ISSN: 3066-7097
Research Article
Journal of Critical Care Research and Emergency Medicine

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

Takayuki Okada

Okada Medical Clinic. Suite 64, level 6, 101 Wickham Terrace, Brisbane,Queensland, Australia, 4000.

***Corresponding author:** Takayuki Okada, Okada Medical Clinic. Suite 64, level 6, 101 Wickham Terrace, Brisbane,Queensland, Australia, 4000.

Submitted: 01 April 2024 **Accepted:** 14 April 2025 **Published:** 21 April 2025

https://doi.org/10.63620/MKJCCREM.2025.1039

Citation: *Okada, T. (2025). Isolation of Streptococcus mobilis from a Japanese couple with Helicobacter pylori-negative chronic gastritis, intestinal metaplasia, and reactive gastropathy. J of Cri Res & Eme Med, 4(2), 01-08.*

Abstract

Reactive gastropathy associated with the infection of gastric microbiota has received limitedattention compared to Helicobacter pylori. We have reported the association between thethree types of classification for reactive gastropathy and unidentified coccoid bacteria. Thisstudy aimed to culture and isolate gastric microorganisms from H. pylori-negative Japanesecouples 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 Streptococcusoralis subsp. tigurinus were isolated from gastric biopsy specimens. After administering asequential combination of antibiotics over six weeks, the couple became asymptomatic withno adverse effects. Given the intracellular and intranuclear invasive characteristics of thebacteria S. mobilis, along with its production of DNase, hydrogen sulfide (H2S), and CHOtoxin, 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 significantlyenhances 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 Cgastritis, is the second most common type after H. pylori gastritis [1, 2]. The definitivediagnosis is made through the histological examination of a gastric biopsy, as endoscopicfindings are often nonspecific. The histological features of reactive gastritis include foveolarhyperplasia with tortuosity of elongated crypts, mucosal and lamina propria edema, capillarycongestion and vasodilatation in lamina propria, smooth muscle hyperplasia in laminapropria, and sparse chronic inflammatory cell infiltration [1, 2]. Reactive gastropathy isassociated with various factors such as bile, pancreatic secretions, alcohol, nonsteroidal anti-inflammatory drugs (NSAIDs), and other chemicals and medications [1, 2].

Althoughmicroorganisms in the gastrointestinal tract produce various chemicals, enzymes, and toxins,the role of gastric microbiota in causing reactive gastropathy, aside from H. pylori, has notbeen extensively studied. We previously reported an association among three classificationtypes of reactive gastropathy with unidentified coccoid bacteria; however, we could notsuccessfully isolate or molecularly identify the organism [3]. Reactive gastropathy has beendescribed as “the constellation of endoscopic and histological changes resulting fromchemical injury to the gastric mucosa” [2, 4]. A thorough evaluation of histological andendoscopic 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 andintranuclear invasive, acid-tolerant, Gram-stain-variable bacterium found in both H. pylori-positive and H. pylori-negative gastropathies. Its involvement in the gastric cancer cascadehas been suggested based on its intranuclear presence and various biochemical products,including CHO cytotoxin, DNase, and H2S, which may also contribute to reactive orchemical gastropathy. This study aimed to isolate gastric microorganisms from patients withreactive 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 dyspepticsymptoms and consented to undergo an esophagogastroduodenoscopy (EGD). During theprocedure, gastric biopsy specimens were collected from the pre-pyloric antrum and the midand proximal antrum for histological and microbiological examinations and from thegastroesophageal junction (GEJ). The gastric biopsy specimens from the stomach wereplaced in a 3 mL chocolate agar slope (bioMérieux) with 0.5 mL of BHI broth (Remel) and a3 mL chocolate agar slope containing 0.5 mL of sterile normal saline. Immediately after thecompletion of EGD, the biopsy specimens on the agar slope with BHI broth and the agarslope 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 theprocedure, the biopsy specimens were returned to their containers. The gastric aspiratecollected 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 containing0.5 mL of sterile normal saline or BHI broth and the chocolate agar plate without biopsyspecimens served as their respective controls. These specimens were incubated for 3~14 daysunder 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 achocolate agar plate with a disposable sterile probe, and incubated under anaerobic conditionsat 37 °C. Isolated bacteria from the biopsy specimens were subcultured under anaerobicconditions for 72 hours on 5% horse blood agar (bioMérieux) at 37°C. CampyGen™ (Oxoid)and AnaeroGen™ (Oxoid) were used for microaerophilic and anaerobicconditions,respectively. This study employed the Gram stain method, utilizing crystal violetandsafranin. 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 andGrampositive bacteria from the husband.

The antibiotics used for diffusion disc tests included azithromycin, chloramphenicol, colistin,gentamicin, doxycycline, erythromycin, kanamycin, metronidazole, suxamethonium, sodiumpolyanetholsulfonate (SPS), tetracycline, and vancomycin. For the E-tests, the antibioticsused were amoxicillin, amikacin, azithromycin, clarithromycin, cefotaxime, gatifloxacin,penicillin, rifampicin, and tetracycline.

According to their protocols, the couple's isolates underwent 16S rRNA gene sequencinganalysis and phylogenetic tree reconstruction at the TechnoSuruga Laboratory in Japan.H&E, Warthin-Starry silver (WSS) stains, and H. pylori immunohistochemistry wereperformed on biopsy specimens fixed in 10% formalin at Sullivan and Nicolades PrivatePathology for histological examination. H. pylori-positive specimens were used as controlsfor the WSS stain and immunohistochemistry methods-Based on their sensitivity responses to the isolated bacteria, the patients were treated withomeprazole 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 thehusband.The WSS stain revealed intracellular and extracellular S. mobilis-like bacteria(SMLB) in the squamocolumnarjunction. Figure 4a illustrates the endoscopic appearance ofthe wife's pre-pyloric antrum. The histologic examination identified reactive gastritis withintestinal metaplasia against a background of chronic gastritis (Fig. 4b). Multipleextracellular 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, andinterstitial space (Fig. 4d). Figure 5a shows the endoscopic appearance of the husband's pre-pyloric antrum. The histological examination revealed reactive gastritis with focal intestinalmetaplasia against mild chronic gastritis (Fig. 5b). SMLB were detected in the mucous andmucosa (Fig. 5c) under WSS stain. Dark-stained coccoid microorganisms, slightly larger thanSMLB, were found in the gastric pits of the specimens (Fig. 5c). The WSS staindemonstrated not only H. pylori but also, incidentally, intracellular SMLB in the controls, asshown in Figure 5d. H. pylori immunohistochemistry was negative in both cases. Bile refluxwas 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-negativereactions (Fig. 6b) and similar biochemical and antibiotic sensitivity results. The phenotypiccharacteristics of the isolates resembled those of S. mobilis: facultative anaerobic, mesophilic, alpha-hemolytic, catalase-negative, oxidase-negative, urease-negative, H2S-positive, andGram-stain-variable coccoid bacteria.

The tests used in this investigation were unable toidentify the isolated bacteria. Additionally, it was unfortunately not possible to continuesubculturing the isolate from her husband due to unknown reasons. The available tests alsodid 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 otherfrom the husband (Gram-positive bacteria) were sent to the TechnoSuruga laboratory in Japanfor molecular identification. 16S rRNA gene sequencing analysis of the bacteria isolated fromthe wife identified it as a new species of S. mobilis (Fig. 7). Furthermore, the analysisrevealed that the unidentified Gram-positive bacteria isolated from the husband represented anovel 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 gastropathyis illustrated in Figure 1. The endoscopic appearance may be normal (Fig. 1a) or show mild tomoderate gastritis without erosion, haematin, or petechial hemorrhage. The histologicaldiagnosis indicates mild reactive gastritis (Fig. 1b). WSS stain reveals numerous coccoidbacteria in the interstitial space, hyperplastic fibroblasts, and smooth muscle fibers (Fig. 1c).

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

Type III reactive gastritis is illustrated in Figure 3. The endoscopic appearance revealserosions, 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 diagnose intestinal metaplasia but not reactive gastritis.Numerous coccoid bacteria are observed on the mucosal cells and intestinal metaplasia usingWSS stain (Fig. 3e).

In previous investigations, intracellular SMLB were consistently found in H. pylori-positivespecimens under WSS staining, as shown in Figure 5d. Based on our studies over the past 30years, SMLB infection consistently coexists with H. pylori infection. However, H. pyloriinfection does not occur in the context of SMLB-associated gastric mucosal pathologies.Biopsy samples obtained from the couple revealed mild gastroesophagitis withoutmacroscopic evidence of bile reflux. In the wife's case, intestinal metaplasia in the cardiacould not exclude Barrett's esophagus. These findings supported the report of a tendency foran increased incidence of reactive gastropathy among patients with inflammation of GEJ [5].

Although the researchers concluded that bile reflux induced inflammation, our findingsobtained through special staining methods and electron microscopy suggested a possibleassociation between SMLB infection, gastroesophagitis, and Barrett's esophagus, which hasbeen presented at international meetings (unpublished). The couple was free from NSAIDs,smoking, and hepatorenal dysfunction. The wife did not drink alcohol but intermittently tookthe 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 bentirely excluded.

In the couple's cases, histological examination revealed areas of focal intestinal metaplasia intheir antral biopsy specimens. Mild reactive gastritis was also observed alongside mildchronic gastritis. Therefore, the classification is Type III reactive gastropathy for both casesSMLB 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 anunknown reason. However, the phenotypic features of the isolates from the couple had beenstudied prior to the loss of the husband's isolate. The results of the Gram stain, biochemicaltests, and sensitivity tests were identical.The phenotypic features of the isolated S. mobilifrom the couple suggested intrafamily transmission. However, this requires investigation andconfirmation through molecular studies.

To verify whether the bacteria found in the biopsyspecimens during the histological examination are S. mobilis, a monoclonal antibodyimmunohistochemistry or FISH (fluorescence in situ hybridization) test would provide theanswer.Considering the improvement in dyspeptic symptoms following sequential treatments, thisevidence further supports theassociation between Type III reactive gastropathy and S.mobilis infection. In addition to our previous efforts to isolate gastric microorganisms, at leasttwo bacteria, S. mobilis IV and a new species closely related to S. oralis subsp. tigurinuscould be associated with reactive gastropathy.

Therefore, further investigations are required toclarify the relationship between gastric microbiota and reactive gastropathy. This mayrepresent a rare case of reactive gastropathy with intestinal metaplasia in the context ofchronic gastritis. Type III re-

active gastropathy should be monitored regularly, as it can lead togastric cancer over time due to preneoplastic mucosal changes, particularly in populationswith a higher incidence of gastric cancer, such as the Japanese.

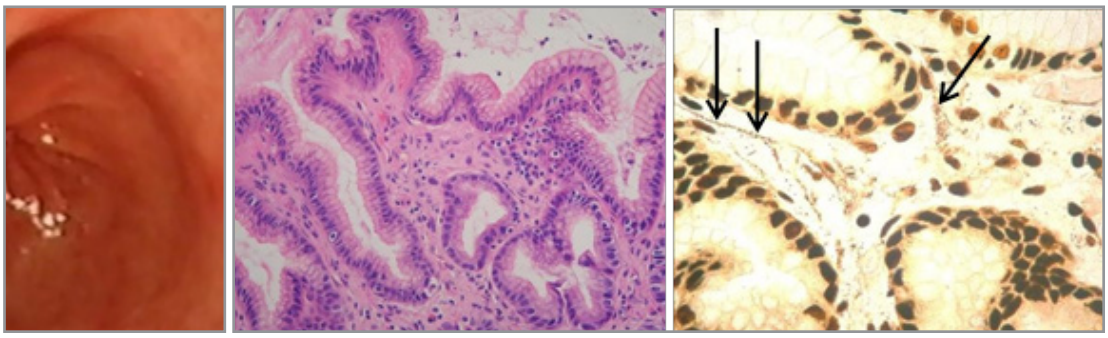


Figure 1: Type I Reactive Gastropathy.

Endoscopic View of The Antrum

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

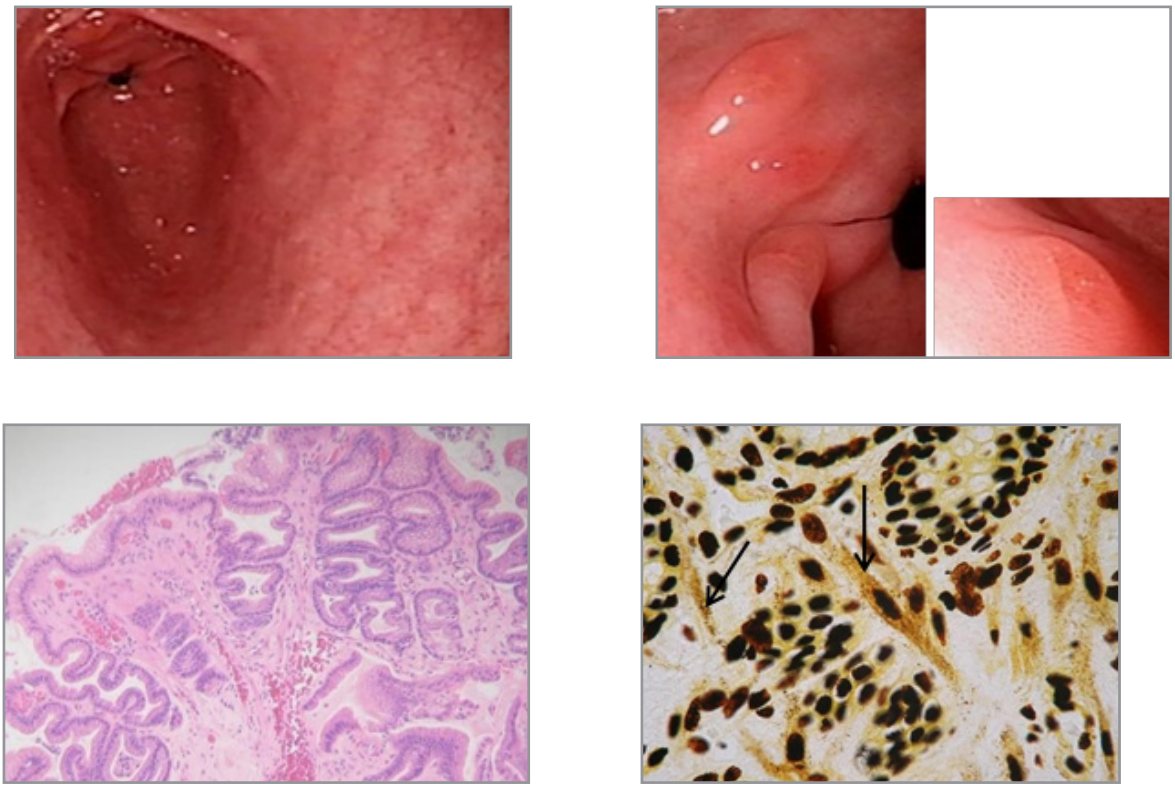
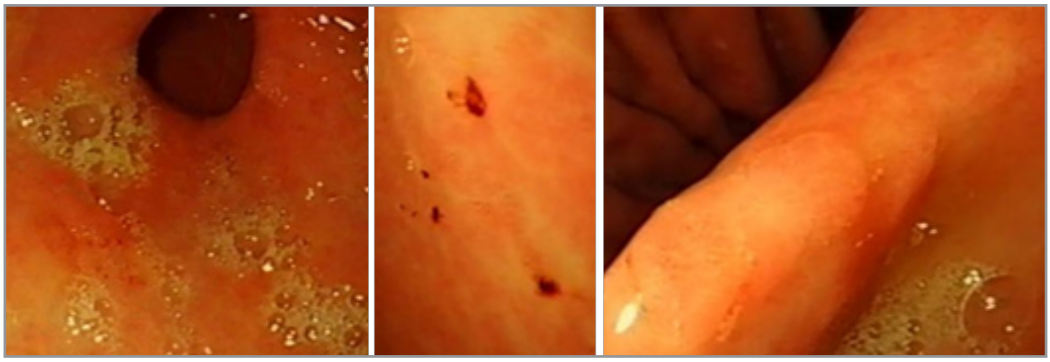


Figure 2: Type II Reactive Gastropathy.

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

- (a), as well as numerous elevated erosive lesions
- (b, c). There is noevidence of macroscopic intestinal metaplasia. Histological examination with H&E staining
- reveals moderate reactive gastritis
- (b). Multiple SMLB (arrows) in the interstitial space areassociated with smooth muscle fibers under WSS staining
- (c). SMLB can be observed in thegastric pit, peri-vascular area, and intravascular site (not shown). X: magnification of the
- objective lens.



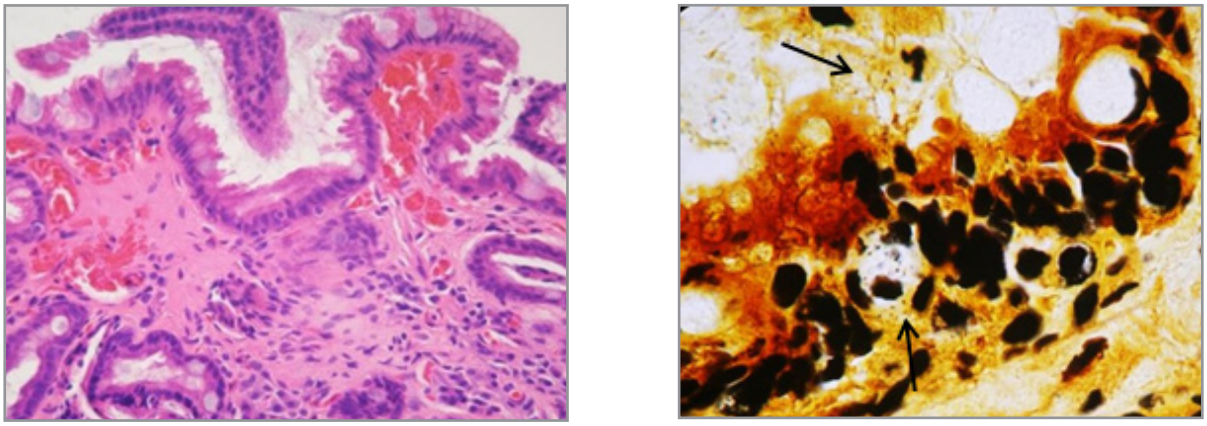


Figure 3: Type III Reactive Gastropathy.

The endoscopic view of the antrum reveals multiple erosive lesions

- The gastric mucosashows several petechial hemorrhages and hematins
- An area of intestinal metaplasia isobserved at the angular notch of the lesser curvature
- Histological examination with H&Estain 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.

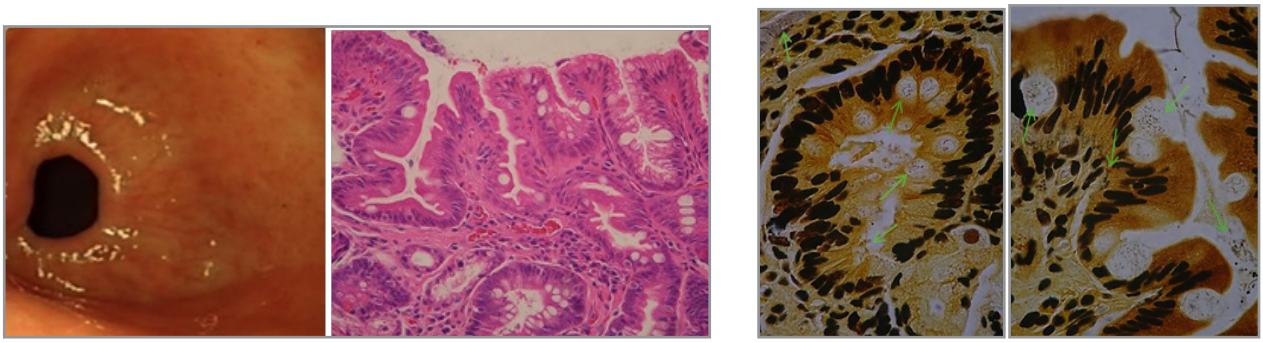


Figure 4: The Case of The Wife.

The endoscopic appearance of the pre-pyloric antrum is unremarkable

- The gastricmucosa has no erosion, petechial hemorrhage, or hematin. There is no macroscopic intestinal
- metaplasia. Histologic examination by H&E stain reveals reactive gastritis with intestinalmetaplasia in the background of mild chronic gastritis
- Multiple extracellular andintracellular SMLB (green arrow) are seen in intestinal metaplasia under WSS stain (c). SML B are found in the vessel
- SMLB (green arrow) are also observed in the lamina
- propria, interstitial space, and gastric pit of intestinal metaplasia
- H. pyloriimmunohistochemistry was negative (not shown). X: magnification of the objective lens.

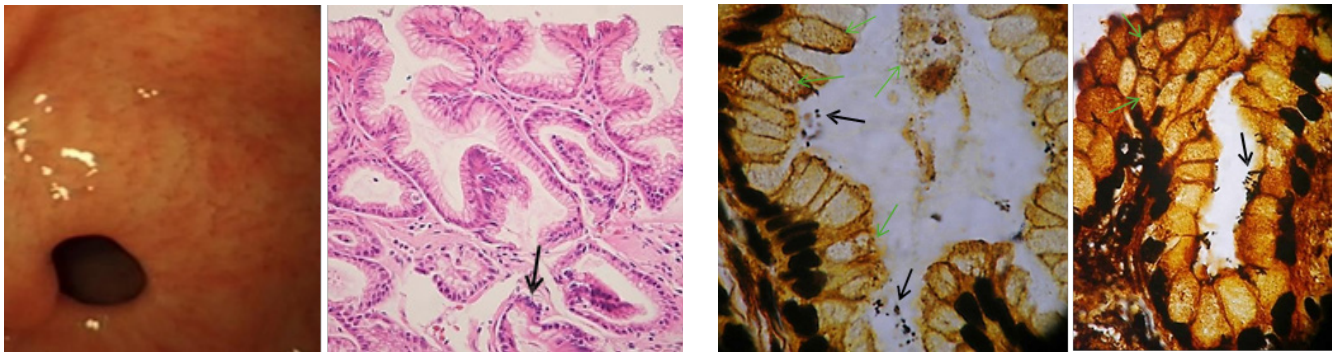


Figure 5: The Case of the Husband.

Endoscopic Appearance of The Pre-Pyloric Antrum

- Although it appears unremarkable,carefully inspecting the mucosa indicates superficial chronic gastritis. The gastric mucosashows 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 slightlylarger than SMLB, are seen in the mucous and on the epithelia of the gastric pits under theWSS stain
- WSS stain demonstrated H. pylori (black arrow) in the gastric pits of thecontrol specimen
- Incidentally, intracellular SMLB (green arrow) were seen in this
- specimen
- H. pylori immunohistochemistry was negative (not shown). X: magnification
- of the objective lens.

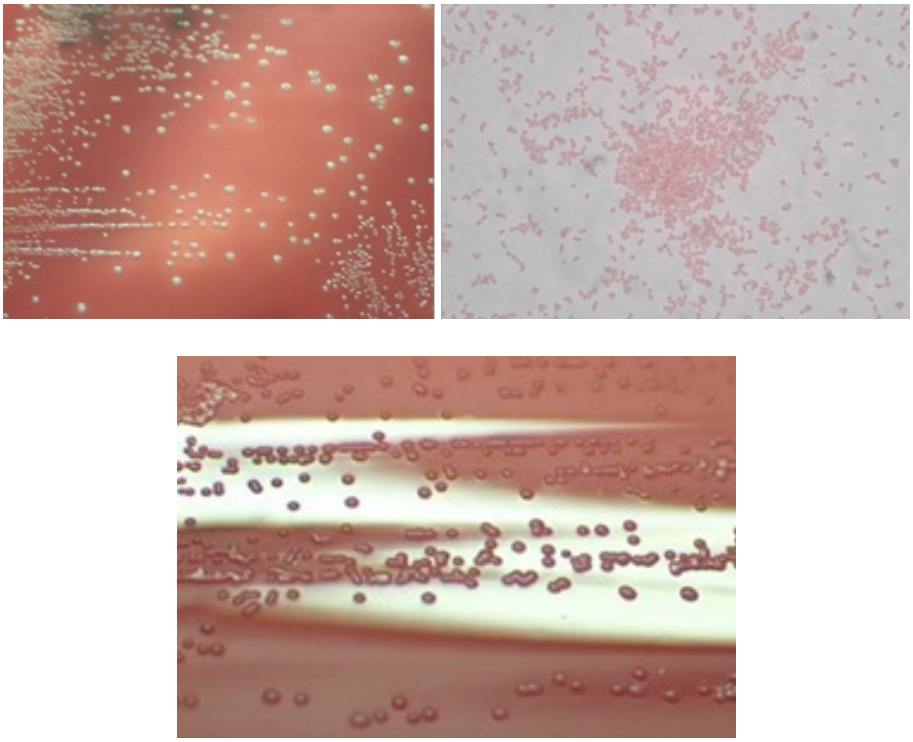


Figure 6: Cultured Colonies of Two Isolated Types of Bacteria.

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).

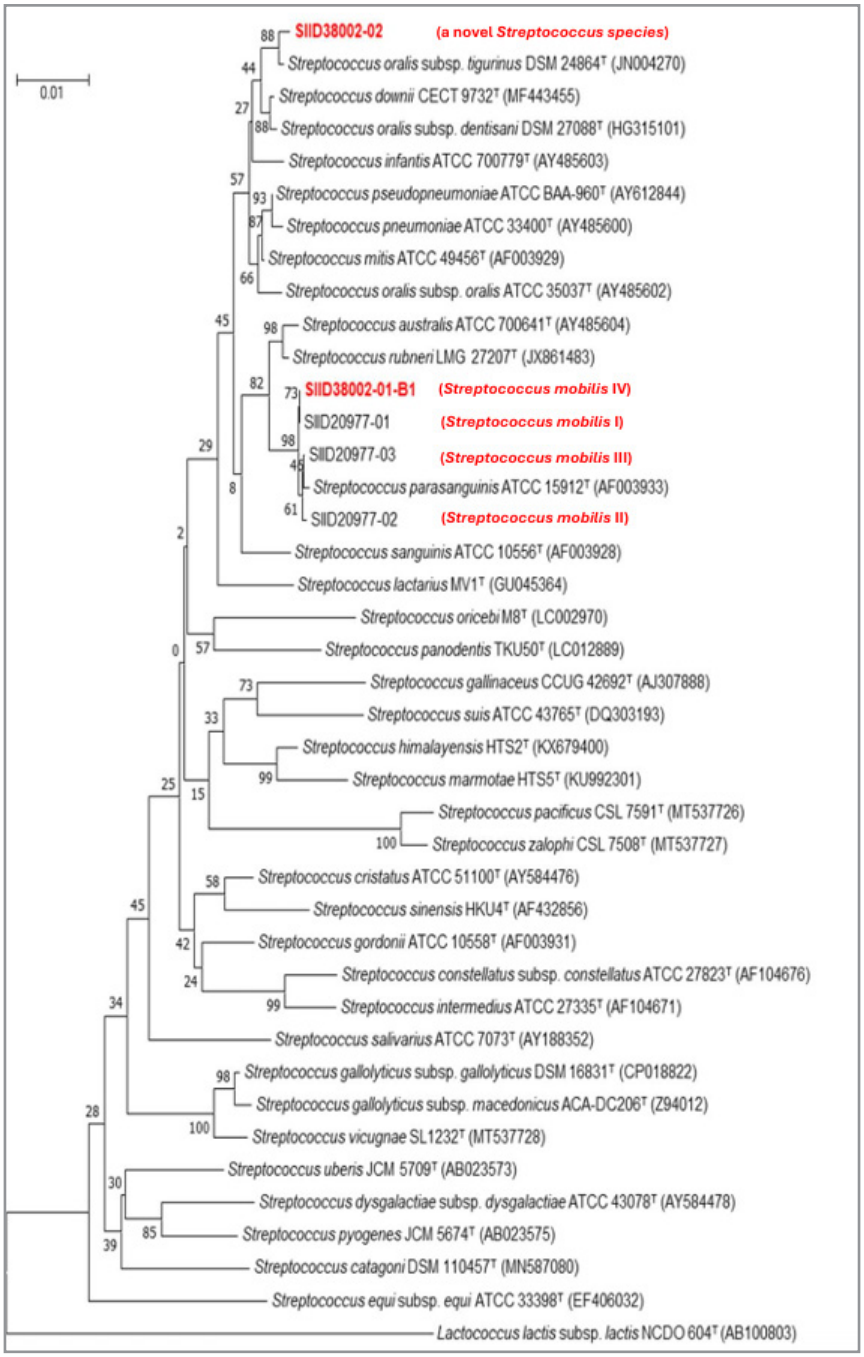


Figure 7: Neighbor-Joining Phylogenetic Tree Based on 16S rRNA Gene Sequences of the Isolates in Figure 6 (a, c).

Molecular studies have identified that the bacteria isolated from the wife represent a newstrain of Streptococcus mobilis IV (SIID38002-01-B1). Unidentified Gram-positive coccoidbacteria isolated from the husband wereidentified as a new species closely related to S. oralissubsp. 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 on1000 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

1. Hassan, O. (2023, May 18). Pathology of reactive gastropathy. Medscape. https://emedicine.medscape.com/pathology
2. Genta, R. M. (2005). Differential diagnosis of reactive gastropathy. Seminars in Diagnostic Pathology, 22(4), 273–283. https://doi.org/10.1053
3. 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).

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.