

A Systematic Review: Fecal Bacterial Profile in Patients with Irritable Bowel Syndrome Analyzed with the GA-Map Dysbiosis Test Based on the 16S rRNA Gene of Bacterial Species or Groups

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Purpose: The diagnosis of irritable bowel syndrome (IBS) is based on symptom-based criteria due to lack of reliable disease-specific biomarkers. Gut microbiota is perturbed in IBS and when comparing different methods used to analyze gut microbiota, the results might be obscured. Therefore, in this systematic review we aimed to investigate the profile of fecal bacterial markers and dysbiosis index (DI) in patients with IBS and IBS subgroups compared to healthy controls (HCs) conducted by the same method (GA-map Dysbiosis Test based on 16S rRNA sequencing).

Material and Method: We searched PubMed, EMBASE (Ovid) and Cochrane Library for case-control studies comparing fecal gut microbiota analyzed with the GA-map[®] Dysbiosis Test (Oslo, Norway) in patients with IBS and HCs. Our outcomes were the difference in fecal bacterial markers and DI in patients with IBS and IBS subgroups compared to HCs.

Results: The search identified 28 citations; five articles were included. Most studies evaluated fecal bacterial markers and DI in patients with diarrhea-predominant IBS (IBS-D). Results of fecal bacteria profile in IBS and IBS subgroups compared to HCs are inconsistent, however, two studies showed increased levels of *Ruminococcus gnavus* in IBS-D compared to HCs and results of DI indicated IBS and IBS subgroups (especially IBS-D) having higher DI compared to HCs.

Conclusion: This systematic review revealed inconsistent findings in respect to differences in bacterial markers between IBS and IBS subgroups with HCs in studies using the GA-map Dysbiosis Test based on 16S rRNA sequencing. However, the test is quite novel, and few studies have used the method so far. More research comparing fecal microbiota profile differences in IBS and IBS subgroups compared to HCs utilizing the same method of analysis is needed to give us further insight into the gut bacteria profile in IBS and the clinical consequences of intestinal dysbiosis.

Keywords: gut microbiota, dysbiosis, diagnostic test, *Ruminococcus gnavus*

Introduction

Irritable bowel syndrome (IBS) is a disorder of the brain-gut axis interaction¹ affecting 4–10% of the general population.² Today, the diagnosis is based on exclusion of organic diseases and fulfilling the Rome IV criteria¹ which is based on symptoms of recurrent abdominal pain at least one day per week associated with stool irregularities.³ IBS is divided in four subtypes based on the predominant stool consistency: IBS with predominantly diarrhea (IBS-D), with predominantly constipation (IBS-C), with a mix of diarrhea and constipation (IBS-M) and unclassified subtype (IBS-U).³ The symptoms are experienced from mild to severe and often comes with somatic and psychiatric comorbidities such as fibromyalgia, fatigue, depression and anxiety, and impacts the quality of life in these patients.^{4–6} It also gives a profound burden on the healthcare system and the society.⁶

The etiology of IBS is complex and multifactorial, including altered brain-gut-microbiota axis, visceral hypersensitivity, low-grade inflammation, intestinal dysbiosis, and impaired gut barrier functions and integrity.^{1,7-9} Dysbiosis is an deviation from normobiosis and is defined as an imbalance in bacterial composition.⁴ Today, the manipulation of gut microbiota is suggested to be a target for therapy of IBS⁷ but the gut microbiota profile in IBS is still not fully understood as the literature reports inconsistent results. Several different methods are available and used for studying the gut microbiota and the costs are becoming more affordable.⁷ However, when comparing the fecal microbiota profile across different methods, the results might be obscured.

Therefore, in this systematic review we aimed to investigate the profile of fecal bacterial markers and dysbiosis index (DI) in patients with IBS and IBS subgroups compared to healthy controls (HCs) conducted by the same method of analysis, GA-map Dysbiosis Test.¹⁰ The test is based on 16S rRNA sequencing, is commercially available and is an easy to use method which aims to identify and characterize dysbiosis by determining deviation from normobiosis in fecal samples in IBS and IBD. Hopefully, this will give us further insight into the gut bacteria profile in IBS and the clinical consequences of intestinal dysbiosis.

Materials and Methods

Search Strategy

We performed a systematic review with relevant literature retrieved from the databases PubMed, EMBASE (Ovid) and Cochrane Library to identify case-control studies comparing fecal gut microbiota analyzed with the GA-map Dysbiosis Test in patients with IBS and HCs. The search terms used to identify potentially related publications included «Genetic analysis AND Dysbiosis test AND Irritable bowel syndrome», with results by year 2015 to January 2023. Two authors independently reviewed the studies retrieved by the search strategy and excluded trials based on titles, abstracts, and full-text analysis. We followed the checklist and flowchart of the Preferred Reporting of Systematic Reviews and Meta-Analyses (PRISMA) for this systematic review.

Study Selection

The inclusion criteria (Table 1) were studies investigating fecal microbiota in adult IBS patients compared with HCs, IBS patients classified according to the ROME II–IV criteria, GA-map Dysbiosis Test to analyze fecal bacterial samples, samples from stool, written in English, published from 2015 to January 2023 and articles published as a full article. The exclusion criteria were literature published before 2015, not written in English, fecal microbiota not analyzed with the GA-map Dysbiosis Test or not case-control studies.

Table 1 Inclusion Criteria for Articles Included in the Systematic Review

Study, First Author, Year	Case-Control (IBS vs HC)	GA-map Dysbiosis Test	Samples of Feces	Adult Population	Written in English	Article Published 2015–2023	Article Available as a Full Article
Ahluwalia et al, 2021 ¹¹	*	*	*	*	*	*	*
Casén et al, 2015 ¹⁰	*	*	*	*	*	*	*
Vasapolli et al, 2021 ¹²	*	*	*	*	*	*	*
Iribarren et al, 2022 ¹³	*	*	*	*	*	*	*
Mazzawi et al, 2018 ¹⁴	*	*	*	*	*	*	*

Choice of Outcome

The primary outcome was differences in fecal bacterial markers and DI in patients with IBS compared to HCs. The secondary outcome was differences in bacterial markers and DI in subgroups of IBS compared to HCs. We classified the primary and secondary outcomes into three categories; significantly increased in IBS patients, significantly decreased in IBS patients and no significant (NS) difference between IBS patients and HCs. The DI was estimated as mean or median.

Fecal Bacteria and Dysbiosis Analysis

GA-map Dysbiosis Test (GA-map[®] Dysbiosis Test, Oslo, Norway)¹⁰ was developed to identify and characterize dysbiosis by determining deviation from normobiosis in fecal samples in patients with IBS and IBD. It gives an algorithmically Dysbiosis Index (DI) from 1 to 5 where a DI value of 2 or lower being non-dysbiotic and 3–5 being dysbiotic. The higher DI above 2, the more it is considered to deviate from normobiosis and being dysbiotic. Transient luminal bacteria with no diagnostic values have the potential to obscure diagnostic results. Therefore, the probe set was based on intestinal microbiota observed in IBS and IBD in the literature and each probe was selected based on the ability to distinguish between IBS and IBD and HCs.¹⁰ The technology uses 54 bacterial markers, based on the 16S rRNA sequences in seven variable regions (V3-V9) and measures relative abundance of bacteria according to strength of fluorescent signal detection. This provides a unique opportunity to investigate changes in gut microbiota profiles which potentially might be associated with GI-related disorders.

Eligibility Assessment and Data Extraction

To reduce the reporting error and bias in data manipulation, both authors independently reviewed the selected studies for complete analysis. One study author extracted the data and entered it into a spreadsheet, while the other study author evaluated the process. When there was a discrepancy between reviewers, we re-checked and discussed the data together to reach an agreement by consensus. The data included are presented in [Table 2](#); distribution of age and sex, country in which the research was conducted, the size of the IBS and HC groups, IBS subtypes, details of intervention, multiple comparison correction, dysbiosis index and bacterial markers difference with a significant P value < 0.05.

Quality Assessment

We applied the Newcastle-Ottawa Scale (NOS) for assessing the quality of included case-control studies in this review (data presented in [Table 3](#)). The Newcastle-Ottawa Scale consists of 3 domains (maximum nine stars); selection (is the case definition adequate, representativeness of the cases, selection of controls, definition of controls); comparability (comparability of baseline characteristics); and exposure (ascertainment of exposure, same method of ascertainment for cases and controls, attrition rate). We decided that sex and age was the most important baseline characteristics to be described and compared in both groups.

Results

Study Selection and Characteristics

Overall, 28 citations were retrieved ([Figure 1](#)): six citations were duplicates and four citations were rejected based on their title. Within the 22 remaining studies, 13 were excluded based on the abstract or full-text review as nine studies had no healthy controls, two studies were conference abstracts and two papers included repetition of data. In total, five studies investigating fecal microbiota composition and/or DI were included in the systematic review.

Study populations across the studies were conducted from Sweden,^{10,11,13} Norway,^{10,14} Denmark,¹⁰ Spain¹⁰ and Germany.¹² All studies had case-control design, were published in the period of 2015 to January 2023 and fecal microbiota was assessed with the GA-map Dysbiosis Test in adults of both genders.

The Newcastle Ottawa Scale ([Table 3](#)) showed all relevant studies provided an adequate explanation in the definition and selection method for IBS patients and HCs with IBS classified according to Rome II–IV criteria by a physician, and HCs did not have any organic disease or gastrointestinal (GI) symptoms. All studies demonstrated comparable data of both sex and age in IBS and HCs, hence, one study had significantly higher median age in IBS compared to HCs¹¹ and as

Table 2 Characteristics of Included Studies in the Systematic Review

Study, first author, year	Country	Type of specimen	IBS patients					Controls						
			IBS subtype	Age (years)	n participants	Female/ Males	DI ^a	Age (years)	n participants	Female/ Males	DI ^a	Microbiome assessment method	Multiple comparison correction	Bacterial markers difference with a significant P value < 0.05
Ahluwalia et al, 2021 ¹¹	Sweden	Stool	IBS, IBS-D, IBS-M, IBS-C, IBS-U	Median (min-max) 52 (24–70)	40	31/9	No	Median (min-max) 26 (19–54)	18	9/9	No	GA-map Dysbiosis Test	NR ^b	Yes
Casén et al, 2015 ¹⁰	Norway, Sweden, Denmark, Spain	Stool	IBS, IBS-D, IBS-C, IBS-M, IBS-U, IBS-A	Mean: 39	109	184/52	Yes	Mean (min-max) 41 (21–70)	43	187/110	Yes	GA-map Dysbiosis Test	NR ^b	Yes
Vasapolli et al, 2021 ¹²	Germany	Stool	IBS-D, IBS-C	Mean (SD) IBS-D: 43.5 (20.6) IBS-C: 50.6 (18.2)	IBS-D: 15 IBS-C: 13	IBS-D: 10/5, IBS-C: 10/3	Yes	Mean (SD) 54.8 (11.2)	22	12/10	Yes	GA-map Dysbiosis Test (Lx)	Yes	Yes
Iribarren et al, 2022 ¹³	Sweden	Stool	IBS-D	Median (range) 31 (26–66)	9	6/3	No	Median (range) 22 (20–36)	7	5/2	No	GA-map Dysbiosis Test	NR ^b	Yes
Mazzawi et al, 2018 ¹⁴	Norway	Stool	Mostly IBS-D	Mean (min-max) 32 (20–44)	13	4/9	Yes	Mean (min-max) 33 (20–42)	13	7/6	Yes	GA-map Dysbiosis Test	Yes	Yes

Notes: ^aDysbiosis Index, ^bNot reported.

Table 3 Quality of Each Study Included by Newcastle Ottawa Scale (NOS)

	Selection				Comparability		Exposure		
Study, first author, year	(1) Is the case definition adequate? (ROME criteria)	(2) Representativeness of the cases	(3) Selection of controls	(4) Definition of controls	(5) Comparability of baseline characteristics 1 (sex)	(6) Comparability of baseline characteristics 2 (age)	(7) Ascertainment of exposure	(8) Same method of ascertainment for cases and controls?	(9) Non-response rate
Ahluwalia et al, 2021 ¹¹	*	*	*	*	*	IBS significantly older than HCs	*	*	Not relevant
Casén et al, 2015 ¹⁰	*	*	*	*	*	*	*	*	Not relevant
Vasapolli et al, 2021 ¹²	*	*	*	*	*	*	*	*	Not relevant
Iribarren et al, 2022 ¹³	*	*	NR ^a	*	*	*	*	*	Not relevant
Mazzawi et al, 2018 ¹⁴	*	*	*	*	*	*	*	*	Not relevant

Note: ^aNot reported.

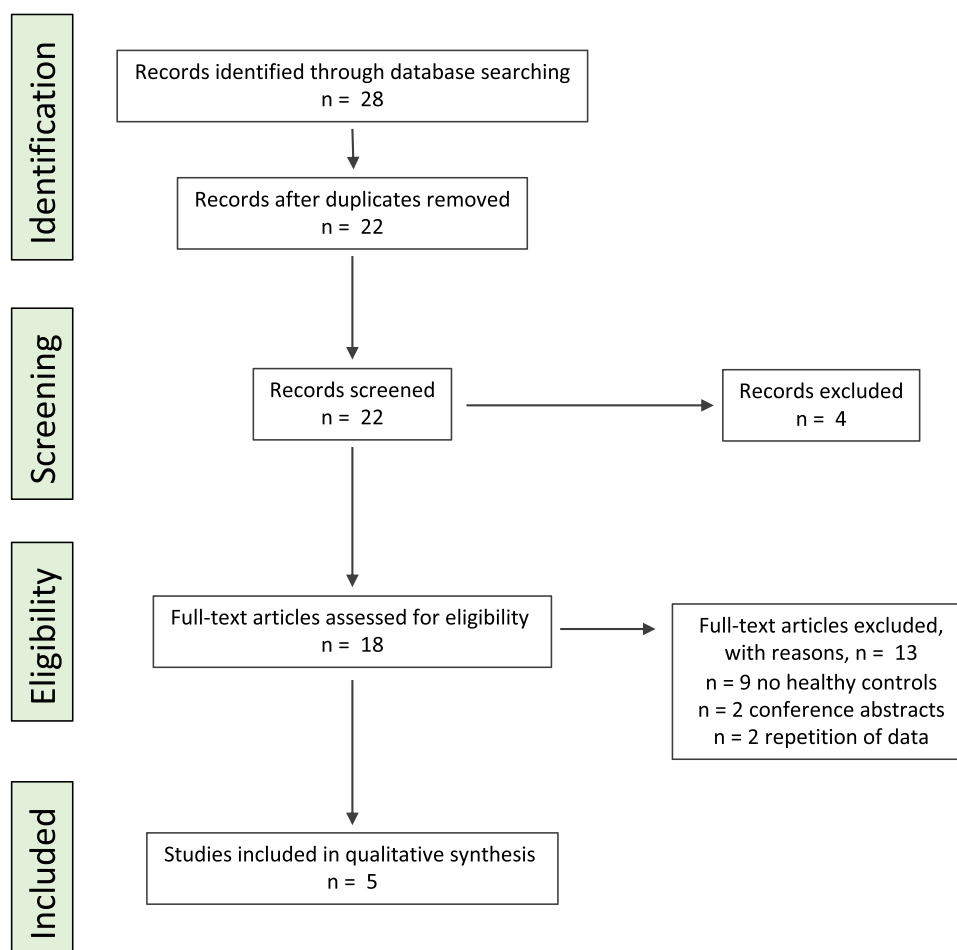


Figure 1 PRISMA flow diagram for depicting the literature search in PubMed (Medline) for this systematic review.

expected, all studies had an overrepresentation of females in both groups except from Ahluwalia et al 2021¹¹ having the same number for males and females in HCs, and Mazzawi et al 2018¹⁴ having more males than females in IBS (Table 2). Two out of five articles reported multiple comparison correction^{12,14} and all studies used a significant p-value < 0.05 for bacterial marker and/or DI difference.

In terms of IBS subtype, three out of five studies included both IBS-D and IBS-C^{10–12} where two also included IBS-M.^{10,11} The two remaining articles included only IBS-D.^{13,14} One study did not investigate fecal microbiota composition between IBS and HCs nor across IBS subgroups but investigated DI.¹⁰ One study investigated the DI for IBS,¹⁰ three studies investigated the DI for IBS-D,^{10,12,14} two studies investigated DI for IBS-C^{10,12} and only one study investigated DI for IBS-M.¹⁰ In respect to bacterial markers, one study investigated IBS compared to HCs,¹¹ three studies investigated IBS-D^{12–14} one study investigated IBS-C¹² and no studies investigated the difference in bacterial markers in IBS-M compared to HCs.

Fecal Microbiota Composition

Findings for fecal microbiota are summarized in Table 4. Among five studies included in this review, the reported main findings are overall inconsistent. For differences between IBS and HCs, one study reported enrichment of *Acinetobacter junii* and *Akkermansia muciniphila* and decreased levels of *Clostridium* sp. in IBS compared to HCs. For differences between IBS subgroups and HCs only IBS-D was reported to have significant findings; two studies reported increased levels of *Ruminococcus gnavus*, one study reported enrichment of *Proteobacteria* and *Dorea* spp. and one study reported decreased levels of *Bacteroides pectinophilus*, Actinobacteria and *Bifidobacteria* compared to HCs.

Table 4 Differences in Bacterial Markers Between IBS and IBS Subgroups and Healthy Controls (HCs)

	Increased in IBS vs HCs				Decreased in IBS vs HCs				No difference in IBS vs HCs			
Study, first author, year	IBS	IBS-D	IBS-M	IBS-C	IBS	IBS-D	IBS-M	IBS-C	IBS	IBS-D	IBS-M	IBS-C
Ahluwalia et al, 2021 ¹¹	<i>Acinetobacter junii</i> ; <i>Akkermansia muciniphila</i>				<i>Clostridium</i> sp.							
Casén et al, 2015 ¹⁰	NR ^a											
Vasapolli et al, 2021 ¹²										NS ^b		NS ^b
Iribarren et al, 2022 ¹³		Proteobacteria <i>Pseudomonas</i> spp.; <i>Dorea</i> spp.; <i>Ruminococcus</i> <i>gnavus</i>				<i>Bacteroides pectinophilus</i>						
Mazzawi, 2018		<i>Ruminococcus gnavus</i>				<i>Actinobacteria</i> ; <i>Bifidobacteria</i>						

^aNot reported, ^bNon-significant.

Dysbiosis Index

Findings for DI are summarized in Table 5. The reported main findings indicate that IBS and IBS subgroups have higher levels of DI compared to HCs. For differences between IBS and HCs, one study found a significant increase in DI in IBS compared to HCs. For differences between IBS subgroups and HCs: two studies found IBS-D with increased^{10,14} and one study with a non-significant increased¹² DI compared to HCs. In IBS-C one study found a significant and one study found a non-significant increase in DI compared to HCs. For the only study investigating IBS-M, the DI was significantly increased in comparison to HCs.

Table 5 Dysbiosis Index in IBS and IBS Subgroups Compared to Healthy Controls (HCs)

	Increased in IBS vs HCs				Decreased in IBS vs HCs				No difference in IBS vs HCs			
Study, first author, year	IBS	IBS-D	IBS-M	IBS-C	IBS	IBS-D	IBS-M	IBS-C	IBS	IBS-D	IBS-M	IBS-C
Ahluwalia, 2021	NR ^a											
Casén, 2015	73% (IBS) vs 16% (HCs) Mean: IBS: 2.98 HCs: 1.72	76% (IBS-D) vs 16% (HCs) Mean: IBS-D: 3.03 HCs: 1.72	67% (IBS-M) vs 16% (HCs) Mean: IBS-M: 3.33 HCs: 1.72	73% (IBS-C) vs 16% (HCs) Mean: IBS-C: 3.00 HCs: 1.72								
Vasapolli, 2021									NS ^b	NS ^b		NS ^b
Iribarren, 2022	NR ^a											
Mazzawi, 2018		Mean±SD: 4 ±0.5 (IBS) vs 2.6±0.2 (HCs)										

Notes: ^aNot reported, ^bNon-significant.

Abbreviations: IBS, irritable bowel syndrome; IBS-D, diarrhea-predominant IBS; IBS-M, IBS with mix of diarrhea and constipation; IBS-C, constipation-predominant IBS; HCs, healthy controls; DI, dysbiosis index; NOS, Newcastle Ottawa Scale; PRISMA, preferred reporting items for systematic reviews and meta-analysis; NR, not reported; NS, non-significant.

Discussion

To our knowledge, this is the first systematic review investigating differences in fecal bacterial profile and DI between IBS and IBS subgroups compared to HCs performed by using GA-map Dysbiosis Test based on the 16s rRNA gene of bacterial species or groups. Based on only five studies included the results was inconsistent. However, two studies showed an increase in *Ruminococcus gnavus* in IBS-D compared to HCs and results of DI indicated IBS and IBS subgroups (especially IBS-D) having higher DI compared to HCs.

Today, there are many methods used to investigate the gut microbiota profile in patients with IBS, and recently we have been able to increase the exploration due to progress in microbial analytic techniques.⁴ Studies have shown that IBS have a different microbiota profile compared to HCs, but the existing literature is inconclusive.¹⁵ There are many factors which makes it challenging to interpret the results of gut microbiota profile. Firstly, the gut microbiota is highly dynamic and is affected especially by diet, medications, ethnicity, and geographical deviations. Secondly, the samples can be taken at different sites such as in stool or mucosa, and thirdly, the results can be obscured when comparing findings across different methods.^{15,16} All these factors might contribute to the inconclusive findings of gut microbiota in IBS and is needed to be taken into consideration when interpreting results of gut microbiota.

In the present review, only one study investigated the difference in fecal bacterial markers between IBS and HCs: increased levels of *Acinetobacter junii* (phyla Proteobacteria) and *Akkermansia muciniphila* (phyla Verrucomicrobiota) and decreased levels of *Clostridium* sp. (phyla Firmicutes) in IBS compared to HCs, and only one study compared the DI between the two groups: DI was significantly higher in IBS compared to HCs.

The observations of differences in bacterial composition are not similar to previous findings conducted with several different methods of bacterial analysis. A systematic review¹⁷ investigating differences in gut microbiota in IBS compared to HCs conducted with 16S rRNA-targeted sequencing observed consistent findings which included increased level of the phyla Firmicutes and reduced level of the phyla Bacteroidetes and increased Firmicutes/Bacteroidetes ratio, higher levels of *Clostridia* and *Clostridiales* (phyla Firmicutes), and lower levels of *Bacteroidia* and *Bacteroidales* (phylum Bacteroidetes) in IBS. The studies were discrepant in results of changes in alpha-diversity (specie richness), observing both higher, lower and no difference between IBS and HCs using the Shannon index and Chao1 index. However, almost similar to our findings, the authors found a trend of IBS having lower alfa-diversity in comparison to HCs. A systematic review conducted by Pittayanon et al 2019¹⁵ based on stool and colonic tissue microbiota analyzed with different methods found that the difference between IBS and HCs included increased levels of *Enterobacteriaceae* (family, phylum Proteobacteria), *Lactobacillaceae* (family, phylum Firmicutes) and *Bacteroides* (genus, phyla Bacteroidetes), and reduced levels of uncultured *Clostridiales* I (phyla Firmicutes), genus *Faecalibacterium* (including *Faecalibacterium prausnitzii*, phyla Firmicutes) and genus *Bifidobacterium* (phyla Actinobacteria) in IBS compared to HCs. One study showed increased levels and another no significant difference in the abundance of *Acinetobacter* (phylum Proteobacteria) in IBS compared to HCs. The diversity of microbiota was either decreased or not different in IBS compared to HCs. The author addressed that there were no two studies reporting the same differences in OTUs.¹⁵ Another systematic review and meta-analysis of fecal microbiota profile in IBS compared to HCs using quantitative real-time PCR analysis found a reduced abundance of *Lactobacillus* (phyla Firmicutes), *Bifidobacterium* (phyla Actinobacteria) and *F. Prausnitzii* (phyla Firmicutes) in IBS compared to HCs.¹⁸

The abovementioned systematic reviews^{15,17} reveal inconsistent results in the findings of *Clostridiales* and *Clostridia*. We observed decreased levels of *Clostridium* sp. (specie in the *Clostridia* class) in IBS. *Clostridia* class is observed to be at higher levels in IBS^{19,20} and IBS-D²¹ compared to HCs and has been speculated to be a part of the pathogenesis in IBS due to the contribution of overactive bile acid secretion²¹ and dysregulation of gut serotonin production.^{22,23} *Clostridia* class contains gut pathogens causing some of the most common clostridial infections in the gut,²⁴ such as *Clostridium difficile* infections which is a common cause of post-infectious IBS.²⁵ But some *Clostridium* species are commensals and might have potentials as probiotics.²⁶ Their cellular components and metabolites play a probiotic role primarily through strengthening the intestinal barrier and interacting with the immune system. Unique patterns of *Clostridium* species in gut can be shaped depending on our diets and physical state of body²⁶ and a balance of *Clostridium* spp. is needed to form a healthy gut. Therefore, both decreased and increased numbers might cause IBS symptoms.

Patients with IBS are a heterogeneous group with different stool habits affecting the fecal and luminal microbiota.^{27,28} This makes the comparison of gut microbiota in IBS across studies challenging as the relative frequency of each subgroup is different in all studies. We should take this into consideration and investigate all subgroups.

In respect to differences in IBS subgroups and HCs, IBS-D had significantly higher levels of Proteobacteria, *Pseudomonas* spp. (phyla Proteobacteria), *Dorea* spp. (phyla Firmicutes) and *Ruminococcus gnavus* (phyla Firmicutes) and significantly lower levels of *Bacteroides pectinophilus* (phyla Bacteroidetes), Actinobacteria and *Bifidobacteria* (phyla Actinobacteria) compared to HCs. *Ruminococcus gnavus* was found to be increased in IBS-D in two studies. Two studies also found DI to be significantly higher in IBS-D, hence, one study found a non-significant increase compared to HCs. DI in IBS-C and IBS-M was found to be significantly increased in comparison to HCs.

The findings of *Ruminococcus gnavus* being increased in IBS-D are directly in line with previous findings. The bacterial marker is a member of the *Clostridia* class (Firmicutes phyla) and it has been suggested to play a pathogenic role in IBS-D.²⁹ Monocolonization of germ-free mice with *Ruminococcus gnavus* induced IBS-D like symptoms, including increased GI transit and colonic secretion by stimulating the production of peripheral serotonin.²⁹ Serotonin (5-HT) has been shown to modulate gut motility and hypersensitivity functions.³⁰ The study of Rajilic-Stojanovic et al 2011²⁰ indicated that phylotypes related to *Ruminococcus gnavus* are significantly increased in patients with IBS and is positively associated with IBS symptoms. Increased levels of *Ruminococcus gnavus* contributed to the increased dysbiosis in patients with IBS in the study of Casén et al 2015,¹⁰ along with increased levels of Proteobacteria (*Shigella/Escherichia*) and Actinobacteria. Our findings also reveal Proteobacteria (phylum) being increased in IBS-D according to Iribarren et al 2022.¹³ An increasing amount of data identifies Proteobacteria as a possible microbial signature of disease as it includes pathogenic bacteria with potential inflammation causing mechanisms.^{31,32} Increased levels in IBS^{17,33} and IBS-D^{31,34} are observed, and several studies have found the bacterial marker associated with IBS¹⁷ and with individuals with high DI scores.¹² We also found *Dorea* spp. (Firmicutes phyla) at higher levels in IBS-D. Proteobacteria and *Dorea* spp. are observed to be increased in IBS-D together with excessive excreted bile acid²¹ and higher levels of *Streptococcus* spp., *Dorea* spp. and *Ruminococcus* are reported to be associated with potentially harmful properties.²⁰ It has been proposed that *Dorea* spp. is associated with increased gas production and increased gut permeability, which is thought to contribute to IBS pathophysiology.³⁵ Altogether, our findings of increased levels of *Ruminococcus gnavus* and Proteobacteria in IBS-D seems to be supported by the literature.

Furthermore, we observed that Actinobacteria and *Bifidobacteria* (phylum Actinobacteria)¹⁴ was decreased in IBS-D compared to HCs. A significant reduction in Actinobacteria has previously been observed in IBS²⁰ and IBS-D.³⁴ A systematic review showed that patients with IBS tend to have decreased levels of *Bifidobacteria*¹⁵ and it is found a 1,5 decrease in *Bifidobacteria* in IBS compared to HCs and a negative association between *Bifidobacterium* spp. and abdominal pain in IBS.²⁰ Actinobacteria is one of the major phyla in the gut microbiota and is pivotal in the maintenance of gut homeostasis. Classes of this phylum are widely used as probiotics demonstrating beneficial effects in many pathological conditions,³⁶ especially *Bifidobacterium* is believed to exert positive health benefits on the host³⁷ and has been commercially exploited as probiotics.³⁸ These characteristics of Actinobacteria and *Bifidobacteria* might explain the observation of low levels of Actinobacteria in IBS-D. However, increased level of Actinobacteria was shown to contribute to the dysbiosis of IBS in the study of Casén et al 2015,¹⁰ making the interpretation inconclusive.

Recent evidence suggests that dysbiosis might play a role in the pathogenesis of IBS^{1,39} and a systematic review and meta-analysis⁴⁰ investigating gut microbial dysbiosis in patients with IBS concludes that IBS, including IBS-D and IBS-C, are characterized by gut microbiota dysbiosis. These findings are in line with the observations of dysbiosis in IBS, IBS-D, IBS-M, and IBS-C.

The above-mentioned studies have aimed to differentiate IBS and/or IBS subgroups from healthy controls based on fecal and/or gut microbiota composition but the results are inconclusive. When adding relevant variables (as Rome criteria, fecal transit time, gut microbiota metabolites and more), and using machine learning techniques the ability to distinguish IBS and/or IBS subgroups from healthy controls has been shown possible and might be a future method for diagnosing patients with IBS.^{41–43}

Limitations

The similarities and differences in fecal bacterial profile across studies using the GA-map Dysbiosis Test in patients with IBS compared to HCs might be explained by several reasons; patients and cohorts have different ethnicities, geographical sites, and different diets. Studies suggest that people from the same ethnicity share the same number of gut microbiota and indicate that the ethnicity selects for specific gut taxa, and diet is reported to have profound effects on the gut microbiota composition.^{16,44} Furthermore, although in the same group of patients, there are interindividual variations in the gut microbiota profile. Such variations are poorly understood, and appears to obscure the microbiota's associations with patients health and impacts the microbiota-based diagnostics of disease.¹⁶ The use of both Rome II, III and IV criteria for participants included in this systematic review might affect the outcome. Rome IV criteria differs from Rome III criteria in different ways. The most distinct difference is the change in the criteria of “abdominal pain or discomfort” in Rome III (and Rome II) to only include “abdominal pain” in Rome IV.⁴⁵ Although Rome II, III and IV was used in the five studies included, three^{11,12,14} studies and some of the participants of Casén et al¹⁰ used Rome III criteria.

The GA-map Dysbiosis Test training and test cohort was included from Norway, Sweden, Denmark, and Spain, in an effort to achieve heterogeneity. Hence, they found a significantly different fecal bacterial profile in the Spanish cohort compared to the Scandinavian, and different bacterial markers contributing to the dysbiosis of the Spanish cohort.¹⁰ The studies included in the present systematic review are conducted at different geographical sites and include cohorts with different ethnicities. Moreover, all participants in the studies were either told to obtain from a special diet or to have a normal Scandinavian diet without any major dietary change months prior to inclusion, or there were not reported any dietary inclusion or exclusion criteria. The diet at the time of inclusion naturally includes variation and were not reported to be controlled for in these studies. In respect to medications, as a minimum criterion, all studies did not allow the use of systemic antibiotics four weeks to six months prior to inclusion, and some studies excluded the use of immunosuppressive and/or glucocorticoids, or large doses of commercially available probiotics, and some, any other medications.

Additional limitations of this systematic review are the low number of studies (n = 5) included. Only one study investigated DI and one study investigated bacterial markers in IBS compared to HCs. Hence, three studies investigated the DI and fecal bacterial markers in IBS-D compared to HCs. In total there was a low volume of cases and controls. A higher number of females in respect to men was included in most of the studies. This was expected as IBS is reported to be more frequent in females.⁴⁶ Ahluwalia et al 2021¹¹ reported IBS patients to be significantly older than HCs.

Conclusion

This systematic review reveals inconsistent findings in respect to differences in bacterial markers between IBS and IBS subgroups with healthy controls in studies using the GA-map Dysbiosis Test. However, IBS-D was observed to have increased levels of *Ruminococcus gnavus* in two studies and IBS and IBS subgroups, especially IBS-D tend to have higher relative frequency of dysbiosis compared to HCs. The GA-map Dysbiosis Test is stated as a precise and easy in use method to identify dysbiosis in IBS and IBD. Comparing gut microbiota profiles in IBS patients across different ethnicities, geographical sites, sample sites, with different diets, medication use and with different methods will obscure the results. Hopefully, future research will take these aspects into account and investing fecal microbiota profile and dysbiotic differences in IBS and IBS subgroups compared to HCs. This will increase our understanding of gut microbiota's associations with IBS pathophysiology and the clinical consequences of intestinal dysbiosis.

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