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***Sarcina* Organisms in the Gastrointestinal Tract: A Clinicopathologic and Molecular Study**

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Abstract

Sarcina organisms were first observed and recorded in the stomach contents of a patient with vomiting by John Goodsir in 1842. Since that time, the fine structure, phylogenetic classification, and biochemical characteristics have been described. While numerous cases of fatal disease have been attributed to this organism in the veterinary literature, only a few human cases have been documented. As a result, whether this organism causes disease in humans has not been definitively established. We report the clinicopathologic findings in a series of 5 patients with *Sarcina*-like organisms identified in upper gastrointestinal endoscopic biopsies with molecular confirmation. Based on our findings, the organism is most commonly found in patients with a history of gastric outlet obstruction or delayed gastric emptying. While many of the patients do not demonstrate direct mucosal injury from the organism, the presence of a concurrent gastric ulcer puts the patient at increased risk for complications such as emphysematous gastritis or perforation. The finding of *Sarcina* organisms should prompt further investigation for functional causes of gastric outlet obstruction and delayed gastric emptying, such as occult malignancy.

Keywords

Sarcina; gastrointestinal tract; gastroparesis; emphysematous gastritis; gastritis

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INTRODUCTION

Sarcina organisms were observed and documented by John Goodsir in 1842 in the stomach contents of a patient with gastric pain secondary to bloating and vomiting.(9) *Sarcina* organisms are nearly spherical cells, 1.8 to 3 micrometers in diameter and occur in tetrads or packets of 8 or more. This characteristic packeting is the result of cell division in at least two planes of growth.(4, 5) The distinctive packeted morphology and cell wall structure was so unusual that it was believed to be disease-causing vegetable matter. This original description of the organism drew the attention of other physicians and scientists who recognized a frothy vomit which was so characteristic of the presence of *Sarcina* that it was termed “sarcinous vomit”.(8) Even Charles Darwin became interested in the organism as possible explanation for his own gastric maladies.(1)

Sarcina was reported in numerous similar human cases(3), though it was not isolated in pure culture from the human stomach until 1911 when it was grown using strictly anaerobic techniques.(2) While scientists questioned the pathogenicity of the organism, it was eventually shown to occur ubiquitously in the air and soil, and has also been isolated from the feces of healthy humans, mainly from those subsisting on a vegetarian diet.(6, 15) Eventually the morphology and fine structure were detailed, and in the 1960’s the organism was definitively classified as a gram positive cocci.(11, 12)

Sarcina has been demonstrated as a causative organism in abomasal bloat and death of livestock, particularly sheep and goats.(7) Authors in the veterinary literature soon followed with descriptions of deadly emphysematous conditions and bloat in other animals.(17, 18) A few cases of human disease have also been associated with *Sarcina* organisms, including cases of emphysematous gastritis, peritonitis following gastric perforation, and gastric ulcer.(13, 16) The association of severe human disease with the *Sarcina* organism raises the question of whether the bacteria are pathogenic in humans. We have collected a series of 5 patients with *Sarcina* identified in endoscopic biopsies and report the clinicopathologic features, as well as perform DNA sequencing to confirm their identity.

METHODS AND MATERIALS

Identification of Cases and Review of Histology

This study protocol was reviewed and approved by the Johns Hopkins Institutional Review Board. Endoscopic mucosal biopsies containing *Sarcina*-like organisms were identified prospectively on routine hematoxylin and eosin stain during a 12-month period at Johns Hopkins Hospital from in-house routine endoscopic biopsies, consultation cases, and referral from colleagues at other academic institutions. Clinical and endoscopic reports on patients with biopsies containing *Sarcina*-like organisms were reviewed (length of follow-up: 3–12 months). Clinical and follow-up data were obtained from the referring pathologists and clinicians, when available.

In addition, a search was performed on all non-consultation biopsies and resections from 1990–2009 using the Johns Hopkins Hospital laboratory information system. A total of 145 sequential cases, including 25 gastric resections, were identified using the keywords “duodenal ulcer,” “duodenal mass,” “pyloric ulcer,” or “pyloric mass.” These cases were reviewed retrospectively for microscopic evidence of *Sarcina*-like organisms.

Microdissection and Polymerase Chain Reaction (PCR)

Sequential unstained 10 micron sections were cut from the corresponding paraffin blocks, yielding 20–50 sections for each case. These sections were deparaffinized and grossly microdissected with the aid of a dissecting microscope in the cases of histologically

suspected *Sarcina*-like organisms. Microdissected tissue was then placed into a sterile eppendorf tube with 340 µl ATL buffer and 60 µl of proteinase K and incubated at 56 °C overnight. The following day, DNA was isolated from the microdissected tissue using the Qiamp DNA Mini kit (Qiagen Valencia CA) and eluted into a final volume of 50 µl dH₂O as per manufacturer's protocol. Primers were designed using sequence data from GenBank to target proximal, mid and terminal regions of the *16S rRNA* gene (AF110272.1) and the pyruvate decarboxylase (*PDC*) gene (AF354297.1) of *Sarcina ventriculi* (Table 2).

Each PCR reaction was carried out in a 52 µl reaction mixture which included 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 45 µl of Platinum Taq Supermix (Invitrogen Carlsbad, CA) and 5 µl of DNA template. Touchdown PCR was performed with the following cycling conditions in an ABI9700 thermal cycler (Applied Biosystems Carlsbad, CA): 95°C, 5 minutes, 3 cycles (95°C, 10 sec; 64 °C, 10 sec; 70 °C, 30 sec), 3 cycles (95 °C, 10 sec; 62 °C, 10 sec; 70 °C, 30 sec), 3 cycles (95 °C, 10 sec; 60 °C, 10 sec; 70 °C, 30 sec), 3 cycles (95 °C, 10 sec; 58 °C, 10 sec; 70 °C, 30 sec), 3 cycles (95 °C, 10 sec; 56 °C, 10 sec; 70 °C, 30 sec), 41 cycles (95 °C, 10 sec; 52 °C, 10 sec; 70 °C, 30 sec), 72 °C, 7 minutes, 4 °C, hold. Confirmation of amplicon size was assessed by running 10 µl of PCR product on an 8% polyacrylamide gel (Invitrogen) with 1 µl of loading buffer (New England Biolabs Ipswich, MA) and visualizing the product under ultraviolet light after staining with ethidium bromide.

DNA sequencing and alignment

Sequencing products were purified using Exo-SAP-IT (Affymetrix Santa Clara, CA), and automated sequencing was subsequently performed using BigDye 3.1 and capillary electrophoresis on an ABI3700 (Applied Biosystems). Sequences were aligned and examined by visual inspection of the electropherogram to *16S rRNA* and *PDC* gene reference sequences, using Sequencher software (Gene Codes Corp., Inc.). Basic local alignment search tool (BLAST) comparisons were then performed to assess extent of homology to the reference sequences.

RESULTS

Clinical Features

The clinical and histologic findings are described in Table 1. Five patients were identified with a total of 6 biopsies containing *Sarcina*-like organisms; two patients were primary in-house cases, and the remaining three patients were referral cases identified by pathologists. The cohort was composed of 4 females and 1 male with an average age of 39 years (range 12–58 years). All patients had significant clinical symptoms of epigastric discomfort (n=5), epigastric pain (n=3), nausea and vomiting (n=2), dysphagia (n=1), and dyspepsia (n=1). In each case the clinical histories of these patients included either gastroparesis (n=2) and/or gastric outlet obstruction (n=4).

Of the four patients with gastric outlet obstruction, one was subsequently diagnosed with an obstructing mass-forming gastric adenocarcinoma (Patient 1); two had undergone prior gastric surgery (Patients 4 and 5); and one (Patient 3) had narcotic-related gastroparesis (Table 1).

The endoscopic findings also showed some similarities. All five patients had retained food at the time of upper endoscopy, including one patient with a bezoar. Other endoscopic findings included pyloric mass (n=3), stricture (n=1), gastritis (n=2), ulcer (n=1), and bile retention (n=1).

Clinical follow-up was available for 4 of the 5 patients and ranged from 3–12 months. One patient (Patient 1) was subsequently diagnosed with gastric adenocarcinoma of the pylorus. One patient (Patient 2) showed symptomatic improvement following a combination of proton pump inhibitor, H-2 receptor antagonist, and antiemetic/prokinetic. Another patient (Patient 3) received a jejunostomy tube for malnutrition and the last patient (Patient 5), did not receive medical intervention and continued to have painful epigastric spasms.

Pathologic Features

All patients showed the classic tetrad morphology associated with *Sarcina*-like organisms in gastric biopsies (Figure 1). Organisms were identified on routine hematoxylin and eosin stained slides and were primarily identified on the luminal mucosal surface epithelium, without direct evidence of mucosal reaction (Figure 2). Only one case showed organisms embedded within the gastric mucosa in the presence of acute inflammation and ulcer (Figure 3). None of the cases showed concurrent *Helicobacter* organisms. There was no common theme in the background gastric and duodenal mucosa. Two cases showed no diagnostic abnormalities aside from the presence of the organisms; two cases showed active or chronic duodenitis and two showed gastric intestinal metaplasia.

Review of Archived Cases

Histologic review of 145 sequential, non-consultation, in-house gastric biopsies and resections from 1990–2009 was performed to search for the presence of *Sarcina*-like organisms. *Sarcina*-like organisms were not identified in any of these retrospective cases.

Molecular Identification

The distinctive tetrad morphology we used to identify cases is not unique to *Sarcina*, as this feature can be seen in other common gastrointestinal microorganism such as *Micrococcus*. While there are other histologic qualities which differ, such as size and pattern of clustering, we sought independent, unbiased confirmation of the organism using *16S rRNA* sequence identification. Tissue was microdissected from the 6 histologically suspected samples and from an additional six stomach biopsies from matched histologically unremarkable control patients. PCR using primers targeting the proximal, middle and terminal *16S rRNA* gene was performed. Sequencing of the terminal *16S rRNA* amplicons from the 6 histologically positive cases revealed four to be most closely related to *Sarcina ventriculi* with three cases (patients 1 (both samples), and Patient 3) having 100% and the other (patient 4) 95% sequence homology. In the remaining two cases, one patient's (patient 5) *16S rRNA* amplified sequence showed 86% homology with *Sarcina*, and the other patient's (Patient 2) was most similar to *Lactobacillus* species. Upon histological reexamination of the latter case, there are multiple rod shaped organisms seen in addition to the *Sarcina*-like organisms. We surmise *Sarcina* organisms were only present in the first H&E reference slide, but not in the deeper unstained tissue sections from which the nucleic acid was isolated. Sequencing of the amplicons from the negative case controls showed no significant sequence homology to *Sarcina*. Sequencing of the proximal and mid *16S rRNA* in the majority of samples was generally mixed and of minimal use for classification purposes.

To further confirm the identity of this organism, we exploited a unique metabolic feature of *Sarcina*. The PDC gene product allows *Sarcina ventriculi* to convert pyruvate to acetaldehyde and carbon dioxide, and is present in few other bacterial species. Five of the histologically positive cases show a distinct 149 base pair amplicon from the *PDC* gene of *Sarcina ventriculi* (Figure 4). Sequencing all five amplicons revealed that each had a 96–97% sequence homology to the *Sarcina ventriculi* *PDC* (NCBI reference sequence AF354297.1). We did not detect this amplicon in any of the 6 histologically negative control cases. One case (patient 5), which showed marginal sequence identity using the *16S rRNA*

showed near perfect identity to the *Sarcina* PDC amplicon, which we took as evidence for the presence of *Sarcina* organisms. Criteria used to establish positivity for *Sarcina* organisms required that, in addition to histological evidence, two of the four amplicons, both that of the PDC and at least one of the *16S rRNA*, showed significant homology to *Sarcina ventriculi's 16S rRNA* and PDC sequence. Using these criteria, we concluded that of the six histologically suspected samples, 4 were positive and 1 was likely positive for the presence of *Sarcina*-like organisms.

DISCUSSION

Since the original description of *Sarcina* organisms in humans by Goodsir in 1842 (9), many observers speculated upon their significance; some have implicated the organism as pathogenic, while others have suggested the stomach as their natural habitat and their existence as a curiosity.(8) Even though there appears to be considerable interest in the organism during this time period, to the best of our knowledge, a definitive series of human cases has not been published. This is the first series to confirm the identity of *Sarcina* by DNA sequencing from formalin-fixed paraffin-embedded tissue, and to describe and correlate the clinical and pathologic findings in the upper gastrointestinal tract.

The scarcity of published literature on this organism raises the question of whether there was a decline in human cases since the original descriptions, followed by a recent resurgence. Our retrospective review of archived institutional pathology material over the past decade did not identify any additional cases of *Sarcina* organisms, supporting that the organism had not gone unrecognized or ignored in the recent past; however, we did identify a cluster of five cases over the relatively short course of 12 months in 2009–2010. In addition, the authors are aware of at least three additional cases that have been identified through consultation, following the completion of this study. This adds to the support of a recent reappearance of *Sarcina* in humans, especially among endoscopic biopsies.

The important clinical consideration is whether *Sarcina* is a contributory factor in the associated ulceration and mass formation, and therefore requires medical intervention, or whether it is simply a bystander in an underlying disease process. Given that two of the 6 biopsies showed no pathologic changes in the gastric mucosa, despite the presence of *Sarcina*, it is unlikely that *Sarcina* is the cause of ulceration or mass formation. However, it is also unlikely that the presence of *Sarcina* is merely an incidental finding and without consequence. All five patients in our series demonstrated retained food in the stomach at the time of endoscopy, either as a result of gastric outlet obstruction (due to mass or stricture) or gastroparesis. While it is unlikely that *Sarcina* is a cause of these functional disorders, the presence of these organisms may be considered a marker of delayed gastric emptying and a search for an underlying reason should be considered. For example, one patient was subsequently found to have an adenocarcinoma of the pylorus.

None of our patients showed the severity of disease that had been seen in earlier case reports of emphysematous gastritis and peritonitis. These three previously reported cases all showed underlying gastric outlet obstruction and ulcer formation.(13, 16) Two cases had prior medical intervention (bowel reduction secondary to malrotation, and a history of gunshot wound to the anterior chest) which might explain the gastric outlet obstruction. One patient developed gastric perforation and peritonitis, while the other two patients developed emphysematous gastritis. These two cases of emphysematous gastritis are the most provoking evidence that *Sarcina* can cause significant disease in humans, as the organisms are gas-forming fermenters and cause lethal gastric bloating-like syndrome in animals. However, given our data, it seems more likely that a pre-existing mucosal defect (such as an

ulcer) provided the nidus for emphysematous gastritis to develop, rather than direct invasion of *Sarcina* into the gastric wall.

Sarcina organisms' characteristic tetrad packaging is the result of cell division in at least two planes of growth.(4, 5) The organism is a gram-positive, non-motile, chemoorganotrophic anaerobe, having exclusively fermentative metabolism and being relatively aerotolerant.(10, 14) The characteristic cell wall is refractile under light microscopy and was the feature that caused the earliest observers to believe these organisms were vegetable matter. The main differential diagnosis on light microscopy is with *Micrococcus* species. *Micrococcus* shows a number of similar features, being a gram positive cocci that also occurs in tetrads or packets.(12) These organisms also have a substantial cell wall, and can resemble *Sarcina* on first glance. However, a number of features are helpful in differentiating the two organisms. For example, at 0.5 microns, *Micrococcus* is considerably smaller than *Sarcina*. In addition, *Micrococcus* species tend to form clusters (Figure 5), a feature not described among *Sarcina*.

The authors are not aware of other microorganisms with similar morphology, size and clustering; we believe the histologic features of *Sarcina* are singular enough that a diagnosis can be made on routine hematoxylin and eosin stain. The molecular confirmation of *Sarcina* in nearly all of our histologically suspected cases provides support for a straightforward histologic diagnosis. Of note, the organisms in all cases were quite sparse, and the disappearance of *Sarcina* from the block upon recut was experienced with at least one case. Additional special stains, such as Browns and Hopps may help to highlight the unique tetrad morphology, but is not necessary for diagnosis.

CONCLUSION

The presence of *Sarcina* organisms in gastric contents have been documented as early as 1842, but the significance of its presence has not been well established. Based on our findings, the organism is most commonly found in patients with a history of gastric outlet obstruction or delayed gastric emptying. While the organism does not appear to cause direct mucosal injury, the presence of a concurrent gastric ulcer may put the patient at increased risk for complications such as emphysematous gastritis or perforation. The finding of *Sarcina* organisms should prompt further investigation for functional causes of gastric outlet obstruction and delayed gastric emptying, such as occult malignancy.

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Figure 1. Sarcina organisms in a biopsy of the gastric antrum

Note the characteristic packeting of tetrads or octets with individual bacterial cells measuring up to 3 microns; the resulting tetrads are comparable to the size of a lymphocyte nucleus. (Brown and Hopps, original magnification 100 $\times$, oil objective.)

Figure 2. Sarcina organisms in the gastric lumen

The distinctive octets are present in the superficial gastric mucin without apparent mucosal reaction or invasion. This was seen in 5 of the 6 biopsies, suggesting that *Sarcina* may be present in many cases without causing mucosal injury. (Hematoxylin and eosin, original magnification 100×, oil objective.)

Figure 3. Sarcina organisms embedded in a gastric ulcer
(Hematoxylin and eosin, original magnification 100×, oil objective.)

Figure 4. Summary of gel electrophoresis of PCR products targeting *Sarcina PDC*
 (Lane 1) The ϕ X174 DNA-HaeIII DNA ladder highlights DNA fragments between 72 and 1,353 base pairs. (Lane 15) The 1kb DNA ladder highlights DNA fragments between 500 (white arrowhead) and 10,000 base pairs. (Lanes 2–7) PCR amplification reactions from patient samples from histologically suspected cases of *Sarcina*. Lanes 2 and 4–7 show the 149 base pair *PDC* amplicon (white arrow). Patient 2 (Lane 3) does not show amplification of the *PDC* target. (Lanes 8–13) PCR amplification reactions from histologically unremarkable gastric biopsy sample serving as negative controls. None of the negative control reactions show amplification of the *PDC* target. In the absence of *PDC* amplification a non-specific amplicon of ~120 bp is seen. (Lane 14) The water template negative PCR control shows no amplification of PCR products.

Figure 5. *Micrococcus* spp

These organisms were identified in a gastric biopsy. While they have similar packet formation as *Sarcina*, the packets are more tightly clustered. In addition, the individual bacterial cells are much smaller, measuring about 0.5 microns. (Hematoxylin and eosin, original magnification 1000 $\times$, oil objective.)

Table 1

Clinical and Pathologic Features of *Sarcina* in the Upper Gastrointestinal Tract

	Age Sex	Clinical Information	Symptoms	Endoscopic Findings	Histologic Findings	Clinical Follow-up	Follow-up Pathology
Patient 1 Biopsy 1	58 F	No significant history.	Nausea and vomiting.	Bezoar. Gastritis. Inflammatory mass (3cm) in pylorus with partial obstruction. Duodenum normal.	<i>Sarcina</i> organisms. Active chronic gastritis.	1 month: Repeat endoscopy with biopsies.	Repeat biopsy reported below.
Patient 1 Biopsy 2 (1 month later)	58 F	Gastric outlet obstruction with markedly distended stomach.	Epigastric pain. Nausea.	Pyloric mass with complete obstruction and retained food; unable to pass endoscope.	<i>Sarcina</i> organisms.	1 month: Partial gastrectomy for obstruction.	Gastric adenocarcinoma of the pylorus.
Patient 2	44 F	Type I diabetes with gastroparesis.	Postprandial dyspepsia and substernal burning.	Gastric ulcer with retained food. 3 pyloric polyps (0.3cm; 0.3cm; 0.8cm).	Non-malignant gastric ulcer with <i>Sarcina</i> organisms. Gastric hyperplastic polyps.	5 months: Omeprazole 20mg BID. Ranitidine 300mg. Metoclopramide 15mg TID. Symptomatic improvement.	No repeat biopsy.
Patient 3	36 M	Narcotic related gastroparesis and gastric outlet obstruction.	Intractable nausea and vomiting. Epigastric pain.	Retained food.	<i>Sarcina</i> organisms. Otherwise, no diagnostic abnormality.	6 weeks: Jejunostomy tube for malnutrition secondary to intractable nausea and vomiting.	Repeat biopsy: Negative for <i>Sarcina</i> organisms.
Patient 4	12 F	History of esophageal atresia, status post gastric pull through, with anastomotic narrowing.	Dysphagia.	Stricture at anastomosis. Retained food.	<i>Sarcina</i> organisms. Reflux esophagitis.	No follow-up available.	
Patient 5	46 F	History of pylorus sparing pancreaticoduodenectomy for pancreatic adenocarcinoma.	Painful epigastric spasms.	Bile and retained food.	<i>Sarcina</i> organisms. Active chronic duodenitis.	1 month: Continued spasms.	No repeat biopsy.

Table 2

Summary of Primer Sequences, Melting Temperatures and Expected Amplicon Sizes

Gene	Forward Primer Sequence (T _m °C)	Reverse Primer Sequence (T _m °C)	Amplicon size (base pairs)
<i>PDC</i>	5' AGCGGTTGCAGCGACAATAGGA 3' (59)	5' CTGCAACCAGCGCTGCACCT 3' (60)	149
Proximal <i>16S rRNA</i>	5' TGGAGAGTTTGATCCTGGCTCAG 3' (58)	5' CTGCTGCCTCCCGTA 3' (53)	343
Mid <i>16S rRNA</i>	5' GCCAGCAGCCGCGGTAA 3' (60)	5' CACGAGCTGACGACA 3' (52)	556
Terminal <i>16S rRNA</i>	5' CACACGCCCCGTACACCAT 3' (59)	5' TGATCCAGCCGAGGTTCTCCT 3' (59)	140