



# Modern concepts of small intestinal bacterial overgrowth

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## Purpose of review

Small intestinal bacterial overgrowth (SIBO) has been a recognized condition for more than half a century. Early descriptions of SIBO were based on the concept of colonic bacteria “backing up” into the small intestine. This was based on techniques using unprotected aspiration catheters and earlier culture techniques. Recent advances in breath testing, small bowel sampling, culture techniques, and next generation sequencing have helped expand our understanding of SIBO.

## Recent findings

“SIBO” is now understood to encompass at least three different types of overgrowth including SIBO, intestinal methanogen overgrowth (IMO) and intestinal sulfide overproduction (ISO). Each has their own unique microbial profile. In addition, next generation sequencing has revealed that SIBO is not a migration of colonic flora into the small intestine, but rather overgrowth of two predominant species/strains from phylum Proteobacteria (*Escherichia coli* and *Klebsiella*). Lastly, results from next generation sequencing of the stool and small intestinal microbiomes have validated breath testing as a diagnostic tool.

## Summary

Together, these advances have allowed the identification of key microbes in overgrowth syndromes, uncovering their relationships to conditions such as irritable bowel syndrome, and paving the way for the development of novel customized treatment options in the future.

## Keywords

breath testing, intestinal methanogen overgrowth, intestinal sulfide overproduction, small intestinal bacterial overgrowth, treatments

## INTRODUCTION

Small intestinal bacterial overgrowth (SIBO) is caused by alterations in the small bowel microbiome. Traditionally, SIBO was considered in the differential diagnosis of unexplained diarrhea, weight loss and malabsorption in patients who had altered anatomy such as Billroth II, antrectomy and blind loop syndromes [1]. In these iatrogenic causes, extraordinarily high bacterial counts were seen. It was unclear whether the malabsorption symptoms seen in these patients were due to the SIBO or the altered anatomy of the intestinal tract [1–4]. Modern thinking suggests it was the postsurgical changes.

Over the ensuing decades, our understanding of SIBO has evolved for a number of reasons. First was the advent of indirect testing such as the hydrogen breath test [5–7]. Later, other gases were added to the breath test, adding new dimensions to our understanding of the gut microbiome and microbial overgrowth [8–10]. In the 2000s, next generation sequencing (NGS) became a powerful tool in understanding the small intestinal microbiome [11,12<sup>\*</sup>]. The combination of

these advanced sequencing techniques and breath testing has redefined our understanding of microbial overgrowth as compared to the older techniques of the 1960s (Table 1). As an example, SIBO was often referred to or defined as a migration of colonic flora into the small intestine. Modern data using NGS refutes this hypothesis by demonstrating that specific organisms such as *Escherichia coli* become opportunistic in SIBO [12<sup>\*</sup>].

Given these exciting new developments in understanding the small intestinal microbiome, we will explore the more modern concept of SIBO and other microbial overgrowth syndromes.

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**KEY POINTS**

- Microbial overgrowth is now understood to encompass small intestinal overgrowth, intestinal methanogen overgrowth, and intestinal sulfide overproduction.
- Each of these is linked to distinct breath gas profiles, and key organisms responsible for the production of these gases in the gut have been identified.
- Understanding the relationships between breath gases, gut microbial profiles, and patient symptoms is key to developing effective treatments.

**THIS IS NOT YOUR GRANDFATHER'S SMALL INTESTINAL BACTERIAL OVERGROWTH**

The concept of SIBO has been recognized for decades. The early definitions for SIBO included indirect assessment via breath testing [5,6], and the more direct technique of culturing small bowel aspirates [13]. In those early days, aspirates were obtained by passing a catheter into the small intestine [14,15]. The sterility of the catheter, the culture techniques, and the lack of endoscopy now call into question the accuracy of those determinations. SIBO was originally defined as coliform counts of  $>10^5$  colony forming units (CFU) per ml of aspirate [1] (Table 1). It is difficult to reconcile whether these findings were accurate or due to contamination. Moreover, in those early years, very little was known about the normal levels of coliforms in the small intestine. Without a full understanding of the healthy foregut, it was impossible to define SIBO. As in most of medicine, abnormality is defined by normality. It was not until the 2000s that a systematic review of the literature was able to summarize the existing literature on the normal upper small intestine [13]. In this review, healthy subjects virtually

never had coliform levels  $>10^3$  CFU/ml. As a result, in 2018, the North American consensus affirmed a new definition of SIBO as  $>10^3$  CFU/ml using an evidence-based medicine approach [9]; this was later confirmed by other consensus guidelines [16,17] (Table 1). While coliform levels in the small intestine can exceed  $10^5$  CFU/ml, this usually happens under extraordinary circumstances such as blind loop syndrome [18].

In the 1980s, hydrogen breath testing became popularized as a means of diagnosing SIBO. Various substrates were used in this test [19,20]. In the end, the two principal substrates that remain are glucose and lactulose [5,6,21]. The principle of hydrogen breath testing is that when an ingested carbohydrate traverses the gut and is encountered by bacteria they will ferment it, producing gases which are then absorbed detected on the breath. In the 1990s, methane was added to breath testing [8]. However, it was unclear what the role of methane was. What was known is that human cells do not produce hydrogen or methane [22], suggesting that the presence of these gases on the breath test had to result from gut microbial sources [23].

Over the decades as breath testing became more commonly used, some have pointed back to culture as the gold standard [24]. We now know that traditional culture was not a good “gold standard” for a number of reasons [25–27], particularly as catheters were not improved to prevent contamination. A recent study from the Mayo Clinic reported that approximately 20% of cultures from the small bowel were contaminated [26]. This level of uncertainty cannot be a gold standard. With this in mind, modern catheters have been developed that are sterile double lumen catheters [11,28], improving the certainty that the aspirates obtained truly represent the small intestine (Table 1). In addition, capsule based technologies, while still nascent, offer hope for further alternatives to older inaccurate techniques [29,30].

**Table 1.** Comparison of antiquated vs. modern understanding of SIBO

Antiquated understanding of SIBO	Modern understanding of SIBO
Coliforms accumulating in the small intestine	Increases in highly specific bacteria in the small bowel
SIBO is defined as $>10^5$ CFU/ml	SIBO defined based on healthy small bowel microbial profiles and is now $>10^3$ CFU/ml
Culture of the small bowel is gold standard	Proper technique with sterile double catheter aspiration and culture is the gold standard
Hydrogen only breath testing is a poor tool for SIBO	Modern three gas breath testing accurately reflects small bowel microbial composition
Breath testing should only be considered if there is malabsorption	SIBO is a common cause of nonspecific symptoms such as bloating, pain, diarrhea and constipation

SIBO, small intestinal bacterial overgrowth.

More recently, breath testing has further advanced with the addition of hydrogen sulfide (H<sub>2</sub>S) [31,32,33<sup>\*\*\*</sup>]. In fact, H<sub>2</sub>S completes the three gases that can be detected on breath test and are produced by the gut microbiome. As we will discuss further later, hydrogen may be the least interesting of these gases. With our more modern understanding of microbial overgrowth, we now know that methane is associated with constipation [34] and hydrogen sulfide, linked in early studies to irritable bowel syndrome (IBS) [35], is associated with diarrhea [10]. These have now been compared to next generation sequencing results from patients, which confirm that the levels of these gases seen on the breath test are an indicator of the degree of colonization of the gut, including the small bowel [36], with organisms that produce these gases (Table 1). In addition, data continue to accumulate that these three gases are in fact associated with functional gastrointestinal (GI) disorders [37,38<sup>\*\*</sup>].

**CAUSES OF SMALL INTESTINAL BACTERIAL OVERGROWTH**

It is important to understand the mechanisms underlying SIBO. Any condition associated with the slowing or delayed transit of the small intestine has the potential to cause SIBO [5,6,13,39,40]. Table 2 highlights some of these mechanisms. These include alterations of motility, mechanical slowing of the small intestine, drugs, immune deficiency, autoimmune diseases, and metabolic disorders. In addition, SIBO has been a notable cause of unexplained diarrhea in the elderly [41] and even hypothyroidism [42–44]. Ironically, one of the clearest associations between a condition and SIBO is IBS [45,46] and yet this association has been contentious.

**DIAGNOSING SMALL INTESTINAL BACTERIAL OVERGROWTH**

The two principal ways of diagnosing SIBO in patients are small intestinal aspirate culture [24] and breath testing [5,6]. Intestinal aspiration is an invasive and expensive procedure involving aspirating from a catheter passed into the lumen of the duodenum or jejunum during endoscopy [15,26]. Alternatively, breath testing is often a preferred method as it is noninvasive, simple, and relatively inexpensive.

Duodenal aspirates are regarded as the gold standard for determining SIBO. While no gold standard is perfect, there are significant limitations to culture of the small bowel. Using microbiological principles, the more sterile you can make the acquisition of your sample the more likely you are to get an

**Table 2.** Causes of SIBO

Category	Condition
Motility	IBS
	Pseudo obstruction
	Lower rate of migrating motor complexes
	Visceral neuropathies/myopathies
Drugs	Narcotics
	GLP-1 agonists
Mechanical	Adhesions
	Bowel obstructions
	Strictures (e.g. Crohn’s disease)
Iatrogenic	Billroth II
	Antrectomy/gastrectomy
	Bariatric Surgery
Autoimmune diseases	Scleroderma
Metabolic conditions	Hypothyroidism
	Diabetes
Cirrhosis	Many types of cirrhosis
Immune deficiency	HIV
	Other immunodeficiencies
Neurological diseases	Parkinson’s
Degenerative conditions	Diarrhea in the elderly
Low acidity in stomach	Achlorhydria/hypochlorhydria
	Possibly Proton Pump Inhibitor (PPI) use
Pancreatic disease	Chronic pancreatitis
Inflammatory disease	Crohn’s disease
	Celiac disease

IBS, irritable bowel syndrome; SIBO, small intestinal bacterial overgrowth.

accurate result. Traditionally, aspirates were obtained by single lumen catheters [14,15]. This open catheter passes through a dirty endoscopy channel getting contaminated as it passes [15], and as a result up to 20% of samples obtained using this technique are contaminated [26]. Newer double lumen catheters carry a lower likelihood of contamination during passage through the instrument. These catheters have a sterile interior and a cap to prevent material from entering. Once in the duodenum, the inner catheter is passed through a membrane and the aspirate is taken [28]. This is a better way to mitigate contamination [11]. However, the second issue with culture is that many centers process samples like a

**Table 3.** Comparison of glucose and lactulose breath testing

Substrate	Pros	Cons
Glucose	<ul style="list-style-type: none"> <li>-Glucose should not get to the cecum so any induction of H<sub>2</sub> is from SIBO</li> <li>-Glucose does not need a prescription</li> <li>-Since it does not reach the colon, it results in better test specificity</li> </ul>	<ul style="list-style-type: none"> <li>-Glucose does not get past the first few feet of small intestine so will not pickup distal/midgut SIBO</li> <li>-Glucose is absorbed by the mouth, mucosa, and stomach so there is individual variation in what truly reaches the small intestine</li> <li>-Since it misses distal SIBO, it has lower sensitivity</li> </ul>
Lactulose	<ul style="list-style-type: none"> <li>-It is not absorbed so encounters bacteria along the entire length of small bowel</li> <li>-Needs a prescription</li> <li>-Since it transits the whole small bowel, it is more likely to find SIBO (higher sensitivity)</li> </ul>	<ul style="list-style-type: none"> <li>-It would be expected to reach the cecum where a large amount of bacteria reside</li> <li>-Not absorbed by the host mucosa so the dose given will transit into the small intestine</li> <li>-Since it will arrive in the cecum, it is likely to demonstrate hydrogen in a false positive fashion in some patients (lower specificity)</li> </ul>

SIBO, small intestinal bacterial overgrowth.

urine culture. That means the clinical lab that receives the sample simply determines whether the sample contains bacteria at  $>10^5$  CFU/ml or not. There are no serial dilutions. However, SIBO is now defined as  $>10^3$  CFU/ml based on the North American Consensus (NAC) [9]. An additional problem with culture is the location of sampling [25]. Since the small bowel is 15 feet long it is possible that SIBO is not occurring in the duodenum but in the jejunum. This could result in sampling error. Finally, with the advent of NGS, it is possible that advanced sequencing techniques could replace culture as a gold standard. This remains to be determined.

Breath testing is the alternative to culture. While breath testing is common practice, there are debates about this technique. One debate is regarding the use of glucose or lactulose as the substrate for testing [47] (Table 3). Since lactulose is nonabsorbed and reaches the cecum, some suggest it is more a measure of orocecal transit than fermentation in the small intestine. One particular, often quoted, study [48] combined lactulose with technetium defining transit when 5% of the technetium/lactulose mixture reached the cecum. They noted that this often happened prior to a rise in hydrogen [48]. Unfortunately, the authors did not take into account microbiological principles. Simply because the lactulose has arrived in the cecum does not imply the hydrogen is coming from the colon. Fermentation takes time to initiate and may take up to 45 min to peak. Secondly, while 5% of the technetium/lactulose mixture was in the cecum, the other 95% was still in the small intestine, making it impossible to conclude the hydrogen is from the colon. Fortunately, modern next generation sequencing is settling the issue. Recent studies comparing breath test results to small

intestinal microbiome composition suggest that a positive hydrogen breath test was associated with elevated Proteobacteria in the small intestine [11] and increases in hydrogen (H<sub>2</sub>) producing microbial metabolic pathways [12<sup>¶</sup>]. Furthermore, a recent study demonstrated that hydrogen sulfide (H<sub>2</sub>S) levels on breath testing correlated with abundance of hydrogen sulfide producing bacteria in the small intestine on the same day of the breath sample [36]. The same is true for methane (CH<sub>4</sub>), as methane levels on the breath correlated with small intestinal methanogen levels [36]. In fact, the breath test maybe a better surrogate of the contents of the small intestine than we once thought.

### THE MICROTYPES OF “SMALL INTESTINAL BACTERIAL OVERGROWTH”

Over the decades, breath testing has evolved and now measures all three gases (H<sub>2</sub>, CH<sub>4</sub>, and H<sub>2</sub>S) produced by gut microbes [10,49]. Each iteration has unlocked new answers about the relationship between the gut microbiome, the gases that are produced, and the symptoms experienced by patients [11]. Combining breath testing with next generation sequencing has demonstrated that the dominant type of gas detected by the breath test predicts an overabundance of specific groups of microbes and their metabolic functions [10,12<sup>¶</sup>]. In addition, some of these gases (such as H<sub>2</sub>S) have a profound effect on intestinal function [50] and could be a missing link in explaining functional gastrointestinal disorders [10,35,38<sup>¶</sup>,51].

The first version of breath testing only measured H<sub>2</sub> [5]. It was understood in the late 1970s and early 1980s that humans do not produce H<sub>2</sub> [22]. Based on this, measuring H<sub>2</sub> over time after a carbohydrate

challenge became the standard approach for identifying SIBO by breath testing. Although elevated H<sub>2</sub> on breath test appears to predict SIBO, there were problems relating H<sub>2</sub> levels to symptoms. Patients with a positive breath test had symptoms but the levels of H<sub>2</sub> didn't appear to have much meaning. For example, a H<sub>2</sub> level of 100 parts per million (ppm) at 90 min on a breath test did not predict greater symptoms than 50 ppm at the same time point, although both predicted SIBO and typical SIBO symptoms. This is now believed to be due to H<sub>2</sub> consumption. In the gut, 4 mol of H<sub>2</sub> are needed by gut methanogens to produce 1 mole of CH<sub>4</sub> [52,53]. Similarly, H<sub>2</sub>S producing organisms consume 5 mol of H<sub>2</sub> to produce 1 mol of H<sub>2</sub>S. This revelation meant that it was difficult to use H<sub>2</sub> as a quantitative measure of the levels of H<sub>2</sub> producing organisms in the gut. Nevertheless, H<sub>2</sub> measurement on breath testing remains a valuable tool.

NGS has taken this to new heights. In a series of papers, sequencing of the small intestine suggests that SIBO is a dramatic aberration of the small intestinal microbiome. One theme of this paper is that this is not your grandfather's SIBO. In your grandfather's version of SIBO, colonic flora move into the small intestine resulting in overgrowth. NGS studies prove that this is incorrect. In fact, SIBO appears to be predominantly an overgrowth of only two species, *Klebsiella pneumoniae* and *Escherichia coli* [12<sup>¶</sup>]. Surprisingly, when SIBO is present by culture, 46% of the entire duodenal microbiome is composed of only these two organisms [12<sup>¶</sup>]. To put a finer point on this, the microbiome of the colon is composed principally of two phyla, Bacteroidetes and Firmicutes, with Verrucomicrobia and Proteobacteria as minor phyla. If your grandfather was right, SIBO would be an overgrowth of Bacteroides and Firmicutes. Instead, Proteobacteria dominate. In addition, it appears that *Klebsiella* and *E. coli* act as disrupters to the rest of the microbiome leading to diminished microbial diversity which progressively decreases in subjects with 10<sup>3</sup> CFU/ml and subjects with 10<sup>5</sup> CFU/ml [12<sup>¶</sup>]. *Klebsiella* and *E. coli* are well known as extremely efficient fermenters and H<sub>2</sub> producers.

The second generation of breath testing involved the addition of methane (CH<sub>4</sub>) measurements during the test. The thinking in the 1990s was that methanogens were exclusively present in the left colon and that CH<sub>4</sub> had no metabolic function in humans. Once again, these older concepts have now been contradicted by modern data. In 2003, a paper examining the relationship between SIBO and IBS contained a small footnote that CH<sub>4</sub> on breath test appeared to be more common in IBS patients with constipation [54]. Since then CH<sub>4</sub> has been shown in numerous studies to be associated with constipation

symptoms [34,55–57]. As mentioned in the previous section on H<sub>2</sub>, methanogens use H<sub>2</sub> to produce CH<sub>4</sub> [58]. Studies infusing CH<sub>4</sub> into live animal models supported the theory that CH<sub>4</sub> slows transit directly and that it may do so by increasing gut contractility [55,59]. More recently, CH<sub>4</sub> has continued to evolve as an important part of the breath test as it is associated not only with constipation [34,36,55–57] but also with other physiologic conditions and events such as obesity [60], aging [61], and even cardiovascular effects such as lower heart rate [62]. As with hydrogen, next generation sequencing has allowed a more complete understanding of the organisms responsible for CH<sub>4</sub> [36]. Once again dispelling a myth, methanogens are now confirmed to be present in the small intestine [36]. This is interesting since methanogens are anaerobes. It is believed that while oxygen levels in the small intestine are greater than in the distal gut [63], radial oxygen levels also vary from lumen to mucosa [63], which may create microenvironments of low oxygen concentration that allow colonization with methanogens. Methane on breath test appears to correlate predominantly with one methanogen, *Methanobrevibacter smithii* [64,65] and the level of methane on breath correlates directly with *M. smithii* levels in the human gut [57,65–67]. Both breath methane levels and levels of *M. smithii* correspondingly correlate with the degree of constipation [10,34,56,68]. Since methanogens are found in both the small intestine [36] and in the colon, the term intestinal methanogen overgrowth (IMO) was proposed to define the condition of having >10 ppm of CH<sub>4</sub> on a breath test [67].

The last addition to breath testing is the measurement of hydrogen sulfide (H<sub>2</sub>S) [10]. H<sub>2</sub>S is proving to be the most interesting gas in breath testing, and the most complicated. There are multiple pathways for H<sub>2</sub>S production, including the assimilatory and dissimilatory pathways. H<sub>2</sub>S production is dependent on the sulfur content of the diet as well as other factors. In contrast to SIBO and IMO, there are a small but greater number of bacteria that uniquely produce H<sub>2</sub>S [69,70]. And finally, while produced in small quantities, human cells are capable of producing H<sub>2</sub>S. This gas may be important for cellular function at specific levels and toxic at higher levels. Since this gas is newly recognized as important in breath testing, large amounts of data are now beginning to emerge [10,36,38<sup>¶</sup>] and the story is likely to change over time. Just at the time of the emergence of biologic agents for inflammatory bowel disease, H<sub>2</sub>S-producing bacteria in the gut were emerging as important mediators of inflammation [50]. What is now known based on recent data is that H<sub>2</sub>S elevations on breath test correlate with a relatively small group of bacteria [10,36]. However, the species are

different in the colon [10] vs. the small intestine [36]. An important recent study suggests that H<sub>2</sub>S on breath correlates directly with the amount of *Proteus mirabilis* in the small intestine [36]. Relating H<sub>2</sub>S to symptoms, this gas correlates directly with the severity of diarrhea [10] (more severe diarrhea than is seen in “traditional” SIBO). In the colon, the main producers of H<sub>2</sub>S linked to the breath test are the genera *Desulfovibrio* and *Fusobacterium*. New terminology which has been used to characterize an abnormally high H<sub>2</sub>S on breath is intestinal sulfide overproduction (ISO) [36]. This term has been used as the H<sub>2</sub>S production may be coming from both the small intestine and the colon.

### SPECIAL CONSIDERATION OF SMALL INTESTINAL BACTERIAL OVERGROWTH IN IRRITABLE BOWEL SYNDROME

It is well known that IBS is one of the most common conditions in gastroenterology. IBS is generally defined or diagnosed based on a constellation of symptoms including abdominal pain, changes in bowel function, and bloating [71–73]. The problem with these criteria for diagnosing IBS is that they likely capture a broader range of conditions not yet determined. Over the last 20 years, data have accumulated that a significant portion of IBS appears related to SIBO and IMO [10,37,74]. While the relationship with IBS was controversial for a number of years, this was related to the fact that breath testing comparisons to the true composition of the microbiome remained lacking. Recent data have clarified these issues. For example, a meta-analysis of 25 studies by Shah *et al.* demonstrated that a positive H<sub>2</sub> breath test was far more common in IBS as compared to healthy controls [74]. The 15 high quality studies among these reinforce this finding.

Emerging breath test data has also helped to expand our understanding of IBS as being not a single disease, but rather a group of conditions with distinct microbial underpinnings. Specifically, IBS has two major subtypes, diarrhea-predominant (IBS-D) and constipation-predominant (IBS-C). IBS-D is associated with SIBO, as diagnosed both via elevated breath H<sub>2</sub> and via small bowel aspirate culture [9,45,46,74]. Moreover, drugs such as rifaximin (which is an FDA approved drug for the treatment of IBS-D [75]) are predicted to yield greater improvements in symptoms in subjects with a positive H<sub>2</sub> breath test [76,77]. In contrast, IBS-C is associated with IMO and elevated breath CH<sub>4</sub> [67]. A recent study comparing breath testing and next generation sequencing of stool samples from IBS subjects from two FDA-approved randomized controlled trials confirmed and expanded the concept of

microbial subtypes in IBS [10]. In this study, IBS-C was associated with elevated breath CH<sub>4</sub> and increased levels of methanogens including *M. smithii* in stool, but IBS-D appeared to be associated with two distinct microtypes, one characterized by elevated breath H<sub>2</sub>, and one characterized by elevated breath H<sub>2</sub>S that correlated with increased relative abundances of H<sub>2</sub>S-producers including the genus *Fusobacterium* and an unknown *Desulfovibrio* species [10]. Moreover, IBS-C subjects exhibited enrichment of microbial metabolic pathways associated with the biosynthesis of F420 (an important enzyme in methane production), whereas IBS-D subjects exhibited enrichment of microbial metabolic pathways associated with H<sub>2</sub>S production, including dissimilatory and assimilatory sulfate reduction pathways [10]. While more recent studies of the small intestine suggest that *P. mirabilis* may be more strongly associated with H<sub>2</sub>S production in the small bowel [36], these findings nevertheless underscore the potential importance of distinct gut gas-producing microbes in the development of distinct IBS subtypes.

One of the interesting aspects of IBS-D is that it can be precipitated by acute gastroenteritis [78–81]. Rat models of infection with *Campylobacter jejuni* (the most common cause of bacterial gastroenteritis [82]) demonstrate that rats develop IBS-D like phenotypes after infection that are dependent on changes in the small intestinal microbiome [78]. Further, rats inoculated with *C. jejuni* cytolethal distending toxin B (CdtB), a toxin common to all pathogens that cause gastroenteritis, develop three distinct small bowel microtypes, including microtypes associated with elevated *E. coli* or elevated *Desulfovibrio*, respectively [83]. These data are consistent with findings in human subjects with different microtypes of IBS-D – *E. coli* is a H<sub>2</sub>-producer seen in human subjects with SIBO and elevated H<sub>2</sub>, associated with IBS-D [12<sup>†</sup>], and *Desulfovibrio* are H<sub>2</sub>S-producers seen in human subjects with IBS-D and elevated H<sub>2</sub>S [10]. Moreover, host ileal transcriptomics analysis revealed that rats with the elevated *Desulfovibrio* microtype exhibited enrichment of pathways linked to circadian rhythm and fibrin clot formation, both of which are partly controlled by H<sub>2</sub>S [84,85], as well as genes encoding sulfur-binding compounds and pathways linked to pain response mechanisms, including serotonin response [83]. Lastly, rats with the elevated *E. coli* microtype exhibited enrichment of pathways linked to more severe immune and acute inflammation response, consistent with the possible role of autoimmunity in IBS-D, and mechanisms associated with gut motility, barrier function, and visceral nociception, previously identified in IBS, were also affected in these rats [83]. These data connecting acute gastroenteritis to the development of IBS-D microtypes tell

a compelling story of how IBS could develop, and may account for up to 60% of IBS-D. Acute gastroenteritis is not linked to constipation or IBS-C, and the mechanisms underlying methanogen elevations and increases in methane remain unknown.

An interesting angle that has emerged from studies of irritable bowel syndrome with SIBO, is that the SIBO could be related to a reduction in migrating motor complexes (MMC). This has been shown in a number of studies [45,78]. In addition, animal models of postinfection IBS suggest a reduction in interstitial cells of Cajal which may substantiate some of the reductions in MMC [78,86,87].

## MANAGEMENT OF MICROBIAL OVERGROWTH

The most important consideration when planning the management of microbial overgrowth is to assess the patient. Is the patient constipated or presenting with diarrhea? Is there a history of pancreatic disease, inflammatory bowel disease, abdominal surgery, autoimmune disease, or immunodeficiency? It is also necessary to get a history of current medication use. Table 2 can be used as a guide to think about the differential diagnosis. Knowing the cause of overgrowth has an impact on the approach to management. For example if a patient has an intestinal adhesion, while antibiotics may benefit temporarily, fixing the adhesion would be curative. Correcting the underlying cause will make treatment easier.

The primary treatment that has been used over the decades for microbial overgrowth is antibiotic therapy. We now know that antibiotics frequently declared as highly useful for microbial overgrowth, such as metronidazole, have low efficacy for microbial overgrowth as a single agent. Conventional antibiotics such as amoxicillin-clavulanate, tetracycline (or doxycycline), fluoroquinolones and rifaximin are better studied. In various studies, there has been relatively good equivalency between these options [88]. However, given concerns about microbial resistance, rifaximin has become a preferred agent. A recent meta-analysis of rifaximin for the treatment of SIBO supports this use [89].

In the case of intestinal methanogen overgrowth (IMO) things are even more complicated. Methanogens are not bacteria and in general antibiotics were designed for bacterial suppression. However, results of a double-blind study suggest that rifaximin in combination with neomycin was superior to neomycin alone in improving constipation and lowering methane [90]. This has become a commonly used combination in practice.

It is less clear what options there are for hydrogen sulfide since the importance of this gas and its

microbiome implications are a relatively new development. One study from the 1990s supports that hydrogen sulfide producing organisms are reduced by the use of bismuth subsalicylate. Although there is little data, some are using a combination of rifaximin and bismuth for this subset. A recent study combining rifaximin with *N*-acetylcysteine demonstrates promise in the treatment of IBS-D, as this combination was shown to reduce small bowel bacterial levels and normalize stool form in a rat model inoculated with *C. jejuni* CdtB [91].

Probiotics have been purported as another mechanism for treating SIBO. Unfortunately, the data to support this are limited and summarized in two recent meta-analyses [92,93]. In the more recent of the two studies, the authors apply more stringent grading and found no benefit to the use of probiotics [93]. Their criteria narrowed the studies to only four good quality studies. The earlier study from 2017 included 22 studies including abstracts ( $n=8$ ) and published papers ( $n=14$ ) [92]. While they showed among users of probiotics no significant effect was seen on the incidence of SIBO, there was a modest effect on correcting SIBO with some nuance. Patients taking probiotics alone for SIBO had a 53.2% normalization of SIBO. However, patients taking antibiotics with probiotics had normalization of SIBO in 85.8%. Notably, this was a mixed group of prospective and retrospective studies with a high heterogeneity [92]. In another meta-analysis, probiotics and synbiotics were noted to be of no significant benefit in managing the SIBO in patients with bariatric surgery (iatrogenic SIBO) [94]. Based on this, the data suggest that there is merit in pursuing better studies to examine this question.

The relationship between diet and microbial overgrowth is, in general, underexplored. Some have proposed using the low FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols) diet, and others suggest a more liberal low fermentation approach. The low fermentation approach combines using less fermentable foods but is less restrictive than low FODMAP, and incorporates meal spacing to allow for the migrating motor complex. The low FODMAP diet is an extreme form of eating that completely restricts poorly digestible food. While this diet is principally used in IBS, studies suggest changes in the breath test in the prediction of response to this diet. In one study, investigators examined a single baseline hydrogen sample prior to implementing the low FODMAP diet in patients with Rome positive functional disorders [95]. They found that 66% of responders to low FODMAP had a baseline hydrogen  $>8$  ppm where only 17% of nonresponders had this threshold. This could suggest that the presence of SIBO or dysbiosis

predicts the benefits of low FODMAP. Similar results were seen in two other studies [96,97]. While data are still modest, there is a good rationale for considering low fermentables in the management of SIBO. Future considerations might include deciding on the diet restrictions depending on the type of breath test finding – for example, restricting sulfur containing foods for patients with high hydrogen sulfide on breath test.

Another dietary approach is the use of an elemental diet as a therapeutic for the induction of remission of SIBO and IMO. Two studies have attempted this. In 2004, a study examined consecutive patients who underwent 14 or 21 days of an exclusive elemental diet [98]. In that study, normalization of the breath test was seen in over 80 of subjects, with 14 days being the ideal amount of time to achieve this. The challenge with elemental diets is that they were designed for enteral feeding. There was no focus on palatability. A newer study examined a palatable elemental diet in the management of 30 subjects with SIBO or IMO. This palatable elemental diet resulted in normalization of breath test results in 73% of subjects overall, and in 100% of subjects with hydrogen on breath test [99\*]. What remains to be determined is whether the benefit of elemental diets is short lived or durable.

## CONCLUSION

This is not your grandfather's SIBO. Modern techniques have improved the understanding of SIBO, and now demonstrate that there are in fact multiple microbial overgrowth syndromes. These result in different phenotypes that could be important in understanding unexplained diarrhea, constipation, bloating, and abdominal pain. Modern concepts of microbial overgrowth now include SIBO, IMO, and the newly identified ISO. Differentiating these different subtypes is already defining new ways of managing these conditions.

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## Conflicts of interest

*M.P. is a consultant for Ferring Pharmaceuticals Inc., Salvo Health, and Cylinder Health Inc. Cedars-Sinai has*

*a licensing agreement with Gemelli Biotech and Hobbs Medical. M.P. has equity in Gemelli Biotech, GoodLFE, Cylinder Health and Salvo Health. G.B. declares no conflicts of interest.*

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