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REVIEW

Small Intestinal Bacterial Overgrowth (SIBO), a clinically overdiagnosed entity?

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Abstract Small intestinal bacterial overgrowth (SIBO) is a clinical entity recognised since ancient times; it represents the consequences of bacterial overgrowth in the small intestine associated with malabsorption. Recently, SIBO as a term has been popularized due to its high prevalence reported in various pathologies since the moment it is indirectly diagnosed with exhaled air tests.

In the present article, the results of duodenal/jejunal aspirate culture testing as a reference diagnostic method, as well as the characteristics of the small intestinal microbiota described by culture-dependent and culture-independent techniques in SIBO, and their comparison with exhaled air testing are presented to argue about its overdiagnosis.

PALABRAS CLAVE
Sobrecrecimiento bacteriano del intestino delgado;
Microbiota intestinal;
Test de aire espirado;
Disbiosis del intestino delgado;
Microbiología

Sobrecrecimiento bacteriano del intestino delgado (SIBO), ¿una entidad clínica sobrediagnosticada?

Resumen El sobrecrecimiento bacteriano del intestino delgado (SIBO), es una entidad clínica reconocida desde tiempos remotos, que representaba las consecuencias del crecimiento bacteriano excesivo en el intestino delgado asociado a malabsorción. Recientemente, el término SIBO se ha popularizado debido a la alta prevalencia reportada en diversas patologías desde que se diagnostica de manera indirecta con las pruebas de aire espirado.

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En el presente artículo, se exponen los resultados de las pruebas de cultivo del aspirado duodenal/yeyunal como método diagnóstico de referencia, las características de la microbiota del intestino delgado descritas mediante técnicas dependientes e independientes de cultivo en SIBO y su comparación con las pruebas de aire espirado, con el fin de argumentar acerca de su sobrediagnóstico.

Introduction

Small intestinal bacterial overgrowth (SIBO), formerly recognised as an objectively defined clinical entity, also called "blind loop syndrome", represented the consequences of excessive bacterial growth in the small intestine, or "contamination" of the small intestine by bacteria from the colon.[1] This definition was not disputed and SIBO was suspected in patients with a syndrome of malabsorption and chronic diarrhoea due to a predisposing surgical or medical cause. Currently, SIBO is defined as when a concentration of $\geq 10^3$ colony forming units per millilitre (CFU/mL) of colonic bacteria are found in duodenal/jejunal aspirate culture.[2,3] However, duodenal/jejunal aspiration for microbial culture is not widely practiced due to its known limitations. Measurement of hydrogen by hydrogen breath test is currently used as an indirect diagnostic method to detect SIBO. Chromatography is used to determine the elimination kinetics of hydrogen (H_2) and methane (CH_4) molecules resulting from the microbial anaerobic fermentation process. These gases are absorbed through the intestinal mucosa, pass into the bloodstream and are finally exhaled through the lungs. SIBO is considered when there is an increase $\geq$20 ppm (ppm) in the concentration of expired hydrogen compared to the baseline 90 min beforehand, in a breath test with an oral challenge with a carbohydrate such as glucose or lactulose as proposed by the North American consensus.[2] It is the most widely used test to detect SIBO in clinical practice due to its ease of use, simplicity and low cost, but with no basis in scientific evidence obtained through clinical trials.[4]

The jejunal epithelium absorbs glucose before it reaches the ileum or colon. Excess jejunal bacteria can cause an early hydrogen surge. However, rapid transit of glucose to the caecum can cause a false positive in the first 90 min due to colonic fermentation. Lactulose is not absorbed in the small intestine and increases hydrogen production in the colon by fermentation. In SIBO, lactulose can generate an early and a late peak of hydrogen, related to bacterial activity in the small intestine and colon respectively. Scintigraphy studies show that the normal increase in H_2 coincides with the arrival of lactulose in the caecum, which can give false positives due to variations in orocaecal transit time (OCTT), limiting the diagnostic accuracy of SIBO by breath tests.

The current definition of SIBO has generated controversy because its spectrum has expanded beyond malabsorption. The term SIBO has become popular and the prevalence is high in patients with gastrointestinal symptoms (up to 68.1% in a French cohort).[5] In addition, breath test results do not always correlate with duodenal/jejunal culture results. This suggests the need for further validation of the breath test as a diagnostic test for SIBO.

In this review we have put the question, *"Is SIBO an overdiagnosed clinical entity?"* The techniques for studying the genetic material of the intestinal microbiota have evolved exponentially in recent decades and the characterisation of bacteria by metagenomics demonstrates the previously unrecognised great complexity of the intestinal microbiota, identifying up to 80% of bacteria which have not been cultured.[6] This review focuses on the study of scientific evidence on the characteristics of the small intestine microbiota in SIBO using culture-dependent and culture-independent techniques and their relationship with the results of breath tests as an indirect diagnostic method of this condition, analysing the sensitivity and specificity of lactulose and glucose breath tests.

Methods

This was a descriptive study based on a review of the literature available from 1968 to July 2023. The information was obtained from the (MEDLINE, PubMed.gov) United States National Library of Medicine database, Google Scholar and ResearchGate. The terms used to narrow the first search were *''small intestinal bacterial overgrowth OR small intestine bacterial overgrowth AND diagnosis''*, in addition to the following terms: *SIBO AND microbiome*; *''Small intestine bacterial overgrowth AND microbiome*»; *SIBO; SIBO "Diagnosis''*; *SIBO ''treatment'', ''antibiotic'', ''probiotic'', ''16S rRNA sequencing''*, «*next generation sequencing''*, «*jejunal fluid culture''; SIBO AND ''Breath Test*». We considered studies in both adult and child populations.

Results

The search strategy initially generated 2089 article titles. After applying the search criteria, 61 published article abstracts were selected for the review. From these, 31 provide complete information about the prevalence and microbiological analysis of duodenal/jejunal aspirate culture in SIBO; five are related to the study of duodenal microbiota by 16S ribosomal RNA sequencing; one shows a subcohort of 38 patients, whose duodenal aspirate was evaluated by whole genome metagenomic sequencing; and

16 articles are on cross-sectional studies, which compare breath testing with duodenal aspirate culture, mentioning sensitivity and specificity.

Analysis of small intestine microbiota in small intestinal bacterial overgrowth, using culture-dependent techniques

The process involving aspiration of fluid from the small intestine (samples from the proximal jejunum or distal duodenum), followed by culture and bacterial count, has been considered the accepted gold standard for the diagnosis of SIBO for many years, although it is not fully validated. Multiple case-control studies with healthy controls have been conducted in this regard. The bacterial count is expressed as CFU/mL, by its logarithmic value 10.

Preliminary studies date back to the 1960s. In a descriptive study in 1966, Tabaqchali et al.[7] reported the changes that occur in the microbiota of the small intestine under conditions of stasis, such as blind loop syndrome secondary to previous surgery, stenosis or jejunal diverticulosis or in the case of gastrocolic or enterocolic fistulas. They explain that an abnormal microbiota can colonise the small intestine of these patients. They described microorganisms such as *Escherichia coli* in high concentrations and anaerobes such as *Bacteroides* and *Lactobacillus*. The number of these microorganisms in the intestinal lumen can reach up to 10^8–10^9 CFU/mL as indicated by Drasar et al. in 1969.[8]

The prospective and retrospective studies which have been analysed for this review in relation to the diagnosis of SIBO by a duodenal/jejunal fluid culture method are shown in Table 1.

Definition of small intestinal bacterial overgrowth

Confirmation of more than 10^5 CFU/mL of colonic-type bacteria in small bowel aspirate culture has long been the established criterion for diagnosing SIBO. Most studies with this cut-off point were conducted before 2010 and their indication was mainly chronic diarrhoea and malabsorption syndrome. The highest counts (10^6–10^9 CFU/mL) were observed in post-surgical patients (Billroth II gastrojejunostomy).[9]

Khoshini et al.[10] published a review of diagnostic tests for SIBO and revealed that small bowel bacterial counts were lower, ranging from 0–10^3 CFU/mL in asymptomatic healthy controls in several case-control studies. Posserud et al.[11] noted that the prevalence of SIBO in patients with irritable bowel syndrome (IBS) was very low (4.3%) and not very different from healthy controls (4%) with a diagnostic threshold $\geq 10^5$ CFU/mL. However, they state that 43% of patients with IBS had a count $\geq 5 \times 10^3$ CFU/mL.

A diagnostic threshold with a bacterial concentration $\geq 10^3$ CFU/mL has been considered appropriate for aspirations obtained from the duodenum given its proximal location, its relative protection against the translocation of bacteria from the colon and its frequent exposure to stomach acid, all of which would decrease the risk of bacterial overgrowth.[12,13] For these reasons, this new count has been considered in recent years as a new diagnostic threshold for SIBO. At least 10 more recent studies have considered this diagnostic threshold, where the indication for culture was the presence of nonspecific gastrointestinal symptoms, dyspepsia, IBS or liver disease, plus a cohort of patients who had undergone a colectomy. There are other definitions for the diagnosis of SIBO in the literature and different authors report on their differences of opinion surrounding the known limitations of duodenal/jejunal aspirate culture.

From a qualitative point of view, the role of coliform bacteria in the aetiopathogenesis of SIBO is clear. Some authors consider a diagnosis of SIBO whenever the microorganisms isolated in the luminal aspirate are those that normally colonise the large intestine.[11,14–16] The growth of colonic bacteria in the small intestine has been associated with absorption defects in classic studies. However, there is uncertainty regarding the clinical relevance of microbial organisms from the upper respiratory and digestive tract in the aetiopathogenesis of SIBO.

Microorganisms identified by culture methods in small intestinal bacterial overgrowth

Most studies mention the results obtained through aerobic and anaerobic culture techniques. There is heterogeneity in sample processing and culture techniques. The concept of SIBO implies that the aetiology is due to one or more bacteria. In general, the bacteria of the Proteobacteria and Firmicutes phyla are the most frequently identified; of the Proteobacteria, the *E. coli* species and *Klebsiella species*, and of the Firmicutes phylum, the species of the *Streptococcus* and *Enterococcus genera*. The most commonly described anaerobic microorganisms are *Bacteroides, Clostridium* and *Lactobacillus*. Fig. 1 shows the bacteria identified in 28 duodenal/jejunal aspirate culture studies.

In several prospective studies,[15–17] in patients with suspected SIBO and IBS, in the duodenal culture in MacConkey agar medium, gram-negative microorganisms were recognised as *E. coli, Enterobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, Citrobacter freundii, Serratia marcescens* and some Gram-positive microorganisms, such as *Staphylococcus aureus* and *Enterococcus faecium were identified in duodenal culture on MacConkey agar medium*.

In other studies, bacteria from genera belonging to the microbiota of the upper respiratory and digestive tract (upper aerodigestive tract [UAT]), such as *Streptococcus* and *Enterococcus*, were isolated and included in the diagnosis.[13,18–24] However, in the study by Cangemi et al.,[25] cultures with growth of Gram-positive bacteria were ruled out for the diagnosis of SIBO.

The results did not show a marked presence of anaerobes, possibly because they do not survive during their manipulation in the culture process or due to lack of anaerobic technique during endoscopic sampling.[15–17] In the study by Choung et al.,[26] 3.7% of the microorganisms detected were anaerobic bacteria and in the study by Erdogan et al.,[13] 3.2% were anaerobic. Cultivation of anaerobes in a Freiter anaerobic chamber contributed to the accurate detection of *Lactobacillus, Bacteroides, Clostridium, Veillonella, Fusobacterium and Peptostreptococcus*.[19]

Table 1 Diagnosis of SIBO by microbiological analysis of duodenal/jejunal aspirate culture.

Author Year	Sample: number of cases (and controls)	Indication	Aspiration site; SIBO microbiological diagnosis threshold	Prevalence (%)	Microbiological analysis
Tabaqchali et al.[7] 1966	Cases: 47 Controls: 16	Blind loop syndrome (14), Partial gastrectomy (33)	Jejunum; Threshold NS	Cases: 100% Controls: 0%	Bacteria count $>10^4$ CFU/mL in 100% cases. **Microorganisms isolated:** *Escherichia coli* 72%, *Streptococcus faecalis* 20%, *Proteus* 10%, *Klebsiella* 8%, *Streptococcus viridans* 12%, *Bacteroides and lactobacillus* 8%, *Alpha-haemolytic streptococcus* 4%
Van Outryve et al.[9] 1978	31	Gastrectomy Billroth I and II	Jejunum or afferent loop; $\geq 10^5$ CFU/mL	80%	Aerobic or facultative aerobic bacteria
Kerlin et al.[18] 1988	45	Chronic diarrhoea, steatorrhoea	Jejunum or afferent loop; $\geq 10^5$ CFU/mL	60%	**Microorganisms isolated:** *Escherichia coli*, *Streptococcus*, *Bacteroides*, *Clostridium*, *candida* sp, *Klebsiella*, *Staphylococcus*, *Pleisiomonas*
Pignata et al.[44] 1990	Cases: 17 Children (2−17 years)	Immunodeficiency syndrome	Jejunum; $\geq 2 \times 10^5$ CFU/mL	41%	Gram-positive oropharyngeal-type bacteria. Genres: *Streptococcus*, *Staphylococcus*
Fried et al.[45] 1994	Cases: 25 Controls: 15	Peptic ulcer disease and treatment with omeprazole	Duodenum; $\geq 10^5$ CFU/mL	Cases: 56% Controls: 0% ($p = 0.0003$)	**Microorganisms isolated:** *Haemolytic Streptococcus* 85%, *Non-haemolytic streptococcus* 71%, *Klebsiella* spp. 35%, *Pseudomonas* spp. 14%, *Escherichia coli* 7%, *Clostridium spp.* 14%, *Bacteroides* 7%, *Enterococcus* 7%
Lewis et al.[46] 1997	Cases: 47 Controls: 23	Chronic diarrhoea, Bowel resection (25); diabetes mellitus and intrinsic bowel disease (21)	Duodenum; $\geq 10^4$ CFU/mL or presence of anaerobes	Cases: 29% Anaerobes: 6.38% Controls: 0%	**Microorganisms isolated:** *Streptococcus* spp., *Staphylococcus* spp., *Enterobacteriaceae*, fungi, anaerobes

Table 1 (*Continued*)

Author Year	Sample: number of cases (and controls)	Indication	Aspiration site; SIBO microbiological diagnosis threshold	Prevalence (%)	Microbiological analysis
Bouhnik et al.[19] 1999	63	Chronic diarrhoea, malabsorption	Jejunum; $\geq 10^5$ CFU/mL	87%	**Microaerophilic bacteria** (100%)**:** *Streptococcus* 71%, *Escherichia coli* 69%, *Staphylococcus* 25%, *Micrococcus* 22%, *Klebsiella* 20%, *Proteus* 11%, **Anaerobes** (93%)**:** *Lactobacillus* 75%, *Bacteroides* 29%, *Clostridium* 25%, *Veillonella* 25%, *Fusobacterium* 13%, *Peptostreptococcus* 13%
Bauer et al.[47] 2000	40	Cirrhosis	Jejunum; $\geq 10^5$ CFU/mL	73%	NS
Ghoshal et al.[48] 2003	Cases: 50 Controles:12	Malabsorption, chronic diarrhoea Controls with IBD	Jejunum; $\geq 10^5$ CFU/mL	Cases: 68% Controls: 25% ($p < 0.05$)	Aerobic bacteria 100% Anaerobic bacteria: 2.9%. **Microorganisms isolated in Malabsorption:** *Escherichia coli* 35%, *Streptococcus* species (non-A, non-B, non-D) 35%, *Klebsiella pneumoniae* 14%, *Enterococcus faecalis* 8%, *Enterococcus faecium* 2.9%, *Staphylococcus aureus* 14%, *Pseudomonas aeruginosa* 8%, *Acinetobacter baumannii* 8%, *Citrobacter freundii* 2,9%, *Proteus mirabilis* 2,9%, **Anaerobes** *Bacteroides melaninogenicus* 2.9%
Posserud et al.[11] 2007	Cases: 162 Controls: 26	IBS (Rome II)	Jejunum; $\geq 10^5$ CFU/mL CB **vs:** $\geq 10^4$ CFU/mL CB and $>5 \times 10^3$ CFU/mL CB	Cases: 4.3% Controls: 4%; Cases: 24% Controls: 4% ($p = 0.02$); Cases: 43% Controls: 12% $p = 0.002$	**IBS-C** *Escherichia coli*, *Enterococcus*, others. **IBS-D** *Enterobacter, Staphylococcus aureus, Klebsiella.* **Mixed IBS** *Escherichia coli*, *Enterococcus*, others

Table 1 (*Continued*)

Author Year	Sample: number of cases (and controls)	Indication	Aspiration site; SIBO microbiological diagnosis threshold	Prevalence (%)	Microbiological analysis
Kerckhoffs et al.[14] 2008	Cases: 12 Controls: 9	Suspected SIBO	Jejunum; $\geq 10^3$ CFU/mL CB (except *Lactobacillus and Streptococcus*)	Cases: 33% Controls: 0%	NS
Rubio-Tapia et al.[49] 2009	149	Non-responding coeliacs	Duodenum; $\geq 10^5$ CFU/mL	9.4%	NS
Choung et al.[26] 2011	675	Diarrhoea, weight loss, dyspepsia, IBS, malabsorption	Jejunum; $\geq 10^5$ CFU/mL Aerobes; **vs:** $\geq 10^4$ CFU/mL Anaerobes; both criteria	7% 0.3% 1%	83.33% aerobic bacteria, 4.6% anaerobic bacteria 12% mixed
Gutierrez et al.[50] 2012	57	Intestinal failure in children and refractory intestinal symptoms	Duodenum $\geq 10^5$ CFU/mL	70%	**Most common:** *Escherichia coli*, *Streptococcus viridans*, *Klebsiella pneumoniae*, *Enterococcus* spp., and *Pseudomonas aeruginosa*
Pyleris et al.[15] 2012	320 112 with IBS	Suspected SIBO. IBS (Rome II)	Duodenum; $\geq 10^3$ CFU/mL aerobic CB **vs:** $\geq 10^4$ CFU/mL aerobic CB $\geq 10^5$ CFU/mL Aerobic CB	19.4% (67.7% with IBS) 16.3% 10.9%	**Microorganisms isolated:** *Escherichia coli* 37.1%, *Enterococcus* spp. 32.3%, *Klebsiella pneumoniae* 24.2%, *Proteus mirabilis* 6.5%, *Acinetobacter baumannii* 4.8%, *Citrobacter freundii* 4.8%, *Serratia marscecens* 4.8%, *Staphylococcus aureus* 2.9%, *Pseudomonas putida* 2.9%, *Pasteurella multocida* 2.9% and *Enterobacter aerogenes* 1.6%
Ghoshal et al.[51] 2014	Cases: 80 Controls: 10	IBS (Rome III)	Jejunum; $\geq 10^5$ CFU/mL	Cases: 18.7% Controls: 0%	NS
Pistiki et al.[17] 2014	Cases: 567 (162 with IBS)	Suspected SIBO, IBS (Rome II)	Duodenum; $\geq 10^3$ CFU/mL	20.6%	**Microorganisms isolated:** *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Citrobacter freundii*, *Serratia marcescens*, *Enterococcus faecium*

Table 1 (*Continued*)

Author Year	Sample: number of cases (and controls)	Indication	Aspiration site; SIBO microbiological diagnosis threshold	Prevalence (%)	Microbiological analysis
Srivastava et al.[52] 2014	82	IBS (Rome II)	Duodenum; $\geq 10^5$ CFU/mL	18.3%	**Microorganisms isolated:** *Pseudomonas aeruginosa, Acinetobacter baumanii, Acinetobacter wofii,* *Staphylococcus* spp., *Enterococcus faecalis,* *Escherichia coli, Enterococcus faecium,* *Klebsiella pneumoniae, Streptococcus* spp.
Erdogan et al.[13] 2015	139	Suspected SIBO	Duodenum; $\geq 10^3$ CFU/mL **vs:** $\geq 10^5$ CFU/mL	44.6% 18%	$\geq$**10^3 CFU/mL:** 74.2% aerobic bacteria 3.2% anaerobic bacteria 22.6% mixed **Most common microorganisms:** *Alpha-haemolytic streptococcus* 30.6% *Escherichia coli* 12.1%, *Klebsiella pneumoniae* 5.6%, *Lactobacillus* spp 6.5%, *Neisseria* spp. 4.8%, *Non-haemolytic Streptococcus* 4.8%
Giamarellos-Bourboulis et al.[16] 2016	897	Suspected SIBO, IBS criteria	Duodenum; >10 CFU/mL CB **Vs:** >10^4 CFU/mL CB >10^5 CFU/mL CB	17.6% 15.6% 10.6%	***With IBS***: *Escherichia coli* 30%, *Klebsiella pneumoniae* 15%, *Enterobacter cloacae* 9.6%, *Staphylococcus aureus* 8.6%, *Enterococcus faecalis* 5.3%, *Enterobacter aerogenes* 5.3%, *Enterococcus faecium* 4.3%. ***More common without IBS:*** *Escherichia coli* 20%, *Klebsiella pneumoniae* 20%, *Staphylococcus aureus* 12.3%, *Enterobacter cloacae* 10.7%, *Enterococcus faecalis* 6.15%

Table 1 (*Continued*)

Author Year	Sample: number of cases (and controls)	Indication	Aspiration site; SIBO microbiological diagnosis threshold	Prevalence (%)	Microbiological analysis
Kapil et al.[20] 2016	32	Non-alcoholic fatty liver disease	Duodenum; $\geq 10^5$ CFU/mL	37.5%	**Most common:** *Escherichia coli*
Ghoshal et al.[53] 2017	Cases: 35 Controles:12	Non-alcoholic fatty liver disease Controls: IBS-constipation	Jejunum; $>10^3$ CFU/mL **vs** $\geq 10^5$ CFU/mL	Cases: 60% Controls: 25% ($p = 0.04$) Cases: 20% Controls: 0%	Cases: Gram-negative bacteria: 61%; Gram-positive: 23%; mixed: 14% Controls: Gram-negative bacteria: 25% **Microorganisms isolated:** *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Streptococcus* spp., *Enterococcus faecalis*
Rao et al.[54] 2018	Cases: 35 Controls: 32	Suspected SIBO in patients with colectomy Controls with gastrointestinal symptoms without colectomy	Duodenum; $\geq 10^3$ CFU/mL and $\geq 10^3$ CFU/mL fungi (SIFO)	Cases: 57.1% Controls: 37.5% SIFO: 28% Controls: 12%	**Most common microorganisms:** Cases: *Streptococcus* spp. 23% *Escherichia coli* 11% *Klebsiella pneumoniae* 11% *Staphylococcus aureus* 6% *Lactobacillus 9%* Controls: *Streptococcus spp.* 26% *Escherichia coli* 5% *Klebsiella pneumoniae* 11% *Lactobacillus*5% **Fungi**: *Candida albicans*, 61% *Candida glabrata* 39%
Bohm et al.[21] 2020	76	Clinically suspected SIBO	Jejunum; $\geq 10^4$ CFU/mL CB and $\geq 10^5$ CFU/mL UAT bacteria	Total 48%; 32% CB 15% UAT	**Colonic bacteria:** *Escherichia coli* 35%, *Klebsiella pneumoniae* 32% *Bacteroides spp.* 11%, *Clostridium spp.* 8% **UAT bacteria:** *Streptococcus viridans* 60% *Prevotella* spp. 15%

Table 1 (*Continued*)

Author Year	Sample: number of cases (and controls)	Indication	Aspiration site; SIBO microbiological diagnosis threshold	Prevalence (%)	Microbiological analysis
Mikolasevic et al.[22] 2020	117	Non-alcoholic fatty liver disease	Duodenum; $\geq 10^5$ CFU/mL	47.2%	**Microorganisms isolated:** *Escherichia coli* 22.2% *Klebsiella pneumoniae* 14.5% *Enterococcus faecalis* 14.5% *Klebsiella oxytoca* 7.7% *Enterococcus faecium* 4.3%
Cangemi et al.[25] 2021	85	Suspected SIBO	Duodenum; $\geq 10^5$ CFU/mL of Gram (-ve)	16.5%	NS
Gkolfakis et al.[23] 2023	Cases: 95 Controls: 30	Non-alcoholic fatty liver disease	Duodenum; $\geq 10^3$ CFU/mL **vs** $\geq 10^4$ CFU/mL $\geq 10^5$ CFU/mL	Cases: 23% Controls: 3.3%; Cases: 18.9% Controls: 0%; Cases: 11.6% Controls: 0%	**Microorganisms isolated:** *Escherichia coli* 45.5% *Staphylococcus aureus* 18.2% *Pseudomonas aeruginosa* 13.6% *Enterococus faecalis* 9.1% *Klebsiella pneumoniae* 4.5% *Providencia alcalifaciens* 4.5% *Acinetobacter* spp. 4.5% **Control:** *Enterobacter cloacae* 3.3%
Siddique et al.[24] 2023	144	Suspected SIBO	Jejunum; $\geq 10^4$ CFU/mL Colonic bacteria $\geq 10^5$ CFU/mL UAT	Total: 49%; 66.6% CB 33% UAT	**Colonic bacteria** *Streptococcus* (22%), *Klebsiella* (14%) *Escherichia* (13%) **UAT bacteria:** *Streptococcus* (26%), *Staphylococcus* (9%) *Prevotella* (7%), *Haemophilus* (7%)

CB: colonic-type bacteria; CFU/mL: colony forming units per millilitre; IBS: irritable bowel syndrome; IBS-C: constipation-type; IBS-D: diarrhoea-type; IBS-M: mixed or alternating; NS: not specified; SIBO: *small intestinal bacterial overgrowth*; SIFO: small intestinal fungal overgrowth; UAT: oropharyngeal or upper aerodigestive tract bacteria.

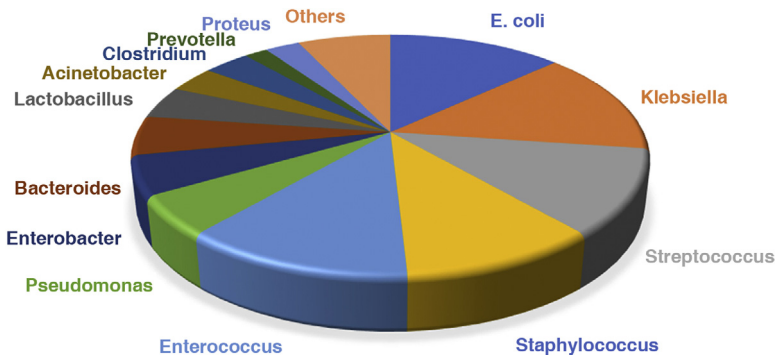

Figure 1 Duodenal/jejunal aspirate culture. Bacterial microorganisms isolated in patients with SIBO. SIBO: small intestinal bacterial overgrowth.

Analysis of the intestinal microbiota in small intestinal bacterial overgrowth by culture-independent techniques

The literature review identified five prospective studies analysing the small intestinal microbiota by sequencing methods in patients diagnosed with SIBO by culture. The characteristics and findings are shown in Table 2. Both the methodology and the results are heterogeneous and discordant.

On the one hand, three studies[27–29] show that there is a decrease in alpha diversity and an increase in beta microbial diversity in the duodenal aspirate of patients with SIBO compared to non-SIBO.

In the studies by Leite et al.,[27,28] patients who warranted an upper endoscopy for any reason were evaluated, diagnosing SIBO with a microbiological threshold >10^3 CFU/mL on MacConkey agar. The validated REIMAGINE (Revealing the Entire Intestinal Microbiota and its Associations with the Genetic, Immunologic, and Neuroendocrine Ecosystem) technique was used, which reduces sample contamination and optimises the recovery of microbial DNA from viscous mucus. Both studies demonstrate a predominance of the phylum Proteobacteria. There is a significant increase in the relative abundance of genera from the *Enterobacteriaceae* families correlated with bloating and *Aeromonadaceae* with defecation urgency. *E. coli* and *Klebsiella* were shown to disrupt duodenal microbial networks, negatively impacting microbial metabolic pathways.

In the most recent study by Leite et al.,[27] whole genome metagenomic sequencing was performed on a subcohort of 38 individuals, 17 of them with SIBO. The findings support results obtained by sequencing the *16S rRNA* gene. SIBO is primarily the result of two strains of *E. coli: E. coli BL21(DE3)* and *K-12* and two species of *Klebsiella*, making up about 40.24% of duodenal bacteria in individuals with SIBO compared to 5.6% in non-SIBO. The metabolic pathways of sugar degradation and fermentation, pyruvate fermentation pathways and sulfate reduction pathways were recognised, which correlate with symptoms such as diarrhoea, bloating and abdominal pain.

The study by Bamba et al.[29] conducted in Japan, with a cohort of 24 patients, 62% of whom had SIBO by microbiological criteria ($\geq 10^3$ CFU/mL), demonstrated remarkable growth of aerobic bacteria in the culture (86%). Significant changes were evident in the microbial composition of the aspirate of patients with SIBO, but different from those of Leite et al.,[27,28] characterised by a significant decrease in bacteria of the Bacteroidetes phylum, a decrease in bacteria of the *Bacteroides, Prevotella and Blautia* genera, and an increase in *Streptococcus, Actinomyces* and *Granulicatella*species.

Studies by Saffouri et al.[30] and Shin et al.[31] failed to demonstrate a correlation between SIBO and dysbiosis in luminal aspirate. No differences were found in alpha and beta microbial diversity in duodenal luminal fluid analysis in patients with SIBO, compared to non-SIBO.

Saffouri et al.[30] identified differences in the microbiota between symptomatic patients and healthy controls, highlighting significant alterations in small intestine microbial diversity in symptomatic patients. The microbial composition did not match the duodenal culture results. Decreases were observed in genera considered "normal" such as *Porphyromonas, Prevotella* and *Fusobacterium* in symptomatic patients. Furthermore, microbial pathways related to oxidative stress, siderophore biosynthesis and simple sugar metabolism were found in these subjects.

In the study by Shin et al.,[31] microbial communities from luminal fluid samples and duodenal biopsies were evaluated in 36 patients with suspected SIBO; 41.6% of those diagnosed by two SIBO cut-off points, $\geq 10^4$ CFU/mL for coliform bacteria and $\geq 10^5$ for UAT bacteria, showed significant changes in microbial alpha diversity only at the mucosal level between patients with SIBO and non-SIBO patients. Subgroup analysis revealed increased β diversity across multiple taxa at the genus level among coliform SIBO compared to mucosal-level UAT SIBO. Furthermore, increased relative abundances of specific bacterial taxa such as *Clostridium* spp. in mucosa and *Granulicatella* spp. in aspirate were observed in coliform SIBO vs non-SIBO subgroups. The findings indicate that microbiota attached to the mucosa and luminal fluid microbiota are distinct microbiological groups that should be examined separately.

Although the studies are very heterogeneous, when a patient has SIBO it is not only due to the increase in the abundance of proteobacteria (aerobic bacteria, especially coliforms), but would include the proliferation of other anaerobic taxa less recognised with traditional culture methods. This suggests that bacterial count by duodenal

Table 2 Metagenomic analysis of small intestine microbiota in SIBO.

Author Year	Sample (*n*); culture indication; microbiological diagnosis of SIBO; prevalence	Metagenomic sequencing analysis (duodenal aspirate, mucosa)	Relative abundance Genus and species	Family	Class	Phylum
Leite et al.[27]	505	**16S rRNA**	↑*Klebsiella*	↑Enterobacteriaceae (adjusted $p = 2.66E-7$)	↑Gamma	↑Proteobacteria
2023	Multiple indications;	**SIBO vs non-SIBO**	(p = <0.0018)		Proteobacteria	
	>10^3 CFU/mL on MCA: 20.3%	α diversity ($p = 0.09E-2$), ↑ β diversity ($p = 0.03$) ↓Connectivity of microbial networks	↑*Escherichia* (p = <0.00001) ↑*Shigella* ↑*Enterobacter*			
			↓*Streptococcus*	↓*Streptococcaceae*		
	34 cases	**Whole genome sequencing**	↑*Escherichia coli BL21(DE3) and K-12* ↑*Klebsiella* spp. ↑*Enterobacter* spp.	↑*Enterobacteriaceae*		
Bamba et al.[29]	24	**16S rRNA**	↑*Streptococcus*			↓Bacteroidetes ($p = 0.02$)
2023	Suspected SIBO;	**SIBO vs non-SIBO**	↑*Actinomyces*			↑Saccharibacteria ($p = 0.07$)
	>10^3 CFU/mL; 62%	↓ α diversity ($p = 0.01$), ↑ β diversity ($p = 0.01$)	↑*Granulicatella* ↓*Bacteroides* ↓ *Blautia* ↓ *Prevotella*			↑ Firmicutes ↑Proteobacteria ↓ Fusobacteria
Leite et al.[28]	140	**16S rRNA**	↑*Klebsiella* (P <0.0001)	↑*Enterobacteriaceae* ($p < 0.0001$)	↑Gammaproteobacteria	↑Proteobacteria
2020	Multiple indications;	**SIBO vs non-SIBO**	↑*Escherichia*/		$p < 0.0001$	(FDR $p = 2.21E-14$)
	>10^3 CFU/mL 30%	↓ α diversity	↑*Shigella* (P < 0.0001)		↑Deltaproteus	↓ Firmicutes

Table 2 (*Continued*)

Author Year	Sample (*n*); culture indication; microbiological diagnosis of SIBO; prevalence	Metagenomic sequencing analysis (duodenal aspirate, mucosa)	Relative abundance Genus and species	Family	Class	Phylum
		($p = 0.0009$), ↑ β diversity Inverse correlation Proteobacteria vs Firmicutes ($p < 0.0001$)	↑*Aeromonas* ↑ *Acinetobacter* ↑ *Moraxella*	↑*Aeromonadaceae* ↑*Moraxellaceae* ($p < 0.0001$)	bacteria $p = 2.07E-7$	($p = 0.0007$) ↓Actinobacteria ↓Fusobacteria ↓Bacteroidetes
Shin et al.[31] 2019	Casos: 36 Suspected SIBO **>10^5 CFU/mL**	**16S rRNA** **SIBO vs non-SIBO** Duodenal aspiration No differences in α or β diversity Mucosa ↓ α diversity **Coliform SIBO vs UAT:** Mucosa: ↑ β diversity ($p = 0.001$), differences in multiple bacterial taxa	**Coliform SIBO:** Aspirate ↑*Granulicatella* spp. Mucosa ↑ *Clostridium* spp.			
Saffouri et al.[30] 2019	Cases: 126 GI symptoms **>10^5 CFU/mL** Controls: 38	**SIBO vs non-SIBO** No differences in α and β diversity. **Symptomatic vs controls** ↓ α diversity, ($p < 0.0001$) ↑ β diversity, phylogenetic ($p < 0.001$) and non-phylogenetic ($p < 0.001$)	Symptomatic: ↓*Porphyromonas* ↓*Prevotella* ↓ *Fusobacterium*			

CFU/mL: colony forming units per millilitre; GI: gastrointestinal; MCA: MacConkey agar; SIBO: small intestinal bacterial overgrowth; UAT: oropharyngeal or upper aerodigestive tract-type bacteria.

Table 3 Sensitivity and specificity of the glucose breath test for the diagnosis of SIBO.

Author	Cases	Indication	GBT Glucose challenge; positivity	Microbiological diagnosis SIBO	Sensitivity (%)	Specificity (%)
Kerlin et al.[18] 1988	45	Suspected SIBO	50 g H_2 ≥12 ppm	≥10^5 CFU/mL	93	78
Pignata et al.[44] 1990	12	Immunodeficiency syndrome in children	Glucose 10% in water: 2 g/kg H_2 >10 ppm	≥10^5 CFU/mL	80	86
Corazza et al.[55] 1990	77	Malabsorption	75g H_2 ≥10 ppm	≥10^6 CFU/mL	63	83
Donald et al.[56] 1992	39	Malnutrition in older adults	50 g H_2 >20 ppm	>10^5 CFU/mL	20	77
Kaye et al.[57] 1995	24	Systemic sclerosis	50 g H_2 >20 ppm	>10^5 CFU/mL	88	100
MacMahon et al.[58] 1996	30	Older adults	50 g H_2 >10 ppm	≥10^5 CFU/mL	75	30
Stotzer et al.[59] 2000	46	Suspected SIBO, diarrhoea	50 g H_2 ≥15 ppm	≥10^5 CFU/mL	58	86
Ghoshal et al.[60] 2006	83	Malabsorption	Challenge NS H_2 >12 ppm	>10^5 CFU/mL	44	80
Berthold et al.[61] 2009	21	Suspected SIBO	50 g H_2 ≥10 ppm	≥10^6 CFU/mL	42	44
Ghoshal et al.[51] 2014	80	IBS	100g H_2 >12 ppm	>10^5 CFU/mL	27	100
Erdogan et al.[13] 2015	139	Suspected SIBO	75g H_2 ≥20 ppm or CH_4 ≥15 ppm	>10^3 CFU/mL	42	84
Rao et al.[54] 2018	15	Previous colectomy	75g H_2 ≥20 ppm or CH_4 >15 ppm or Both >15 ppm	≥10^5 CFU/mL	38	83
Sundin et al.[35] 2018	18	Suspected SIBO	90g H_2 >20 ppm or CH_4 >10 ppm	≥10^5 CFU/mL	20	75

CH_4: methane; GBT: glucose breath test; H_2: hydrogen; IBS: irritable bowel syndrome; NS: not specified; ppm: parts per million; SIBO: small intestinal bacterial overgrowth.

aspirate culture is not a 100% reliable marker as a reference diagnostic standard.

Lactulose and glucose breath tests as indirect methods for diagnosing small intestinal bacterial overgrowth

This literature review included investigating the diagnostic performance (sensitivity and specificity) of breath testing compared to jejunal aspirate culture (with a count of >10^5 CFU/mL and >10^3 CFU/mL).

Thirteen studies were selected in which the breath test was performed with glucose and six with lactulose. Their characteristics are shown in Tables 3 and 4 respectively.

The glucose dose used varied between 50, 75 and 100 g. The most commonly used cut-off value for test positivity is H_2 ≥10−12 ppm above the baseline value.

The dose used for lactulose ranged from 10 to 12 g in six studies and a positive test result was associated with the elevation of the double peak or a single elevation greater than 20 ppm above the baseline value.

The results of the mean sensitivity and specificity based on the diagnosis of SIBO by culture are shown in Table 5.

As can be seen in this table, the sensitivity and specificity of these tests are well below the standards required for a diagnostic test to be reliable. Added to this is the fact that the lactulose test is actually an instrument for estimating OCTT. The criteria for differentiating between SIBO and OCTT are arbitrary and not validated.

Discussion

The definition of SIBO continues to be the subject of debate. The current overdiagnosis of this condition may be partly due to a decrease in the cut-off point >10^3 CFU/mL for bacteria in duodenal aspirate culture, with the premise that bacterial populations are approximately $10^{4,5}$ CFU/mL in the duodenum, according to published reviews on small intestine bacterial populations by Booijink et al.[32] and Kastl

Table 4 Sensitivity and specificity of the lactulose breath test for the diagnosis of SIBO.

Author	Cases	Indication	LBT; lactulose challenge; positivity	Culture (SIBO diagnosis)	Sensitivity (%)	Specificity (%)
King et al.[62] 1986	30	Malabsorption, chronic diarrhoea, GI resection	10 g H_2 >10 ppm in two peaks One peak >20 ppm	>10^6 CFU/mL	55	100
Corazza et al.[55] 1990	77	Malabsorption	12 g H_2 ≥20 ppm or Two peaks >10 ppm	≥10^6 CFU/mL	68	44
Riordan et al.[63] 1996	28	Chronic diarrhoea, dyspepsia, anorexia and weight loss	10 g Double peak. First peak: H_2 >10 ppm, 15́before the second **Scintigraphy**: Two H_2 values >10 ppm First 10́before >5% caecal radioactivity	≥10^5 CFU/mL	16 38.9	70 100
Ghoshal et al.[62] 2006	83	Malabsorption	H_2 ≥20 ppm or double peak	>10^5 CFU/mL	31	86
Ghoshal et al.[60] 2014	80	IBS	10 g H_2 >20 ppm or double peak	>10^5 CFU/mL	33	65
Cangemi et al.[25] 2021	106	Suspected SIBO and GI Surgery (49)	10 g H_2 ≥20 ppm CH_4 ≥10 ppm	≥10^5 CFU/mL Gram (+) bacteria	69	29

CH_4: methane; GI: gastrointestinal; H_2: hydrogen; IBS: irritable bowel syndrome; LBT: lactulose breath test; ppm: parts per million; SIBO: small intestinal bacterial overgrowth.

Table 5 Sensitivity and specificity of the breath test for the diagnosis of SIBO.

Carbohydrate, dose in grams (g)	Sensitivity (%) Range	Specificity (%) Range
Glucose 50 g (6)	63 (20—93)	69 (30—100)
Glucose 75—100 g (5)	44.1 (20—75)	87.5 (75—100)
Lactulose 10—12 g (6)	45 (16—69)	65 (29—100)

SIBO: small intestinal bacterial overgrowth.

et al.[33]. Barlow et al.[34] evaluated absolute microbial loads by digital polymerase chain reaction (PCR) of patients with SIBO and reported higher (but not statistically significant) loads in patients with SIBO (>10^3 CFU/mL) compared to those without SIBO. The metagenomic study of the small intestine by Sundin et al.[35] in healthy individuals indicates that some had much higher bacterial loads, but this was not correlated with decreases in bacterial species diversity or evidence of dysbiosis. Bacterial populations in this region of the intestinal tract have a lower biomass, are less diverse, but are more dynamic, given the need to respond to light conditions and rapidly changing factors such as transit time, digestive enzymes and bile, intermittent delivery of food substrates, and oxygen use by bacteria.[33] The diagnosis of SIBO by culture can be related to factors such as diet and fibre intake, as reported by Saffouri et al.,[30] who found that a subgroup of healthy individuals on a diet high in complex carbohydrates had SIBO according to their culture results.

Microbiota analyses in SIBO highlight Gram-negative bacteria such as *E. coli* and *Klebsiella*, but also Gram-positive bacteria such as *Streptococcus* and *Enterococcus*, which are part of the normal microbiota of the upper gastrointestinal tract. The overgrowth of these bacteria has been linked to predisposing factors such as intestinal slowing by narcotics,[36] hypochlorhydria[21,37] and the ageing process.[38] In the metagenomic study by Barlow et al.,[34] differences in undefined *Streptococcus* species were found between saliva and duodenum in 21 individuals with SIBO, results that provide evidence against oral contamination. The role of these bacteria in SIBO and the development of gastrointestinal symptoms remains to be defined. The results of next-generation sequencing studies are heterogeneous and too inconsistent to draw definitive conclusions. The American Gastroenterological Association (AGA) proposes the term SIBO to describe a clinical disorder in which the symptoms, clinical signs and/or Laboratory abnormalities are attributed

to changes in the number of bacteria or in the composition of the bacterial population in the small intestine.[1] Accurately defining the characterisation of the small intestinal microbial population associated with SIBO is challenging, and future microbiota studies using next-generation sequencing are expected to provide a new concept of SIBO, both in quantitative and qualitative terms.

Overdiagnosis of SIBO may arise from the low sensitivity and specificity of the lactulose and glucose breath test, especially in disorders such as IBS without evident malabsorption. The prevalence of abnormal lactulose test results is high in people with IBS, reaching up to 84%.[39] A positive glucose breath test probably means SIBO. However, it cannot detect distal SIBO. The lactulose test does not differentiate between SIBO and rapid transit, leading to overdiagnosis and unnecessary treatment with antibiotics. The United European Gastroenterology[4] guidelines suggest considering the double peak of H_2 on the graph generated by lactulose and combining the test with scintigraphy to evaluate OCTT when possible. This is in contrast to the North American Consensus,[2] which does not consider the double peak in the breath test to be required for diagnosis and does not recommend performing nuclear scintigraphy to increase specificity.

One cause for confusion is the measurement of CH_4 gas in exhaled air, the elevation of which is associated with constipation in various studies, and has long been included in the diagnosis of SIBO. CH_4 is produced by a group of strictly anaerobic colonic (nonbacterial) microorganisms of the Archaea domain. Kim et al.[40] demonstrated that subjects with significantly higher stool counts of the Archaea *Methanobrevibacter smithii* were correlated with positive CH_4 breath test results. Similar findings were described by Villanueva-Millan et al.[41] Elevated CH_4 in breath tests is now referred to as *intestinal methanogen overgrowth*.[42]

The efficacy of rifaximin in improving gastrointestinal symptoms and normalising breath tests in 70.8% of patients with SIBO[42] could be related to dysbiosis in the small intestine or colon. However, it is important to evaluate the clinical context, history, and risks before diagnosing and treating SIBO with antibiotics. These diagnostic limitations highlight the importance of a more detailed characterisation of the small intestinal microbiota and the development of new diagnostic tools.

Advances are currently being made with new technology, such as the gas-sensing capsule (Atmo Gas-Sensing Capsule; Atmo Biosciences, Melbourne, Victoria, Australia) which, *in vivo*, can measure hydrogen and carbon dioxide, temperature, orientation, and changes in the physical and electromagnetic properties of the environment surrounding the capsule, providing insight into the fermentation activity in the gastrointestinal tract following ingestion of a source of carbohydrates. It also provides information on general and regional intestinal transit in a similar way to the wireless motility capsule; this was demonstrated in a validation study, in which both capsules were evaluated in tandem.[43] The performance and accuracy of the gas-sensing capsule has been demonstrated, showing results consistent with the wireless motility capsule in defining key anatomical reference points such as the gastroduodenal and ileocaecal junctions. This capsule needs to be tested for diagnosing SIBO, as if effective, it would be a better alternative to current breath hydrogen measurement techniques.

Conclusion

This review highlights the current difficulties in accurately determining SIBO, due to limitations with culture methods. Alterations in the microbiota, analysed by metagenomics, require contextual interpretation in clinical practice. Further studies are needed to detail the intestinal microbiota and its relationship with gastrointestinal symptoms in SIBO. Breath tests lack sufficient sensitivity and specificity, limiting their clinical utility, and the diagnosis of SIBO should therefore be approached with caution. We suggest the need to continue developing diagnostic methods, with the gas-sensing capsule being a promising option for the future.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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