal metaplasia. Histologic examination by H&E stain reveals reactive gastritis with intestinal metaplasia in the background of mild chronic gastritis

- Multiple extracellular and intracellular SMLB (green arrow) are seen in intestinal metaplasia under WSS stain (c). SMLB are found in the vessel
- SMLB (green arrow) are also observed in the lamina propria, interstitial space, and gastric pit of intestinal metaplasia
- H. pylori immunohistochemistry was negative (not shown). X: magnification of the objective lens.

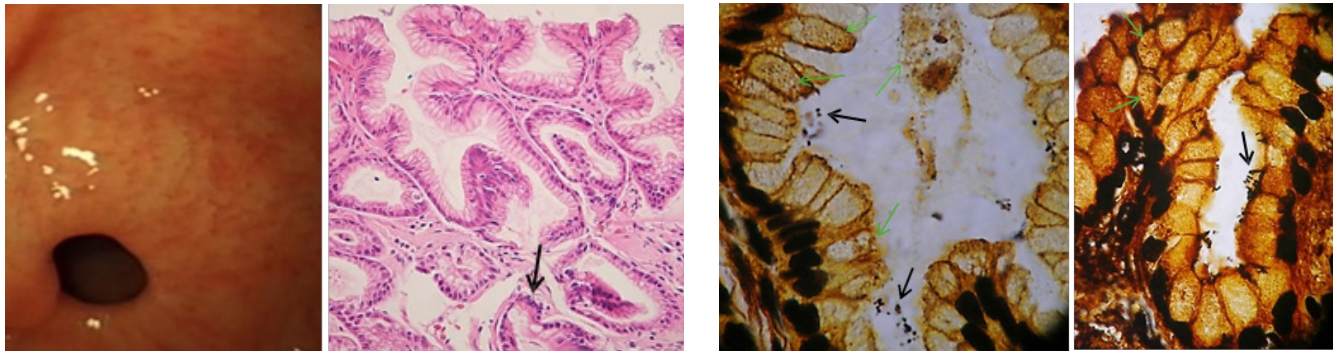


Figure 5: The Case of the Husband.

Endoscopic Appearance of The Pre-Pyloric Antrum

- Although it appears unremarkable, carefully inspecting the mucosa indicates superficial chronic gastritis. The gastric mucosa shows no signs of erosion, petechial hemorrhage, or hematin. There is no macroscopic
- intestinal metaplasia. Histologic examination by H&E stain reveals mild reactive gastritis
- with focal intestinal metaplasia (arrow) in the background of mild chronic gastritis
- Multiple intracellular SMLB (green arrow) are seen in the gastric epithelia and mucous layer of the gastric pits
- Dark-stained coccoid bacteria (black arrow), which appear slightly larger than SMLB, are seen in the mucous and on the epithelia of the gastric pits under the WSS stain
- WSS stain demonstrated *H. pylori* (black arrow) in the gastric pits of the control specimen
- Incidentally, intracellular SMLB (green arrow) were seen in this
- specimen
- H. pylori* immunohistochemistry was negative (not shown).
- X: magnification of the objective lens.

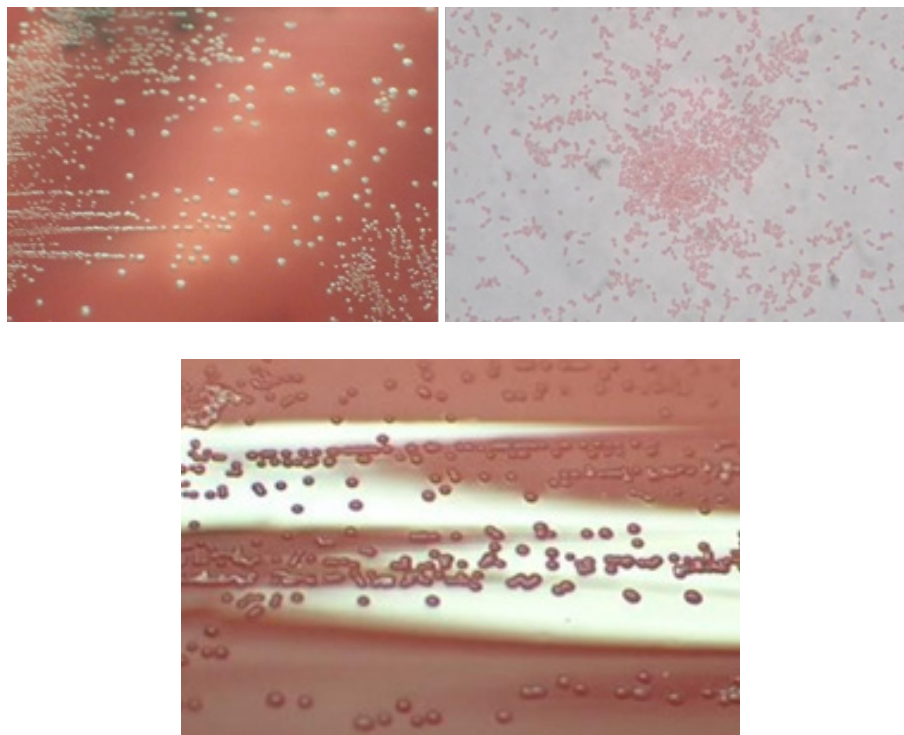


Figure 6: Cultured Colonies of Two Isolated Types of Bacteria.

Cultured colonies of *s. Mobilis* Isolated from The Wife

- and its Gram-negative stain reaction
- Cultured colonies of unidentified coccoid bacteria isolated from the husband (c).

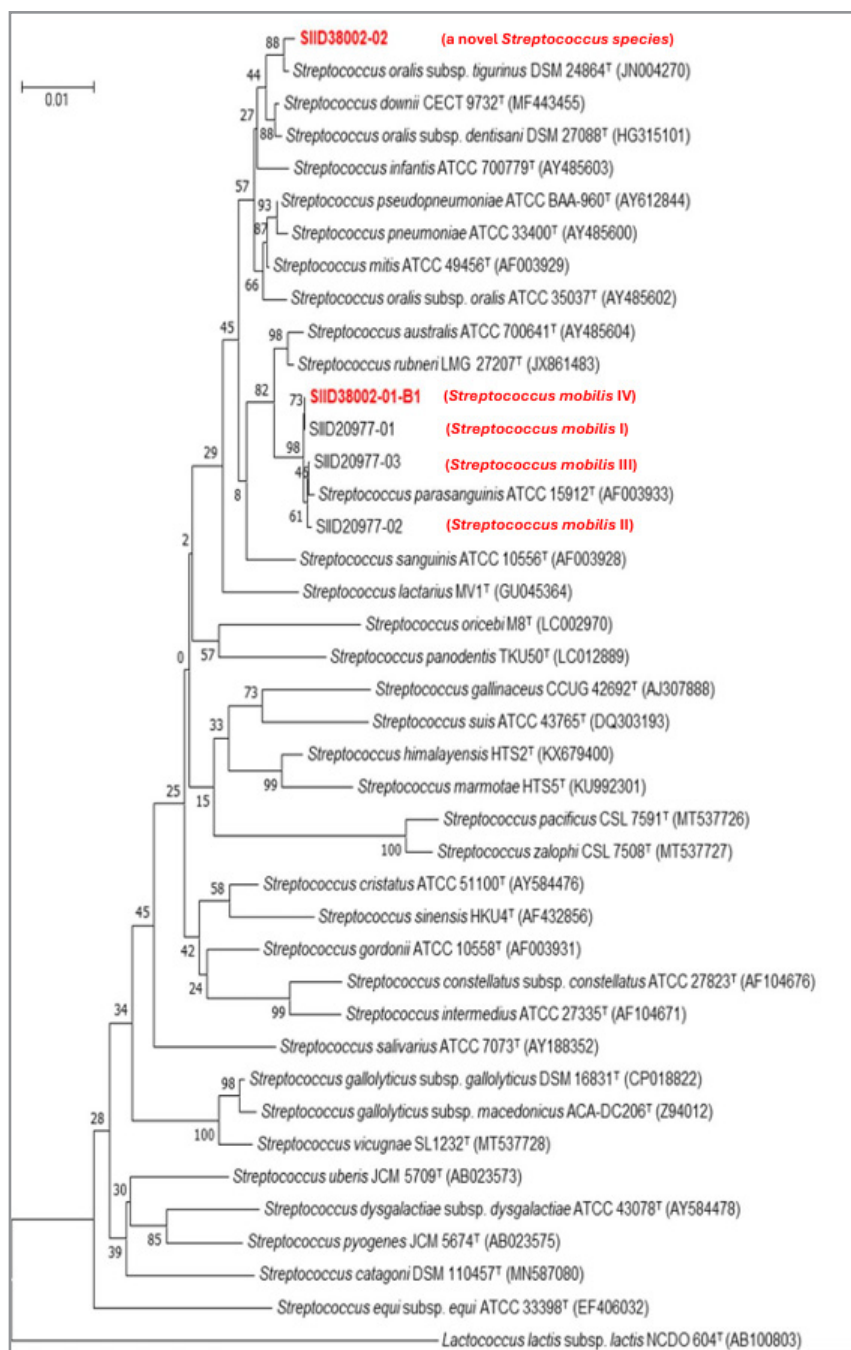


Figure 7: Neighbor-Joining Phylogenetic Tree Based on 16S rRNA Gene Sequences of the Isolates in Figure 6 (a, c).

Molecular studies have identified that the bacteria isolated from the wife represent a new strain of *Streptococcus mobilis* IV (SIID38002-01-B1). Unidentified Gram-positive coccoid bacteria isolated from the husband were identified as a new species closely related to *S. oralis* subsp. *tigurinus* (SIID38102-02). *S. mobilis* I (SIID20977-01), *S. mobilis* II (SIID20977-02), and *S. mobilis* III (SIID20977-03) are previous isolates. (Bootstrap percentages based on 1000 replications are given at nodes. Bar 10 changes per 1000 nucleotide position.) [5].

Conflict of Interest

The author declares that there are no conflicts of interest related to the publication of this article, nor any sources of support or funding.

Ethical Statement

The study was performed under the WMA Declaration of Helsinki and the WMA International Code of Medical Ethics.

References

- Hassan, O. (2023, May 18). Pathology of reactive gastropathy. Medscape. <https://emedicine.medscape.com/pathology>
- Genta, R. M. (2005). Differential diagnosis of reactive gastropathy. *Seminars in Diagnostic Pathology*, 22(4), 273–283. <https://doi.org/10.1053>
- Okada, T., & Adkins, G. (2017). Okadaella gastrococcus-like bacteria positive reactive gastropathy: Endoscopic, histochemical, immuno-histochemical, and electron microscopic studies. *Gastroenterology*, 152(Suppl. 1), S814 (Mo1899).

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4. Dixon, M. F., Genta, R. M., Yardley, J. H., & Correa, P. (1996). Classification and grading of gastritis: The updated Sydney System. *American Journal of Surgical Pathology*, 20(10), 1161–1181.
 5. Vyas, M., Celli, R., Singh, M., Patel, N., Aslanian, H. R., Boffa, D., ... & Jain, D. (2020). Intestinal metaplasia around the gastroesophageal junction is frequently associated with antral reactive gastropathy: implications for carcinoma at the gastroesophageal junction. *Human pathology*, 105, 67-73.