Gastroenterology 2019;157:97–108

Gut Microbiota in Patients With Irritable Bowel Syndrome—A Systematic Review

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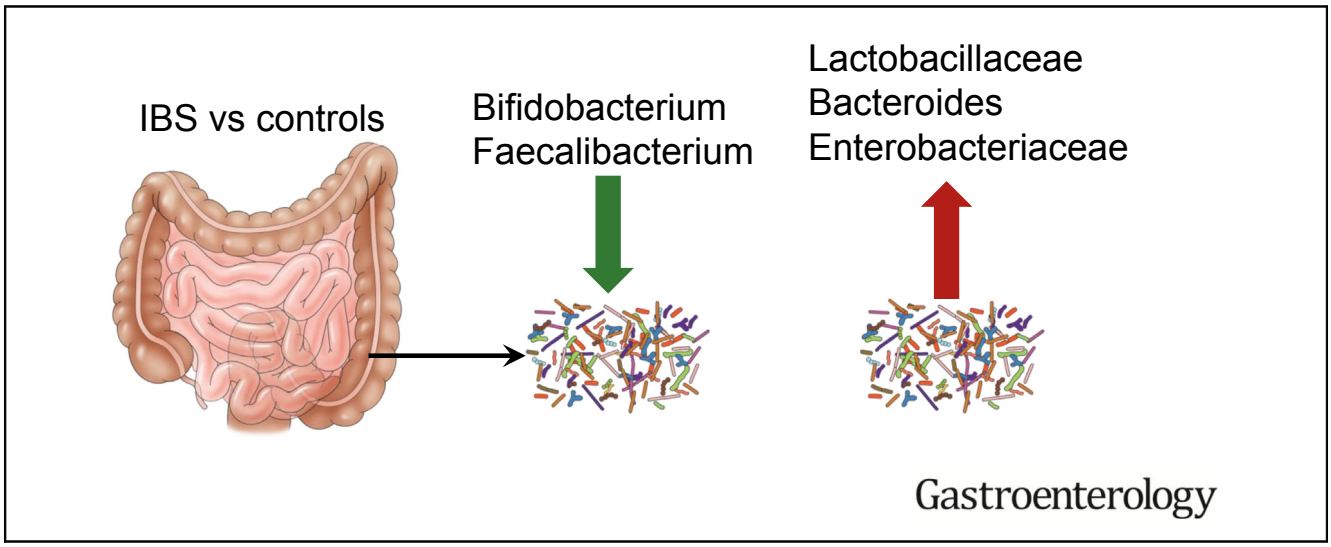

See Covering the Cover synopsis on 1.

BACKGROUND & AIMS: Irritable bowel syndrome (IBS) is common but difficult to treat. Altering the gut microbiota has been proposed as a strategy for treatment of IBS, but the association between the gut microbiome and IBS symptoms has not been well established. We performed a systematic review to explore evidence for this association. **METHODS:** We searched databases, including MEDLINE, EMBASE, Cochrane CDSR, and CENTRAL, through April 2, 2018 for case–control studies comparing the fecal or colon microbiomes of adult or pediatric patients with IBS with microbiomes of healthy individuals (controls). The primary outcome was differences in specific gut microbes between patients with IBS and controls. **RESULTS:** The search identified 2631 citations; 24 studies from 22 articles were included. Most studies evaluated adults presenting with various IBS subtypes. Family *Enterobacteriaceae* (phylum Proteobacteria), family *Lactobacillaceae*, and genus *Bacteroides* were increased in patients with IBS compared with controls, whereas uncultured Clostridiales I, genus *Faecalibacterium* (including *Faecalibacterium prausnitzii*), and genus *Bifidobacterium* were decreased in patients with IBS. The diversity of the microbiota was either decreased or not different in IBS patients compared with controls. More than 40% of included studies did not state whether cases and controls were comparable (did not describe sex and/or age characteristics). **CONCLUSIONS:** In a systematic review, we identified specific bacteria associated with microbiomes of patients with IBS vs controls. Studies are needed to determine whether these microbes are a product or cause of IBS.

Keywords: Inflammation; Intestine; Comparison; Infection.

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder that affects 10%–20% of the population[1] and up to 50% of referral cases are referred to gastroenterologists.[2] Thus, IBS is a significant socioeconomic burden to society.[3,4]

Therapies for IBS are only modestly effective, as the pathophysiology is not completely understood and is

Abbreviations used in this paper: **IBS, irritable bowel syndrome; IBS-C, constipation-predominant irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; IBS-M, mixed or alternating bowel habit irritable bowel syndrome; OTU, operational taxonomic unit; PI-IBS, post-infectious irritable bowel syndrome; rRNA, ribosomal RNA.**

Most current article

0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2019.03.049

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

The pathophysiology of Irritable bowel syndrome (IBS) is complex and not fully understood. The intestinal microbiota possibly influences IBS symptom by altering the brain-gut axis. However, there is still a lack of data to support this hypothesis.

NEW FINDINGS

Uncultured Clostridiales I, genus *Faecalibacterium* including *Faecalibacterium prausnitzii* and genus *Bifidobacterium* were decreased in IBS patients compared to controls.

LIMITATONS

The heterogeneity of microbiota assessment methods in included studies.
Some included studies were at unclear risk of bias due to selection of the control group and/or lack of data in baseline characteristic of cases and controls comparability.

IMPACT

The microbiomes of patients with IBS differ in several ways from comparison patients. These differences can inform future studies that target individual bacterial species/genera for IBS treatment.

believed to be multifactorial.[5] In the last decade, an altered microbiota in the human gastrointestinal tract has been proposed as one of the possible causes of IBS from epidemiologic studies in patients with post-infectious IBS (PI-IBS).[6–8] The previous observational studies have demonstrated that the change of intestinal microbiota as a result of acute gastroenteritis is associated with an increased risk of subsequent development of IBS.[6,7] Additionally, antibiotic therapy for extra-intestinal infections, which may alter the intestinal microbiota, is significantly associated with IBS (odds ratio, 2.30; 95% confidence interval, 1.22–4.33).[9] However, the data on specific bacterial groups in IBS are conflicting and still inconclusive. We have therefore conducted a systematic review of case–control studies evaluating the microbiota in patients with IBS compared to healthy controls to summarize the current evidence in the relationship between individual members of the microbiota and overall IBS symptoms.

Methods

Search Strategy

We performed a systematic search of MEDLINE (OvidSP); and Embase (OvidSP), the Cochrane Central Register of Controlled Trials (CENTRAL) and the Cochrane Database of Systematic Reviews (CDSR) from inception to April 2, 2018 to identify case–control studies comparing gut microbiota in patients with IBS and normal healthy controls.

Study Selection and Patient Population

The inclusion criteria were: intestinal microbiota studies comparing IBS patients with healthy controls; and fecal or colonic tissue sample. Both adult and pediatric studies were included. Studies were excluded if they did not provide the data for individual bacterial groups, were not in English, were published before 2010, were not a case–control design, or were only available as conference proceeding abstracts.

Choice of Outcome

The primary outcome was the difference in every individual intestinal bacterial species, operational taxonomic units (OTUs) or other taxonomic classification reported in patients with IBS compared to controls. OTUs are clusters of organisms grouped by DNA sequence in terms of their similarity to a given taxonomic marker gene. The secondary outcomes were a difference in microbial diversity, average number of OTUs, and total number of bacteria. We classified the primary and secondary outcome into 3 categories; significantly increased in IBS patients, significantly decreased in IBS patients, and no significant difference between IBS patients and controls. We collected the stool microbiota data from each study, but if these were not available, we used the colonic tissue microbiota instead. We included all measurement methods except those that relied solely on terminal restriction fragment length polymorphisms because it provides limited taxonomic resolution.

Eligibility Assessment and Data Extraction

Four authors (RP, FT, GL, and PM) independently reviewed studies retrieved by the search strategy and excluded trials based on titles, abstracts, or both. At least 2 study authors independently reviewed selected studies for complete analysis. One study author extracted data and entered it into a spreadsheet. The other study author evaluated the accuracy of this process. When there was a discrepancy between reviewers, the data were re-checked and there was a discussion to reach an agreement by consensus. If the authors were unable to reach a consensus, the senior author (PM) arbitrated. The data collected included the following: participant characteristics, including age group, country, IBS subtype, number of patients; details of interventions, including type of specimen (stool or tissue biopsy), microbiota assessment method, multiple comparison correction; microbial diversity (which included α-diversity, which is the diversity within a sample, and β-diversity, which is the diversity between samples); mean number of OTUs; total number of bacteria and individual bacterial species; and OTU or other taxonomic classification difference with significant P value $<.05$.

Quality Assessment

The authors applied the Newcastle-Ottawa Scale for assessing the quality of included case–control studies in this review. The Newcastle-Ottawa Scale consists of 3 domains (maximum 9 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 should be the 2 most important baseline characteristics that needed to be described and compared in both groups.

Results

Study Selection

Overall, 2631 citations were retrieved; 2559 were rejected based on title, abstract relevance, or duplication; 72 articles were fully reviewed. After further review, an additional 50 full-text articles were excluded (Figure 1). Final analysis included 24 studies from 22 articles.[8,10–30] Of these, 19 studies from 18 articles[8,10–13,15,17,20–22,24–30] assessed the microbiota in adults (1 article reported 2 studies[11]), 3 studies from 2 papers were pediatric articles,[18,19] and in 2 studies, the age of the included population was not stated.[14,16] Two studies from China assessed the microbiota from colonic tissue[24,30] and the remaining 22 studies evaluated intestinal microbiota from stool. In terms of IBS subtype, 8 studies from 7 articles recruited patients with diarrhea-predominant IBS (IBS-D),[10,11,13,18,24,25,30] 1 with constipation-predominant IBS (IBS-C),[12] 1 with IBS-D or PI-IBS[8] and 14 studies from 13 articles with the combination of IBS-D, IBS-C, and mixed or alternating bowel habit IBS subtype (IBS-M)[14–17,19–23,26–29] (Table 1).

Microbiome Assessment Methods

Of the 24 studies included in this systematic review, 1 study[10] used terminal restriction fragment length polymorphisms to assess the microbiome, 11 studies from 9 articles used 16S ribosomal RNA (rRNA) gene sequencing,[11,14,18,19,21,23,24,26,29] 11 used quantitative polymerase chain reaction,[8,11,13,15,17,18,20,22,28,29] 3 used fluorescent in-situ hybridization,[12,18,30] 2 used bacterial culture,[12,20] 7 studies from 6 articles used microarrays,[8,16–19,23] and 2 used denaturing gel gradient electrophoresis.[15,16] One study[25] did not specify the method used to assess the microbiome.

From the 11 studies that employed sequencing of bacterial 16S rRNA genes, there were differences in the variable region sequenced. Two studies used V1–V2,[14,23] 6 studies from 5 articles sequenced V1–V3 (this was the most commonly studied variable region),[11,18,19,21,24] 2 studies from 1 article used V3–V5,[19] and 1 study each looked at V4,[26] V5–V6,[29] and V6.[11] Additionally, only 6 of 24 studies reported the use of multiple comparison correction when analyzing differences in the IBS microbiota compared to controls.[8,15,16,23,27,28]

Primary Outcomes

At the phylum level, 4 studies from 3 articles[11,19,27] showed increased amounts of Proteobacteria in IBS patients, whereas 2 studies[21,22] showed no difference compared to healthy controls. There were conflicting results of cumulative evidence for Bacteroidetes, Actinobacteria, and Firmicutes and lack of data for Fusobacteria and Verrucomicrobia (Table 2).

In terms of lower taxonomic levels (Table 3), there were 4 studies demonstrating significantly increased number of bacteria in the family *Enterobacteriaceae* (phylum Proteobacteria)[11,12,16] and genus *Bacteroides* (phylum Bacteroidetes)[25,28–30] in patients, with 79 and 246 IBS compared to 82 and 122 healthy controls, respectively (Table 1) whereas another 2 papers each[13,20–22] showed non-significant differences between the 2 groups. The data from 3 papers[11,16,23] in the United States and Italy demonstrated significant increase of family *Lactobacillaceae* in 98 IBS patients, compared to 67 controls; however, another

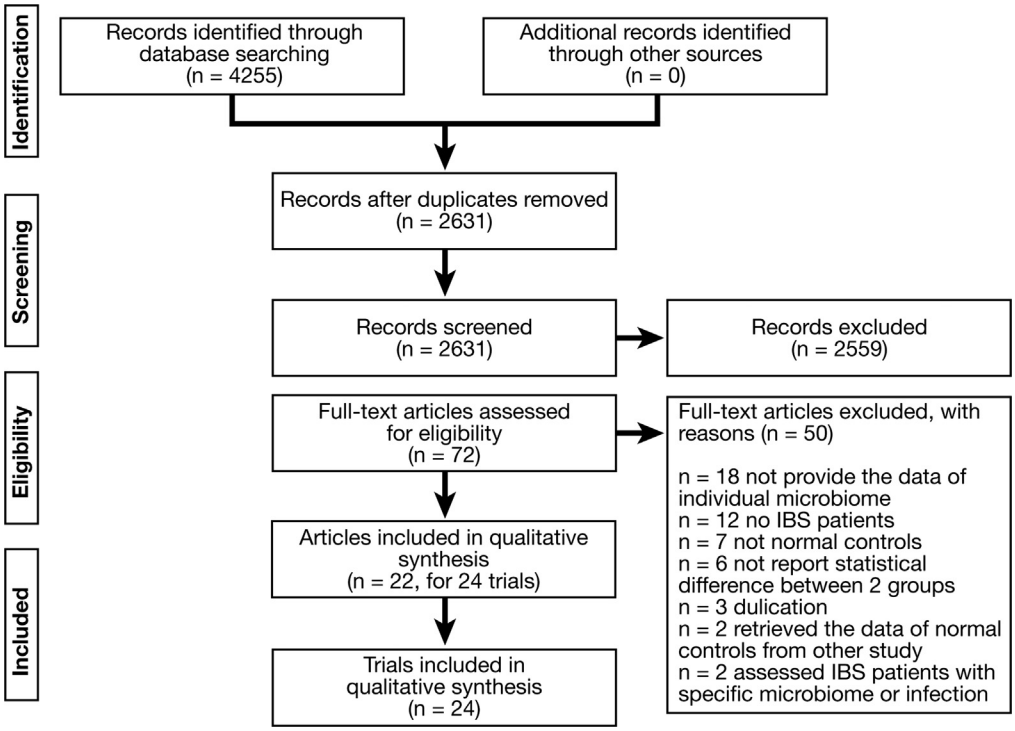

Figure 1. Flow diagram of trial selection.

Table 1. Characteristic of Included Studies

Study, first author, year	Country	Type of specimen	IBS patients			Controls		Microbiome assessment method	Multiple comparison correction
			IBS subtype	Age group	No. of participants	Age group	No. of participants		
Adults									
Carroll, 2011[10]	United States	Stool	IBS-D	Adult	16	Adult	21	16S rRNA gene T-RFLP	NR
Carroll, 2012[11]	United States	Stool	IBS-D	Adult	23	Adult	23	V1–V3 16S rRNA gene 454 sequencing, 16S rRNA gene qPCR with group and species-specific primers	NR
Carroll, 2012[11]	United States	Stool	IBS-D	Adult	23	Adult	23	V6 16S rRNA gene 454 sequencing, 16S rRNA gene qPCR with group and species-specific primers	NR
Chassard, 2012[12]	France	Stool	IBS-C	Adult	14	Adult	12	FISH, bacterial culture	NR
Chung, 2016[21]	Taiwan	Stool	IBS-C	Adult	7	Adult	19	16S rRNA gene V1–V3 regions	NR
			IBS-D		14				
			IBS-M		7				
Dior, 2016[22]	France	Stool	IBS-C	Adult	15	Adult	15	qPCR with group specific primers	NR
			IBS-D		16				
Duboc, 2012[13]	France	Stool	IBS-D	Adult	14	Adult	18	16S rRNA gene RT-qPCR with group and species-specific primers	NR
Jalanka-Tuovinen, 2014[8]	Finland	Stool	PI-IBS	Adult	11	Adult	11	16S rRNA gene phylogenetic microarray analysis with HITChip, 16S rRNA gene qPCR with group and species-specific primers	Yes
			IBS-D		12				
Kerckhoffs, 2011[15]	Netherlands	Stool	IBS-C	Adult	11	Adult	20	V6-8 16S rRNA gene DGGE, qPCR for *Pseudomonas aeruginosa*	Yes
			IBS-D		13				
			IBS-A		13				
Liu, 2016[25]	China	Stool	IBS-D	Adult	40	Adult	20	Not specified	NR
Pozuelo, 2015[26]	Spain	Stool	IBS-C	Adult	32	Adult	66	16S rRNA gene V4 region	NR
			IBS-D		54				
			IBS-M		27				
Rajilic-Stojanovic, 2011[17]	Finland	Stool	IBS-C	Adult	18	Adult	46	qPCR for Archaea, 16S rRNA gene phylogenetic microarray analysis with HITChip	NR
			IBS-D		25				
			IBS-A		19				
Rangel, 2015[27]	Sweden	Stool	IBS-C	Adult	17	Adult	16	Phylogenetic microarray with 16S rRNA gene V1 and V6 region	Yes, used false discovery rate
			IBS-D		5				
			IBS-A		12				
			IBS-U		1				
Ringel-Kulka, 2016[23]	United States	Stool	IBS-C	Adult	21	Adult	20	16S rRNA gene V1–V2 regions	Post-hoc Tukey's test
			IBS-D		21				
			IBS-M		14				

Table 1. Continued

Study, first author, year	Country	Type of specimen	IBS patients			Controls		Microbiome assessment method	Multiple comparison correction
			IBS subtype	Age group	No. of participants	Age group	No. of participants		
Shukla, 2015[28]	India	Stool	IBS-C IBS-D IBS-U	Adult	20 20 7	Adult	30	qPCR with group specific primers	Yes, some use post hoc Bonferroni correction
Tana, 2010[20]	Japan	Stool	IBS-C IBS-D IBS-M	Adult	11 8 7	Adult	26	16S rRNA gene qPCR with group and species-specific primers, culture, microscopy	NR
Tap, 2017[29]	Sweden	Stool	IBS-C IBS-D IBS-M IBS-U	Adult	21 57 52 9	Adult	56	16S rRNA gene V5–V6 region, qPCR for Methanobacteriales	NR
Li, 2018[24]	China	Rectal biopsy	IBS-D	Adult	33	Adult	15	16S rRNA gene V1–V3 regions	NR
Zhong, 2017[30]	China	Colonic biopsy	IBS-D	Adult	20	Adult	16	FISH	NR
Children									
Rigsbee, 2012[18]	United States	Stool	IBS-D	Child	22	Child	22	16S rRNA gene microarray (microbiota array), V1–V3 16S rRNA gene 454 sequencing, FISH, 16S rRNA gene qPCR	NR
Saulnier, 2011[19]	United States	Stool	IBS-C IBS-D IBS-U Other IBS	Child	13 1 7 1	Child	22	16S rRNA gene Phylochip microarray, V1–V3 and V3–V5 16S rRNA gene 454 sequencing	NR
Saulnier, 2011[19]	United States	Stool	IBS-C IBS-D IBS-U Other IBS	Child	13 1 7 1	Child	22	16S rRNA gene Phylochip microarray, V1–V3 and V3–V5 16S rRNA gene 454 sequencing	NR
Unknown population									
Durban, 2012[14]	Spain	Stool	IBS-C IBS-D	NR	3 13	NR	9	V1–V2 16s rRNA gene 454 sequencing	NR
Maccaferri, 2012[16]	Italy	Stool	IBS-C IBS-D IBS-M	Adult	4 10 5	NR	24	16S rRNA gene microarray (HTF-Microbi.Array), V2–V3 16S rRNA gene DGGE	Yes

DGGE, denaturing gel gradient electrophoresis; FISH, fluorescent in situ hybridization; HITChip, human intestinal tract chip; HTF, high taxonomic fingerprint; IBS-A, irritable bowel syndrome alternating between diarrhea and constipation; IBS-M, mixed irritable bowel syndrome; IBS-U, irritable bowel syndrome unsubtyped; NR, not reported; qPCR, quantitative polymerase chain reaction; RT-qPCR, reverse transcription quantitative polymerase chain reaction; T-RFLP, terminal restriction fragment length polymorphism.

Table 2. The Result of Microbiota in Phylum Level

Variable	3 or more papers with increased in IBS	2 papers with increased in IBS	1 paper with increased in IBS	1 paper with no difference	2 papers with no difference	3 or more papers with no difference
3 or more papers with decreased in IBS						
2 papers with decreased in IBS		Bacteroidetes[a] (4 neutral)				
1 paper with decreased in IBS						
1 paper with no difference						
2 papers with no difference	Proteobacteria (4)					
3 or more papers with no difference			Actinobacteria (5) Firmicutes (4)			

NOTE. Values in parentheses are the actual number of papers in the aspect of 3 or more.
[a]Had no difference result in that phylum.

study from Taiwan[21] did not find significant differences in family *Lactobacillaceae* between 28 IBS patients and 19 healthy controls.

Three European studies[8,17,27] (120 IBS patients and 72 controls) assessed uncultured Clostridiales I and consistently demonstrated decreased amount of these bacteria in patients with IBS (Table 4). Four studies evaluated genus *Faecalibacterium* (order Clostridiales) in 119 IBS-D (81 healthy controls) and showed a significant decrease in 3 papers[11,24,25] (2 of 3 using V1–V3 16S rRNA gene sequencing) and non-significant decrease in 1 article[11] (using V6 16S rRNA gene sequencing). Additionally, *Faecalibacterium prausnitzii* was assessed in 5 studies, where 2 articles[11,27] demonstrated a significant decrease, 2 studies[12,13] reported an insignificant decrease and 1 study[22] showed no difference (Table 3).

Genus *Bifidobacterium* (family Bifidobacteriaceae) was evaluated in 7 studies[13,17,20,22,25,28,30] and of those, 5 studies[13,17,25,28,30] showed significant decrease amount of Bifidobacterium in IBS patients, whereas 1 study[20] showed a non-significant trend for decreased amount and the other[22] revealed non-significant difference. Genus *Tannerella* (phylum Bacteroidetes) was significantly decreased in 2 studies[17,27] and not different in another 2 studies.[8,18]

Five studies assessed genus *Alistipes* (phylum Bacteroidetes) and found significantly decreased amount of this genus in 3 studies[17,25,27] of IBS-D or IBS-M patients. One study[8] showed no difference result in IBS-D adult patients and another[14] demonstrated significant increase of Alistipes in IBS-C pediatric patients. The overall results are described in Supplementary Table 1.

Diversity in Irritable Bowel Syndrome

Fewer than half (9 from 24 papers) provided α-diversity. Of those, 5 articles[10,11,25–27] (55.6%) showed a significant decrease in the α-diversity in patients with IBS, whereas 4 articles[11,14,18,29] revealed no difference compared to healthy controls. Additionally, 1 study[15] reported no significant difference between 2 groups in non-specific microbial diversity. Another 2 studies independently demonstrated insignificance difference in β-diversity (Jensen-Shannon distance and Bray-Curtis distance)[29] and mean number of OTUs.[23] Almost all studies[11–13,19–22] (9 from 10), which assessed total number of bacteria demonstrated neutral result in IBS patients and healthy control. Only 1 Chinese paper showed significant increase number of bacteria from colonic tissue in patients with IBS.[30]

Microbiome Analyses According to Irritable Bowel Syndrome Subtype

There were 14 studies described in 13 papers[14–17,19–23,26–29] that recruited more than 1 IBS subtype for evaluation. Four studies reported in 3 papers[19,20,27] evaluating 83 participants with IBS assessed all subtypes as 1 group and made no attempt to analyze the subgroups separately. Ten studies[14–17,21–23,26,28,29] compared IBS-C with IBS-D in the same population and did describe results for each subgroup (142 with IBS-C and 234 with IBS-D) (Supplementary Table 1). Five studies[15–17,23,29] found that any difference between IBS and healthy controls was similar in both IBS-D (n = 117) and IBS-C (n = 65) subgroups with no inconsistency between the groups. The other 5 studies[14,21,22,26,28] involving 117 IBS-D and 77 IBS-C participants did report discrepancies in microbiome profiles between the 2 IBS subtypes, but no theme emerged regarding differences in the microbiome between IBS-C and IBS-D. Each study reported different discrepancies in OTUs with no difference occurring more than once.

Six studies[15–17,23,26,29] described the microbiome in 130 participants with IBS-M (Supplementary Table 1) and all found no differences between this subtype and IBS-C or IBS-D. In all cases, any differences between IBS and healthy controls were the same in the IBS-M group compared with the IBS-C or IBS-D subgroups.

Table 3. The Result of Microbiota in Order, Family, Genus, and Species

Variable	3 or more papers with increased in IBS	2 papers with increased in IBS	1 paper with increased in IBS	1 paper with no difference	2 papers with no difference	3 or more papers with no difference
3 or more papers with decreased in IBS			*Alistipes*[a] (3,1) *Bacteroides vulgatus* (4)	*Faecalibacterium* (3)	*Bifidobacterium* (5)	
2 papers with decreased in IBS			*Bacteroides intestinalis* *Bacteroides ovatus* *Bacteroides plebeius* *Bacteroides uniformis* *Odoribacter* *Prevotella tannerae*		*Tannerella*	*Faecalibacterium prausnitzii* (3)
1 paper with decreased in IBS	*Veillonella*[a] (5,3)	*Fuso bacterium* (–,1)[a]	*Anaerovorax* (–,3)[a] *Bacteroides fragilis* (–,1)[a] *Bacteroides stercoris* *Blautia* *Dialister* *Prevotella* (–,2)[a] *Prevotella ruminicola* *Roseburia* (-,1)[a]	Uncultured *Bacteroidetes* *Christensenellaceae* *Clostridium cluster XIVb* *Clostridium leptum* (–,2)[a] *Erysipelotrichaceae* *Eubacterium* *Lachnospiraceae* *Oscillibacter* *Parabacteroides* *Parabacteroides distasonis* *Prevotella oralis* *Ruminococcaceae*	*Bacteroides-Prevotella*	
1 paper with no difference	*Lactobacillaceae* (3)		*Acinetobacter* *Actinomycetaceae* *Bifidobacteriaceae* *Brachyspira* *Butyricimonas* *Catenibacterium* *Catenibacterium mitsuokai* *Enterococcus* *Enterococcus faecium* *Fusobacteriaceae* *Streptococcus thermophiles*			
2 papers with no difference	*Bacteroides* (4) *Enterobacteriaceae* (4)	*Gamma proteobacteria*				
3 or more papers with no difference			*Collinsella* (n = 3) *Lactobacillus* (n = 5)			

NOTE. Values in parentheses are the actual number of papers in the aspect of 3 or more, number of no difference papers.
[a]Had no difference result in that organism.

Table 4. The Consistent Result of Microbiota

Variable	Bacteria in IBS patients
2 papers with increased in IBS	*Aeromonas, Aneurinibacillus, Bacillus, Campylobacter, Clostridium difficile, Enterobacteriales, Helicobacter, Lactobacillus salivarius, Pediococcus, Psuedomonas aeruginosa, Ruminococcus gnavus*
3 papers with decreased in IBS	Uncultured Clostridiales I
3 or more papers with no difference	*Clostridium coccoides* (4 papers)

There were 8 studies in 7 papers that evaluated IBS-D alone.[10,11,13,18,24,25,30] In terms of IBS-D, 3[25,28,30] of 5 articles assessing genus *Bacteroides* in 80 patients and 66 controls demonstrated significant increase of this genus in IBS-D patients, whereas another 2[13,22] showed insignificant results compared to controls. In contrast, the majority of studies evaluating the genus *Bifidobacterium* (3 from 4 studies with 74 IBS-D patients and 54 controls) showed a significant decrease in IBS-D patients.[13,25,30] The data from 3 papers with genus *Faecalibacterium*[11,24,25] assessed patients with IBS-D and showed decreasing number of this bacteria in those patients as mentioned in the primary outcome.

Only 1 study evaluated IBS-C[12] alone. This compared 14 participants with IBS-C compared to 12 healthy controls and found differences between the groups (Supplementary Table 1), but it is difficult to draw conclusions from 1 study.

Post-infectious Irritable Bowel Syndrome

There was only 1 study[8] that evaluated 11 PI-IBS participants compared with 11 healthy controls and 12 with IBS-D. There were differences between PI-IBS and healthy controls but these largely mirrored differences seen with IBS-D (Supplementary Table 1).

Eastern vs Western Studies in Irritable Bowel Syndrome

The majority of included studies were from Western countries (5 in United States,[10,11,18,19,23] 3 in France,[12,13,22] 2 in Finland,[8,17] 2 in Sweden,[27,29] 2 in Spain,[13,26] 1 in The Netherlands,[15] and 1 in Italy[16]) involving 632 IBS participants and 401 healthy controls. Six studies were conducted in Asia (3 in China,[24,25,30] 1 in Japan,[20] 1 in Taiwan,[21] and 1 in India[28]) evaluating 145 IBS cases and 126 healthy controls. To evaluate whether there were any differences between studies from the East and the West, we selected genus *Bifidobacterium* (family Bifidobacteriaceae), which was reported on most consistently. Seven studies reported on this and, of these, 3 studies from the East[25,28,30] and 2 studies from the West[13,17] reported a significant decreased amount of *Bifidobacterium* in IBS patients, whereas 1 Eastern study[20] showed a non-significant trend for decreased amount and the other Western study[22] revealed no statistically significant difference.

Quality of the Evidence

The Newcastle Ottawa Scale showed all relevant studies provided an adequate explanation in the definition and selection method for IBS patients, whereas only 16 of 24 (66.67%) did the same process for controls. Around 60% (14 from 24 studies) demonstrated comparable data of both sex and age in IBS patients and controls. Neither sex nor age data were statistically compared in 6 studies. Almost all (23 from 24 studies) did not state that the microbiologist was blinded. The rate of untested microbiota for any reason in both groups was the same in all studies (Table 5).

Discussion

To our knowledge, this is the most comprehensive systematic review in microbiota and IBS, as we extracted the data of every available bacterial group using the lowest taxonomic level of each included study. We believe that the results reflect the best available current evidence demonstrating the relationship between individual bacterial groups and IBS symptoms.

Most studies assessed adults with a combination of IBS subtypes and collected microbiota from stool. Although the microbial diversity and composition in luminal- and mucosal-associated flora are different,[10,27,31,32] fecal sampling was used in nearly all studies because of convenience of collection compared to obtaining mucosal associated microbiota. Both types of collection are relevant as both fecal and mucosal flora can affect the host via immune–microbial interactions and subsequently influence IBS symptoms.[33]

None of the studies were multicenter. This review showed that there is evidence that the diversity of the stool microbiota from patients with IBS was either decreased or unchanged. From a previous IBD study, microbial diversity was remarkably reduced in patients with Crohn's disease.[34] Therefore, this information raised the possibility of overlapping pathophysiology between IBS and IBD, including inflammatory processes, causing decreased diversity of microbiota.[35,36]

This review demonstrated the potentially harmful microbiota in patients with IBS, including phylum Proteobacteria, family *Enterobacteriaceae* (phylum Proteobacteria), family *Lactobacillaceae* and genus *Bacteroides* (phylum Bacteroidetes). The *Enterobacteriaceae* family contains several pathogenic bacteria; for instance, *Escherichia*, *Shigella*, *Campylobacter*, and *Salmonella*. This finding

Table 5. Quality of Each Included Study by the Newcastle Ottawa Scale

	Selection				Comparability		Exposure		
Study, first author, year	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of baseline characteristic 1 (sex)	Comparability of baseline characteristic 2 (age)	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-response rate
Carroll, 2011[10]	*	*	*	*	*	*	NR	NR	*
Carroll, 2012[11]	*	*	*	*	*	*	NR	NR	*
Carroll, 2012[11]	*	*	*	*	*	*	NR	NR	*
Chassard, 2012[12]	*	*	*	*	*	NR	NR	NR	*
Chung, 2016[21]	*	*	*	*	*	*	NR	NR	*
Dior, 2016[22]	*	*	NR	NR	*	*	NR	NR	*
Duboc, 2012[13]	*	*	NR	NR	*	*	NR	NR	*
Durban, 2012[14]	*	*	NR	NR	NR	NR	*	*	*
Jalanka-Tuovinen, 2014[8]	*	*	*	*	*	*	NR	NR	*
Kerckhoffs, 2011[15]	*	*	*	*	*	NR	NR	NR	*
Li, 2018[24]	*	*	*	*	NR	*	NR	NR	*
Liu, 2016[25]	*	*	*	*	NR	*	NR	NR	*
Maccaferri, 2012[16]	*	*	NR	NR	*	*	NR	NR	*
Pozuelo, 2015[26]	*	*	*	*	*	*	NR	NR	*
Rajilic-Stojanovic, 2011[17]	*	*	NR	NR	*	*	NR	NR	*
Rangel, 2015[27]	*	*	*	*	NR	NR	NR	NR	*
Rigsbee, 2012[18]	*	*	*	*	NR	NR	NR	NR	*
Ringel-Kulka, 2016[23]	*	*	*	*	NR	NR	NR	NR	*
Saulnier, 2011[19]	*	*	NR	NR	NR	NR	NR	NR	*
Saulnier, 2011[19]	*	*	NR	NR	NR	NR	NR	NR	*
Shukla, 2015[28]	*	*	NR	NR	*	*	NR	NR	*
Tana, 2010[20]	*	*	*	*	*	*	NR	NR	*
Tap, 2017[29]	*	*	*	*	*	*	NR	NR	*
Zhong, 2017[30]	*	*	*	*	*	*	NR	NR	*

NR, not recorded.

could reflect previous intestinal infection in those patients. The other possible hypothesis to explain our finding is the change in the intestinal environment, including a decrease in strictly anaerobic bacteria, and potential inflammation and increased gastrointestinal motility, could allow for the expansion of facultative, non-fastidious bacteria like *Enterobacteriaceae*. For the family *Lactobacillaceae*, *Lactobacillus* can produce organic acids, including lactic acid and/or acetic acid from glucose or fructose, depending on patterns of fermentation[37] and associated with abdominal pain and bloating in IBS patients.[20] Lastly, *Bacteroides* was increased in IBS patients, especially in IBS-D patients. This may be due to low-grade inflammation as some species, including enterotoxigenic strains of *Bacteroides fragilis,* can produce toxins.[38,39] *B fragilis* toxin can dissolve mucosal glycoproteins, thus affecting the microenvironment, colonic mucosal production, and intestinal motility, which can cause abdominal pain and diarrhea.[40] However, most supported studies (approximately 72%) were concern for bias in either control selection or comparability of demographic data.

The most consistent finding of a potentially "protective" bacterial group in IBS patients was found in the group uncultured Clostridiales I (order Clostridiales). Association is not causation, but if there is a protective effect in IBS symptoms the mechanism is unclear. The genus *Faecalibacterium*, especially *Faecalibacterium prausnitzii*, which belongs to similar order as uncultured Clostridial I (order Clostridiales), have been associated with maintaining gut mucosal health. This bacterium was considered as a main butyrate-producing and anti-inflammatory organism[41] and reduced IBS symptoms via mediation of interleukin-17 expression in a rat model,[42,43] as well as maintained gut–barrier integrity.[44] Genus *Bifidobacterium* was decreased significantly in IBS patients regardless of IBS subtype. Therefore, it was another promising potential genus in ameliorating IBS symptoms. This observation is supported by the placebo-controlled trial of *Bifidobacterium longum* treatment in patients with IBS, which concluded that this bacterium can reduce depression scores and improve quality of life in affected patients by decreasing 4-cresol sulfate levels.[45] This substance is produced from host–bacterial interactions[46] and influences the dopamine/noradrenaline pathway in depression.[45,47] Furthermore, a systematic review of probiotics in IBS has highlighted that *Bifidobacterium*-containing interventions reduce IBS symptoms, which is not seen in products that contain *Lactobacillus* alone.[48]

Overall, the most striking observation is the lack of consistency in results between studies. This probably relates to the limitations of the studies included in this review. Heterogeneity between studies is often a problem in systematic reviews, but this is particularly marked in evaluations of the microbiota and IBS, with no 2 studies reporting exactly the same differences in OTUs. Several different methods were used to assess the microbiota, which makes it difficult to compare results between studies and likely contributes to the differences in results. Bacterial profiling involves amplification and sequencing a portion of the 16S rRNA gene for all bacteria present in a sample and provides comprehensive measure of the proportions of all bacterial taxa present. Alternatively, quantitative polymerase chain reaction can provide a measure of overall bacterial abundance, or estimate the concentration of specific taxonomic groups using group specific primers. 16SrRNA gene profiling is the more common approach to studying microbiomes. Even among the 11 studies that employed 16S rRNA gene sequencing, there were differences in the variable region sequenced, and PCR amplification of different variable regions can result in taxonomic bias,[49,50] leading to batch effects between studies. The differences in methodology used are compounded by a lack of description of antibiotics or probiotic use as well as any diet modification. Future studies should describe, for cases and controls, any prior antibiotic and/or probiotic use, as well as details of type of antibiotic or probiotic, when it was last used, and over what time period. Dietary intake should also be recorded in cases and controls. For robust microbiota results that are comparable among studies, there needs to be efforts for standardization of sample storage, DNA extraction, sequencing, and analysis methods among groups undertaking gut microbiota studies. Finally, longitudinal studies would allow for more robust association of changes in the microbiota to changes in IBS symptoms.

Another major limitation of these studies is that fewer than half reported the use of multiple comparison correction, which is important when analyzing data as complex as microbiome sequencing data. All studies reported differences between cases and controls and yet the median sample size is only approximately 20 per group. It is hard to believe that all studies would be positive with such modest sample sizes. The explanation for this finding likely relates to multiple testing. The traditional P value assumes one hypothesis being tested and, if the possibility that a given result occurred by chance is $<5\%$, convention suggests that something other than chance explains the difference observed. If the researcher is testing multiple hypotheses, then a P value $<.05$ becomes meaningless, as this is almost bound to be seen if you make enough comparisons. There are more than 100 OTUs that can be evaluated, so it is almost certain that some of the findings reported in these studies occurred by chance. This is exemplified by the comparison between different IBS subtypes. In these comparisons, 50% of studies found no difference between groups and in the other 50% the differences seen were not replicated in any other study.

Conclusions

This review highlights the heterogeneity of the microbiota in IBS patients; however, proposes some evidence that certain bacteria may be helpful in IBS treatment.

Supplementary Material

To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://doi.org/10.1053/j.gastro.2019.03.049.

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Received January 23, 2019. Accepted March 15, 2019.

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Acknowledgments
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Conflicts of interest
The authors disclose no conflicts.

Funding
This study was funded by Canadian Institutes of Health Research (RN279389–358033). The Inflammation, Microbiome, and Alimentation: Gastro-Intestinal $12,450,000 and Neuropsychiatric Effects: The IMAGINE network—A Strategy for Patient-Oriented Research CIHR Chronic Disease Network.