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Risk factors associated with upper aerodigestive tract or coliform bacterial overgrowth of the small intestine in symptomatic patients

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Abstract

The clinical relevance of bacterial types identified in small bowel aspirate cultures during diagnostic evaluation of small intestinal bacterial overgrowth (SIBO) is unclear.

Aim: To assess associations between risk factors for upper aerodigestive tract (UAT) or coliform SIBO and SIBO diagnosis by culture.

Methods: Small bowel aspirates were cultured in patients with suspected SIBO, defined as ≥ 10^4 colony forming units (CFU)/mL coliform or ≥ 10^5 CFU/mL UAT bacteria. History was reviewed for risk factors and potential SIBO complications. Symptoms, quality of life (QOL), psychological traits and laboratory values were assessed. We compared groups by two-sample t-test, Wilcoxon rank sum test, and Fisher's exact test. Overall associations of primary and secondary endpoints with type of bacterial overgrowth were assessed by ANOVA F-test, Kruskal-Wallis test, and Fisher's exact tests. Associations of risk factors with type of overgrowth were explored using multinomial logistic regression.

Results: Among 76 patients, 37 had SIBO (68% coliform, 33% UAT) and 39 did not. Conditions (p=0.02) and surgery (p<0.01) associated with decreased gastric acid were associated with SIBO. In multinomial logistic regression, conditions of decreased acid was associated with UAT SIBO [OR=5.8, 95% CI 1.4 – 33.3]. Surgery causing decreased acid was associated with UAT [OR=9.5 (1.4 – 106)] and coliform SIBO [OR=8.4 (1.6 – 86.4)]. Three patients with discontinuous small bowel had coliform SIBO [OR=17.4, (1.2 – 2515]. There were no differences in complications, overall symptoms, QOL or psychological traits.

Conclusions: Conditions or surgeries associated with decreased gastric acid are associated with SIBO diagnosis by culture.

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Keywords

risk factors; bacterial overgrowth; coliform

INTRODUCTION

Bacterial composition of the gut varies by location with the number of bacteria being greatest in the colon [10^{11} to 10^{12} colony forming units (CFU) per mL] and much lower in the small intestine (less than 10^3 CFU/mL).[1] Abnormal bacterial proliferation in the small bowel may give rise to small intestinal bacterial overgrowth (SIBO), a heterogeneous syndrome in which clinical manifestations may vary depending on disease severity.[2, 3] Alarm symptoms can include weight loss, iron deficiency anemia, fat-soluble vitamin deficiencies and severe diarrhea causing dehydration. Patients may also be asymptomatic or present with non-specific symptoms including abdominal pain, intermittent diarrhea, excessive flatulence, bloating and abdominal distention. It has been hypothesized that SIBO may play in an important role in gastrointestinal and hepatobiliary disease through multiple mechanisms including altered mucosal immunity, intestinal permeability, motility,[4] serotonin levels,[5] luminal sensing and nutrient digestion, and low-grade intestinal inflammation.[6,7] However, symptoms of SIBO may often overlap with associated conditions, and whether this relationship is a result of the primary condition that subsequently predisposes to SIBO or SIBO as the driving pathophysiologic abnormality causing gut dysfunction remains unclear.

Although SIBO is traditionally defined as positive bacterial cultures from small bowel aspirates with bacterial counts ≥ 10^5 CFU/mL, some experts have suggested that a lower cutoff of 10^3 CFU/mL be used, particularly when there is a predominance of colonic-type bacteria.[8, 9] However, review of the literature has demonstrated inconsistency across studies with some investigators using cultures with ≥ 5×10^3 CFU/mL [10] or even > 10^4 CFU/mL [8, 11] to define SIBO. Controversy in establishing an optimal cutoff arises from the known limitations of culturing small bowel aspirates including possible contamination by oropharyngeal flora, poor reproducibility and potential for false negatives with variable sampling of the small bowel.[9, 12–14] Alternate diagnostic evaluation includes breath testing, a noninvasive method that relies on the principal of hydrogen (H_2) and methane gas production from intestinal microbial fermentation with subsequent diffusion through the systemic circulation into the exhaled breath. [9, 15] It too, is plagued by shortcomings such as unclear recommendations for interpretation in the setting of elevated baseline H_2, inability to detect hydrogen sulfide by standard gas chromatography, alteration of H_2 levels with smoking and exercise and possibility of false positives due to rapid intestinal transit[16] or immediate microbial metabolism by oropharyngeal flora. [9, 17] Sensitivity and specificity of glucose hydrogen breath testing has been reported to range from 20–93% and 30–100% respectively while sensitivity and specificity of lactulose hydrogen breath testing has been reported to range from zero to 68% and 44 to 100%. [9, 18, 19] Thus, despite its known limitations, culture of small bowel aspirates currently remains the “gold standard” for diagnosis of SIBO until a truly validated gold standard is established.

The rationale for emphasizing the presence of coliform bacteria lies in the uncertainty regarding the clinical relevance of microbial organisms associated with the oropharynx and upper respiratory tract.[10, 20] Growth of upper respiratory tract or oral flora has not been clearly associated with symptoms in SIBO, while growth of colonic bacteria has been associated with absorptive defects in classical studies.[21] Factors predisposing to development of bacterial overgrowth derived from the oropharynx include intestinal slowing from narcotics[2] and gastric hypochlorhydria from proton-pump inhibitors (PPI) use or atrophic gastritis.[17] In some cases, overgrowth of gram-positive upper respiratory flora may occur as a normal process in aging. [22] Meanwhile, overgrowth of mixed or coliform bacteria may occur in states of intestinal stasis including small intestinal motility disorders (e.g. neuromuscular disorders, connective tissue disorders involving the small bowel, chronic intestinal pseudo-obstruction), abnormalities of small bowel anatomy (e.g. strictures, diverticula, discontinuous blind limb, and resection of the ileocecal valve).[2, 7]

Relatively little work has been performed examining differences in the clinical presentation of patients with colonic-type vs. oropharyngeal type bacterial overgrowth. The objectives of this study were to: (a) evaluate the frequency of SIBO, oropharyngeal or upper aerodigestive tract (UAT) SIBO and coliform SIBO in patients presenting with compatible symptoms, (b) investigate the association between type of bacterial overgrowth and traditional risk factors associated with SIBO, and (c) investigate the association between type of bacterial overgrowth and symptoms, quality of life and presence or absence of clinical complications related to SIBO.

MATERIALS AND METHODS

Study Design:

We conducted a single center prospective study among patients seen at the GI Motility Clinic at Indiana University from March 2013 to November 2015 and undergoing small bowel enteroscopy for diagnostic evaluation of suspected SIBO. The study was approved by the Institutional Review Board at Indiana University School of Medicine. Informed consent for study participation was obtained at the time of the upper enteroscopy.

Study participants and Eligibility Criteria:

All patients with suspected SIBO based on clinical presentation at the GI Motility Clinic were eligible for inclusion regardless of etiology. We excluded patients if they used prebiotics, probiotics, antibiotics, or bowel cleansers within the preceding 30 days, were pregnant, or unable to provide informed consent.

Experimental Protocol:

Demographic data and symptom assessments were obtained upon enrollment. Clinical history was obtained during medical assessment to determine presence or absence of traditional risk factors associated with SIBO and to identify the presence or absence of clinical complications associated with SIBO. Serum samples were obtained prior to or at the time of upper enteroscopy as part of routine clinical care for laboratory assessments.

Subjects underwent upper enteroscopy for luminal aspiratory and mucosal biopsies as part of routine diagnostic evaluation.

Assessment of risk factors:

Risk factors for UAT SIBO included prior surgery associated with decreased acid production (e.g. Billroth I or II, vagotomy, gastric bypass for obesity) or conditions associated with decreased acid production (e.g. *H. pylori* infection, atrophic gastritis, and daily use of PPIs). Risk factors for coliform SIBO included history of neuromuscular and connective tissue disorders (e.g. scleroderma, polymyositis, mixed connective tissue disease, systemic lupus erythematosus, chronic intestinal pseudo-obstruction, radiation enteropathy, visceral neuropathy, myopathy based on pathology), prior ileocecal valve resection or discontinuous small bowel (e.g., true blind limb or small bowel diverticulum).

Clinical complications of SIBO and laboratory assessments:

Presence of any of the following in the prior 6 months were determined at the time of enrollment: a) unintentional weight loss >10% from baseline, b) evidence of iron, fat-soluble vitamin or B12 deficiencies, c) unintentional weight loss requiring nutritional support via total parental nutrition (TPN) or enteric feeding tube, d) diarrhea causing dehydration requiring intravenous fluids, e) diarrhea causing electrolyte abnormalities or f) diarrhea causing acute renal failure. Serum IgA, anti- transglutaminase IgA antibody, fat-soluble vitamin levels, Vitamin B12, folate, protime (INR), ferritin and iron levels were measured. Fat-soluble vitamin deficiencies of A, D, E, or K were defined by a serum level less than our laboratory's lower limit of normal.

Characterization of symptoms:

A validated Patient Assessment of Gastrointestinal Disorder-Symptoms Severity Index (PAGI-SYM) questionnaire was used to quantify symptoms of functional dyspepsia, gastroparesis and gastroesophageal reflux.[23] Patients were queried about the presence of abdominal distension, defined as daily sensation of increased abdominal girth that progressed from morning to evening despite fasting and symptoms of functional diarrhea [i.e. loose (mushy) or watery stools > 75% of the time], based on Rome III criteria.[24] Patients also completed the Rand 36-item SF quality of life (QOL) health survey (SF-36),[25] the Hospital Anxiety and Depression Scale (HADS), [26] and the System Checklist 90R (SCL90).[27]

Upper Enteroscopy with Luminal Aspiration and Mucosal Biopsy:

Patients were allowed to continue prescribed PPIs and histamine blockers and instructed to fast for 12 hours prior to upper enteroscopy. Those with gastroparesis were on a full liquid diet for 48 hours before testing. Prior to enteroscopy, subjects rinsed their mouths with 20 ml of sodium fluoride (21.6% alcohol) to minimize contamination from oral flora. A pediatric colonoscope (11.3 mm diameter) or a small caliber upper enteroscope (9.2 mm diameter) was advanced past the ligament of Treitz into the jejunum without attaching the suction tubing to minimize contamination. An aspiration catheter was introduced through the working channel and attached to suction to collect at least 2 ml of luminal fluid. No fluid

was added to the lumen to increase the quantity of our aspirate. Aspiration was aborted and suction tubing reattached if undigested food was encountered. Insertion distance into the jejunum was recorded after careful reduction of looping of the enteroscope. Fluid samples were drawn into a sterile syringe. Air collected within the syringe was expelled and the syringe was capped and transported to the microbiology laboratory within 1 hour of collection. Upon endoscope withdrawal, six mucosal biopsies were taken from the proximal jejunum and duodenum to assess for celiac disease or malabsorption and two mucosal biopsies each from the antrum and gastric body to assess for *H. pylori*. Insertion distance to the pylorus or gastro-enteric anastomosis was recorded and small bowel insertion length was defined as the insertion distance to the jejunum minus the insertion distance to the pylorus or gastro-enteric anastomosis.

Microbiological analysis of small bowel aspirates:

Bacteria were cultured for aerobic and anaerobic bacteria from small bowel aspirates using standard techniques. Aliquots were plated on blood agar, MacConkey agar, chocolate agar and colistin and nalidixic acid (CNA) agar plates and incubated for a minimum of 48 hours. Quantification was performed by counting total CFU per mL of individual bacterial species in cases of growth. Culture-verified coliform SIBO was defined as $\geq 10^4$ CFU/mL of colonic-type bacteria (*Escherichia, Klebsiella, Proteus, Acinetobacter, Enterobacter, Citrobacter, Bacteroides or Clostridium* spp). The $\geq 10^4$ CFU/mL cut-off was chosen to maximize sensitivity for diagnosis of coliform SIBO. Lower cutoff values have been associated with increased SIBO prevalence compared to $\geq 10^5$ CFU/mL[28] UAT SIBO was defined as $\geq 10^5$ CFU/mL of oropharyngeal or aerodigestive tract bacteria (*Streptococcus, Staphylococcus, Enterococcus, Lactobacillus, Fusobacterium or Peptostreptococcus* spp). If the culture results revealed both coliform and UAT SIBO, then the subject was classified as having coliform SIBO.

Assessment of small bowel biopsies:

Mucosal biopsy specimens were formalin-fixed, paraffin-embedded and then stained with hematoxylin and eosin for histological examination of villus height, crypt depth and intraepithelial lymphocyte counts. Presence or absence of *H. pylori* was assessed on gastric biopsies by histopathologic exam and addition of immunohistochemical stain when indicated.

Statistical Analyses:

The primary endpoint was presence or absence of predisposing risk factors associated with coliform and UAT SIBO. Secondary endpoints were demographics, endoscopic characteristics, presence or absence of clinical complications, symptoms, laboratory assessments, QOL scores and HADS scores.

Data are summarized using mean (±standard deviation, SD) values for normally distributed continuous variables, median (interquartile range, IQR) values for skewed continuous variables, and frequency (proportions) for categorical variables. Comparisons between SIBO and no SIBO groups were performed using the two-sample t-test for normally distributed continuous variables, Wilcoxon rank sum test for skewed continuous variables, and Fisher’s

exact test for categorical variables. Univariate associations of secondary endpoints with patient group (coliform SIBO, UAT SIBO, and no SIBO) were also performed using the ANOVA F-test for normally distributed continuous variables, the Kruskal-Wallis test for skewed continuous variables, and Fisher's exact test for categorical variables. Missing data were excluded.

To examine the associations of each individual risk factor, any coliform risk factor and any UAT risk factor with final diagnoses of coliform or UAT SIBO, we performed exploratory multinomial logistic regression with a generalized logit link after adjusting for age due to the difference in age across the three groups (coliform SIBO, UAT SIBO, no SIBO). Parameter estimation was obtained using the penalized approach due to the small sample size.[29–31] All statistical analyses were 2-sided at the 5% significance level, performed using SAS 9.4 (*SAS* Institute, Cary NC) and the PMLR package in R (Version 1.0, 2010).

RESULTS

Baseline characteristics and bacterial cultures:

Eighty-six patients signed informed consent. Luminal aspiration was not obtained for 10 patients (12%), due to gastric bezoars found during enteroscopy (n=6), duodenal stricture (n=1), refusal of enteroscopy (n=2) and altered anatomy with inability to identify the efferent small bowel limb (n=1). In the cohort of 76 patients (92% females, 95% Caucasians, mean age 50.4±12.7 years) who completed upper enteroscopy with luminal aspiration, 37 patients had culture verified SIBO (25 coliform SIBO, 12 UAT SIBO) and 39 patients did not have evidence of SIBO by culture. The most common bacterial species cultured were *Streptococcus viridans* for UAT SIBO and *Escheria coli* or *Klebsiella pneumonia*e for coliform SIBO (Figure 1). There was a borderline association between age and diagnosis of SIBO (p=0.07), with SIBO patients being older than non-SIBO patients. There were no significant differences in gender, ethnicity, or body mass index between SIBO patients and non-SIBO patients (Table 1). The most common chief complaints were bloating or distention (50%), abdominal pain (39%), nausea (41%), vomiting (29%) and early satiety (18%). No patients had celiac disease by anti-transglutaminase IgA antibody or small intestinal mucosal atrophy. Seven patients had increased intraepithelial lymphocytes of unknown significance. Mean small bowel insertion length was 43 cm (range 10 to 85 cm). There were no endoscopic complications.

Risk factors for coliform and UAT SIBO:

The proportion of SIBO and non-SIBO patients with traditional risk factors associated with coliform and UAT SIBO are summarized in Table 1. Among patients (n=41) who had conditions associated with decreased acid secretion, 37 were on a daily PPI, three had *H. pylori* and none had atrophic gastritis. Presence of a condition associated with decreased acid was observed more frequently in patients' with SIBO vs. no SIBO (67.6% vs. 41%, p=0.024). Exploratory analysis with multinomial regression after adjusting for age demonstrated an increased odds ratio (OR) of having UAT SIBO relative to no SIBO (OR=5.8, p=0.01) in those with conditions associated with decreased acid (Table 2). There

was no significant increase in the likelihood of coliform SIBO relative to no SIBO and patients were no more likely to have UAT SIBO relative to coliform SIBO.

Prior surgery associated with decreased acid was also correlated with SIBO status, occurring more frequently in those with SIBO vs. no SIBO (27% vs. 2.6%, $p<0.01$). In multinomial logistic regression, increased ORs of UAT (OR=9.5, p=0.02) and coliform SIBO (OR=8.4, p=0.01) relative to no SIBO were observed in patients with prior surgery associated with decreased acid. However, there was no significant difference in the likelihood of UAT vs. coliform SIBO (OR=0.9 p=0.89). Presence of any traditional risk factor previously associated with UAT SIBO was associated with a diagnosis of SIBO by culture ($p<0.01$). Multinomial regression analysis revealed significantly increased ORs of UAT (OR=9.6, $p<0.01$) or coliform (OR=2.9, $p<0.05$) SIBO relative to no SIBO with the presence of any traditional UAT risk factor, but no significant difference in the likelihood of UAT vs. coliform SIB0 (OR=0.3, p=0.19).

A trend towards an association between discontinuous small bowel and SIBO status was observed, with discontinuous small bowel present in 3 patients with SIBO but zero patients without SIBO (p=0.11). In multinomial regression analysis, discontinuous small bowel was significantly associated with coliform SIBO (OR=17.4, p=0.033) relative to no SIBO but not with UAT SIBO. No other risk factors traditionally associated with coliform SIBO were associated with SIBO status in this study cohort.

Clinical complications and laboratory assessments:

Clinical complications associated with SIBO were observed in both SIBO and non-SIBO groups with at least one complication present in 50% of patients. In the entire study cohort, there were 22 patients with unintentional weight loss > 10%, 19 with vitamin deficiency, seven with weight loss requiring enteric feeding or TPN, 10 with diarrhea causing dehydration, five with diarrhea causing electrolyte abnormalities and one patient with diarrhea causing acute renal failure. Twenty-one patients were on daily supplements for vitamins A, D, E, B12 or iron. There were no significant differences in the frequency of any of the clinical complications between SIBO and non-SIBO groups. Univariate analyses revealed no significant association between any of the individual clinical complications and SIBO diagnosis by type of bacterial overgrowth.

Laboratory assessments revealed a borderline association between serum immunoglobulin A (p=0.05) and a significant association between ferritin levels ($p<0.01$) and SIBO status with higher IgA and ferritin levels observed in patients with SIBO. Both levels; however, were still within the normal ranges. Univariate analyses between laboratory values and SIBO group by type of bacterial overgrowth showed a significant association between immunoglobulin A and ferritin levels and SIBO group by type (p=0.03 and $p<0.01$, respectively) with highest mean values observed in patients' with coliform SIBO.

Symptoms, quality of life and psychological traits:

Self-reported PAGI-SYM total and subscale scores, symptom duration, presence of diarrhea and presence of daily abdominal distension for each SIBO group by type of bacterial overgrowth are presented in Table 3. There were no significant associations between

symptom duration, diarrhea, or abdominal distention and SIBO diagnosis or SIBO group by type. There were no significant associations between total PAGI-SYM and SIBO diagnosis or SIBO group by type. No significant differences were seen in PAGI-SYM subscale scores between SIBO and non-SIBO groups. However, in univariate analyses of subscale scores with SIBO group by type (UAT, coliform and no SIBO), PAGI-SYM subscales scores for postprandial fullness/early satiety were significantly associated with SIBO group by type ($p<0.05$) while a borderline association was observed for PAGI-SYM subscale scores for heartburn/regurgitation ($p=0.06$) and bloating ($p=0.06$). Results of HADS, SF-36 QOL, and SCL90 for each SIBO group by type of bacterial overgrowth are presented in Table 4. There were no significant associations between HADS, SF-36 QOL or SCL90 scores and SIBO diagnosis or SIBO group by type.

DISCUSSION

In our study of 76 patients with suspected SIBO, 49% of patients with compatible symptoms had evidence of SIBO based on culture of small bowel aspirates. Conditions associated with decreased gastric acid were associated with UAT SIBO. Surgery associated with decreased acid was associated with both UAT and coliform SIBO, while discontinuous small bowel was associated with coliform SIBO. These findings are consistent with existing literature implicating structural causes and reduced gastric acid secretion in SIBO pathogenesis.[2, 7, 32] Our findings suggest that not only is SIBO common among patients presenting to our tertiary referral center, but hypochlorhydria and abnormal small bowel anatomy may perhaps be among the more relevant factors predisposing individuals to bacterial overgrowth.

Among patients with a condition associated with decreased acid, the majority (39 of 41) were on a daily PPI. Thus, the observed association likely reflects the relationship between hypochlorhydria caused by PPI-use and UAT SIBO. Three patients had documented evidence of *H. pylori*, but none had atrophic gastritis. We are not able to exclude the possibility antral-predominant infection that has be associated with increased acid secretion. [33] However, no patients were noted to have peptic ulcer disease at the time of endoscopy, suggesting that acid levels were not pathologically excessive. Previous studies have shown similar findings supporting the relationship between SIBO and PPI-use including a recent meta-analysis of 11 studies.[34] Lombardo et al. also found SIBO to occur more frequently in patients treated with PPI, with increasing prevalence associated with longer duration of use, [35] the latter which was not specifically examined in our study. Others have shown both oral and fecal-type SIBO in patients treated with omeprazole, [36] and an association between PPI-use and SIBO by culture of small bowel aspirates.

Surgeries reducing acid secretion, such as Roux en Y gastric bypass (RYGB), may modify small intestinal microbiota due to induction of bacterial stasis and decreased acid secretion from reduced gastric size leading to a loss of antimicrobial effects.[37] Our findings are in line with such proposed mechanisms as surgery associated with decreased acid was associated with both UAT and coliform SIBO in our cohort. Prior evaluation of RYGB patients with breath test demonstrated increases in SIBO prevalence after RYGB from 15 to 40%. This increase was associated with decreased weight loss, but not with presence of vitamin deficiencies or symptoms.[38] Others have suggested SIBO prevalence rates as high as 81% in

symptomatic RYGB patients.[39] The correlation with symptoms remains unclear as digestive symptoms in patients after bariatric surgery are common, while asymptomatic SIBO may occur in both obese and post-bariatric populations.[40]

There were only three patients with discontinuous small bowel and all had coliform SIBO. Given the small sample size, OR estimates should be interpreted with caution. However, findings suggest evidence towards an association that will require further validation in larger study cohorts. Of the risk factors identified in our study, it is yet to be determined which has the greatest impact on clinically relevant SIBO and what potential interactions may exist between them.

Given the lack of data comparing symptoms and clinical presentation between UAT and coliform SIBO, we attempted to explore these concepts. We were unable to show significant differences in these outcomes by SIBO diagnosis (SIBO vs. no SIBO). Also, we did not specifically measure treatment effects or clinical response in SIBO patients who were given antibiotics by their treating gastroenterologist. However, assessment of associations of PAGI- SYM subscale scores with the SIBO groups by type of bacterial overgrowth revealed a significant difference in postprandial fullness/satiety, although highest scores were noted in the non-SIBO group. There was also a trend towards higher bloating and heartburn/ regurgitation scores in the UAT SIBO group. It is important to note that our study was not powered nor designed for these endpoints. A cause and effect relationship between SIBO diagnosis and symptoms is difficult to prove. Although our results may suggest that symptoms do not accurately predict SIBO diagnosis by culture, our inability to show significant differences may also be related selection bias. All patients undergoing luminal aspiration were referred for testing due to a high index of clinical suspicion, which may have affected our ability to differentiate appreciable differences between groups. It is also plausible that the observed lack of difference may suggest that differentiation between UAT and coliform SIBO may not be as clinically relevant as previously assumed, although the true challenge may lie in differentiating the genera that colonize the UAT vs. lower GI tract as overlap may occur and many genera may occupy both sites. Furthermore, only 30% of intestinal bacteria can be cultured, and current techniques may not accurately represent microbial diversity.[7, 41] Future studies utilizing culture-independent techniques may serve to address these gaps.

Despite a lack of differences in symptoms or clinical complications, one interesting observation in our study was increased serum IgA levels in SIBO patients. Several patients also had elevated IELs, although the association with SIBO group was not specifically analyzed. Riordan et al. previously reported increased plasma IgA cell counts within the lamina propria of SIBO patients as well as increased IEL counts in subjects with colonic-type overgrowth. However, investigators did not compare symptoms between subjects with oropharyngeal-type vs. coliform SIBO. Whether these findings may suggest differential immune-mediated effects of UAT vs. coliform SIBO is yet unknown.

Strengths of this study include standardization of endoscopic techniques and collection of luminal aspirate by two experienced gastroenterologists, in-depth prospective assessment of clinical history, symptoms, QOL, psychological traits and laboratory assessments. It is the

first study attempting to carefully characterize clinically important outcomes in patients with evidence of UAT vs. coliform SIBO.

Study limitations include potential exposure of anaerobic culture specimens to an aerobic environment, lack of enrollment of healthy volunteers, possible referral bias, small sample size and lack of direct assessment of gastric acid. Lack of anaerobic technique during endoscopic sampling, may have actually led to an underestimation of SIBO prevalence in our study cohort. We did not recruit healthy volunteers due to ethical considerations of performing an invasive endoscopic procedure for the purposes of a pilot investigation. Enrollment of controls should be included in next steps with study of larger patient cohorts of adequate power. In addition, we did not use hydrogen breath testing as a comparator, which is the more common technique used in clinical practice as it is less invasive and more cost effective. We elected to utilize small bowel aspirates only due concerns regarding the poorer sensitivity and specificity of breath testing for SIBO. Although referral bias may imply that findings are not be generalizable to patients in the community, our results are consistent that those reported in the literature and thus, we suspect the role of referral bias to be of limited consequence. Furthermore, we acknowledge the inherent limitations of current culture techniques as previously discussed. We elected to define coliform SIBO by bacterial counts $\geq 10^4$ CFU/mL. A wide range of bacterial counts have been used to define SIBO[8] and the validity of the $\geq 10^5$ CFU/mL threshold has been questioned by many. Some have used bacterial counts $> 10^3$ CFU/mL, [42] $\geq 5 \times 10^3$ CFU/mL [10] or even $> 10^4$ CFU/mL [8, 19] to define positive results. Thus, we attempted to maximize test specificity by defining coliform SIBO as the presence of coliform bacteria in quantities of $\geq 10^4$ CFU/ml. It is possible that using a more stringent cutoff of 10^5 CFU/mL to diagnose coliform SIBO could impact results. However, others who have reported similar associations between risk factors such as PPI-use or dysmotility and SIBO diagnosis found these factors to be significant with both the lowest (10^3 CFU/mL) and highest (10^5 CFU/mL) bacterial counts.[28]

In conclusion, our findings suggest that hypochlorhydria from PPI-use, prior gastric surgery associated with decreased acid production and discontinuous small bowel increase the risk of UAT and coliform SIBO. We were unable to show significant differences in symptoms or clinical presentation based on the final diagnosis by culture of proximal jejunal aspirates. Understanding the relevance of type of bacterial overgrowth remains challenging, particularly given the limitations of available techniques. Future studies will require larger study cohorts with the utilization of novel techniques with incorporation of detailed clinical and symptom assessments to understand the role of SIBO in GI disease.

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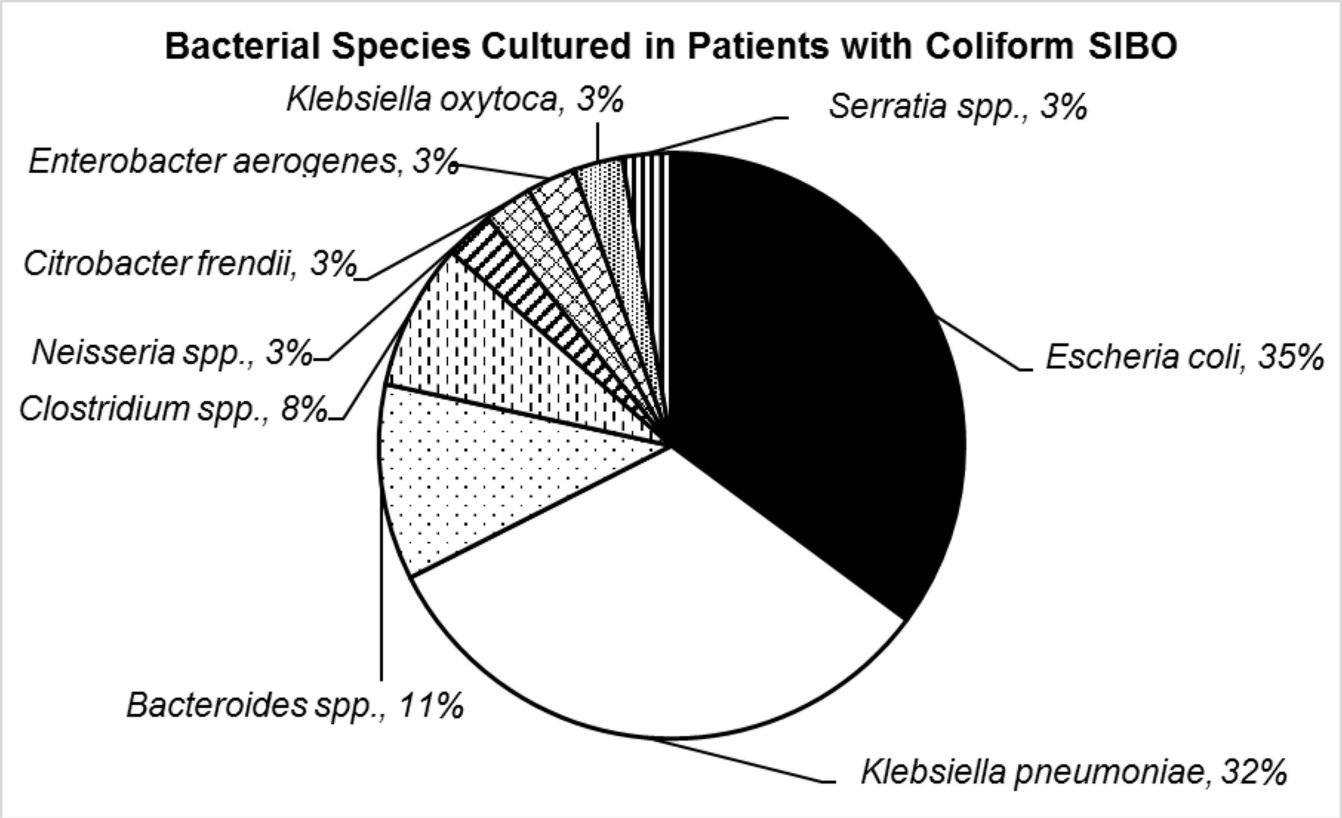

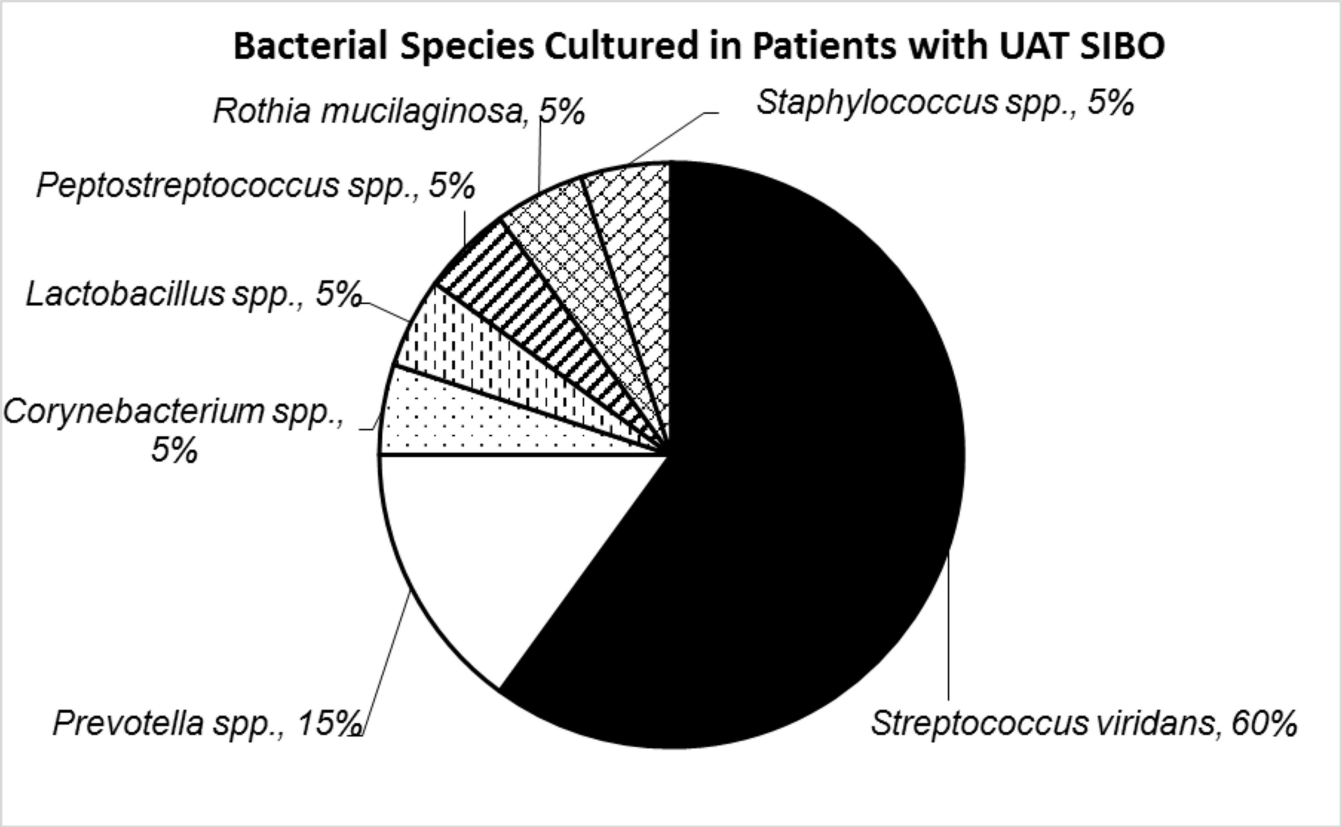

Figure 1:
Bacterial species culture in (panel A) 25 patients with coliform small intestinal bacterial overgrowth (SIBO) and (panel B) 12 patients with upper aerodigestive tract (UAT) SIBO.

Table 1:

Demographics and traditional risk factors among patients with suspected small intestinal bacterial overgrowth (SIBO) in patients with and without SIBO by culture

	Total (N = 76)	No SIBO (N = 39)	SIBO (N = 37)
Baseline characteristics			
Age *	50.4 (12.7)	47.8 (11.4)	53 (13.6)
Sex (% female)	92	95	90
Race (% Caucasian)	95	97	92
Body mass index, kg/m^2	26.2 (6.9)	25.9 (6.7)	26.5 (7.2)
Established risk factors for SIBO			
Risk factor for Coliform SIBO	34 (44.7%)	15 (38.5%)	19 (51.4%)
Risk factor for URT SIBO #	46 (60.5%)	17 (43.6%)	29 (78.4%)
Connective tissue disorder	10 (13.3%)	5 (12.8%)	5 (13.9%)
Resection of ileocecal valve	17 (22.4%)	7 (18%)	10 (27%)
Small bowel motility failure	10 (13.3%)	5 (12.8%)	5 (13.9%)
Discontinuous small bowel	3 (4%)	0 (0%)	3 (8.1%)
Surgery with decreased gastric acid exposure &	11 (14.5%)	1 (2.6%)	10 (27%)
Condition with decreased gastric acid ^	41 (54%)	16 (41%)	25 (67.6%)

* UAT=upper aerodigestive tact; Data presented as mean values (standard deviation) and proportions. Statistical analysis by the two-sample t-test for normally distributed continuous variables, Wilcoxon rank sum test for skewed continuous variables, and Fisher's exact test for categorical variables:

* p=0.07

# p<0.01

& p<0.01

^ p=0.02, all other p-values ns

Table 2:

Association between traditional risk factors for coliform and upper aerodigestive tract (UAT) colonization and final SIBO group by type of bacterial overgrowth in 76 patients

	UAT SIBO vs. No SIBO	Coliform SIBO vs. No SIBO	Coliform SIBO vs. UAT SIBO
	Odds Ratio (95% CI)	**Odds Ratio (95% CI)**	**Odds Ratio (95% CI)**
Traditional risk factors for coliform bacterial colonization			
Connective tissue disorder (scleroderma, polymyositis, mixed CT disease, lupus)	0.3 (0.002 – 2.5)	1.8 (0.5 – 7.2)	7.2 (0.7 – 982.7)
Resection of ileocecal valve	1.0 (0.2 – 4.6)	1.7 (0.5 – 5.9)	1.7 (0.3 – 10.8)
Failure of small intestinal motility (chronic intestinal pseudo-obstruction, radiation enteropathy, visceral neuropathy or myopathy)	0.81 (0.1 – 4.7)	1.5 (0.3 – 6.1)	1.8 (0.3 – 20.5)
Discontinuous small bowel (blind limb, small bowel diverticulum)	2.9 (0.02 – 553.2)	**17.4 (1.2 – 2515)**	6 (0.4 – 883.5)
At least one of the above	0.6 (0.1 – 2.1)	2.8 (1.0 – 8.5)	**4.9 (1.1 – 25.2)**
Traditional risk factors for UAT bacterial colonization			
Surgery causing diminished gastric acid exposure to small bowel (Billroth I or II, vagotomy, gastric bypass)	**9.5 (1.4 – 106)**	**8.4 (1.6 – 86.4)**	0.9 (0.2 – 4.7)
Condition with decreased gastric acid secretion (*H. pylor*i or daily proton pump inhibitor-use)	**5.8 (1.4 – 33.3)**	2.1 (0.8 – 6.4)	0.4 (0.1 – 1.7)
At least one of the above	**9.6 (2.0 – 94.1)**	**2.9 (1.0 – 9.1)**	0.3 (0 – 1.7)

Data presented as odds ratio (95% confidence intervals). Statistical analysis by penalized multinomial logistic regression adjusted for age

Table 3:

Symptoms and Patient Assessment of Gastrointestinal Disorders-Symptom Severity Index (PAGI-SYM) in patients with suspected small intestinal bacterial overgrowth (SIBO) stratified by type of bacterial overgrowth

	No SIBO (N = 39)	UAT SIBO (N = 12)	Coliform SIBO (N = 25)
Symptom duration (months)	36 (12 – 108)	15 (6 – 36)	30 (18 – 60)
Diarrhea	12 (30.8%)	5 (41.7%)	11 (44%)
Daily abdominal distention	21 (53.8%)	8 (66.7%)	10 (40%)
PAGI-SYM total score	3.2 (2.3 – 3.6)	3.4 (2.6 – 3.8)	2.8 (2.3 – 3.2)
PAGI-SYM subscale scores			
Heartburn/regurgitation*	2 (0.7 – 3.1)	3.4 (1.4 – 3.7)	1.9 (0.5 – 2.3)
Nausea and vomiting	3.3 (1.7 – 4)	3 (1.7 – 3.3)	2.7 (1.9 – 3.7)
Postprandial fullness/early# satiety	4 (3 – 4.3)	3.8 (3 – 4.3)	3.3 (2.3 – 3.7)
Bloating&	3.5 (3 – 5)	5 (3.5 – 5)	3.5 (2.3 – 4.5)
Upper abdominal pain	3.5 (3 – 4)	4 (3 – 5)	3.5 (2.5 – 4)
Lower abdominal pain	3 (2 – 4)	2.5 (1 – 3)	3 (2 – 3)

*UAT=Upper aerodigestive tract; Data presented as median (interquartile range) and frequency (proportion). Statistical analysis by Kruskal-Wallis test for skewed continuous variables and Fisher's exact test for categorical variables for assessing overall group differences:

*p=0.06

#p=<0.05

&p=0.06, all other p-values ns

Table 4:

Quality of life and psychological traits from patients with suspected small intestinal bacterial overgrowth (SIBO) stratified by type of bacterial overgrowth

	No SIBO	UAT SIBO	Coliform SIBO
Hospital Anxiety and Depression Scale	N=28	N=6	N=14
Anxiety scale	5.5 (3 – 13.5)	5.5 (3 – 13)	8 (4 – 13)
Depression scale	7.5 (3 – 10)	7 (2 – 12)	6.5 (3 – 9)
RAND 36-item Short Form survey	N=28	N=6	N=15
Physical functioning	45 (17.5 – 67.5)	35 (20 – 60)	35 (15 – 80)
Role limitations due to physical health	0 (0 – 0)	0 (0 – 0)	0 (0 – 25)
Role limitations due to emotional problems	33.3 (0 – 100)	66.7 (0 – 100)	33.3 (0 – 100)
Energy/fatigue	20 (7.5 – 37.5)	27.5 (20 – 45)	35 (15 – 40)
Emotional well being	64 (44 – 82)	66 (44 – 92)	52 (36 – 64)
Social functioning	37.5 (25 – 75)	43.8 (25 – 50)	50 (12.5 – 50)
Pain	32.5 (22.5 – 45)	22.5 (12.5 – 22.5)	45 (22.5 – 45)
General health	25 (15 – 47.5)	22.5 (10 – 55)	30 (20 – 35)
Symptom Check List-90	N=26	N=6	N=15
Somatization	1.4 (1 – 2)	1.3 (0.7 – 2.1)	1.3 (0.9 – 2.1)
Obsessive-compulsive symptoms	0.9 (0.1 – 2.3)	1.9 (0.1 – 2.3)	1.2 (0.9 – 1.7)
Interpersonal sensitivity	0.3 (0 – 0.8)	0.4 (0.1 – 1)	0.6 (0 – 1.2)
Depression	1.2 (0.4 – 2.3)	1.4 (0.2 – 2.2)	0.9 (0.5 – 2.2)
Anxiety	0.6 (0.1 – 1.6)	1 (0.2 – 2.2)	0.4 (0.2 – 1.5)
Hostility	0.3 (0 – 0.3)	0.4 (0.2 – 0.5)	0.3 (0.2 – 0.7)
Phobic-Anxiety	0 (0 – 0.4)	0.1 (0 – 0.9)	0 (0 – 0.4)
Paranoid ideation	0 (0 – 0.5)	0.5 (0 – 1)	0 (0 – 0.2)
Psychoticism	0.2 (0 – 0.4)	0.5 (0 – 0.9)	0.3 (0 – 0.6)
Global severity index	0.8 (0.3 – 1.4)	1.1 (0.2 – 1.7)	0.7 (0.4 – 1.2)

Data presented as median (interquartile range). Statistical analysis by the Kruskal-Wallis test assessing overall group differences: all p-values ns.