Bifidobacteria Was Decreased in Adult Patients With Irritable Bowel Syndrome Based on PCR and Bacterial Culture: A Systematic Review and Meta-Analysis

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Cite this article as: Bu Z, Ye X, Huang B, Liu R, Peng L. *Bifidobacteria* was decreased in adult patients with irritable bowel syndrome based on PCR and bacterial culture: A systematic review and meta-analysis. *Turk J Gastroenterol.* 2022;33(5):368-376.

ABSTRACT

The causes of irritable bowel syndrome remain unknown. Studies and meta-analyses revealed that intestinal microbiota disturbance was one of the causes of irritable bowel syndrome, but the results remained controversial. Therefore, we performed a systematic review and meta-analysis to identify the association between them. We performed a systematic meta-analysis of case–control studies from January 2000 to December 2020 to compare fecal microbes based on polymerase chain reaction and bacterial culture between adult irritable bowel syndrome patients and healthy controls. The standardized mean difference value and a 95% CI were calculated. Two professional researchers used Newcastle–Ottawa Scale to reassess selected literature and extract high-quality studies. Six studies were included in our analysis. When all eligible studies were pooled into the meta-analysis, compared with healthy controls, the standardized mean differences of Bifidobacteria (standardized mean difference = −1.01, 95% CI =: −2.01 to −0.01) in irritable bowel syndrome patients decreased significantly, whereas the standardized mean differences of Enterococcus, Enterobacter, Lactobacillus, Bacteroides, and Escherichia coli did not change significantly in irritable bowel syndrome patients. However, heterogeneity was significant to perform sensitivity analysis and stratified analysis in all these special intestinal microbes. In summary, this study indicated that only Bifidobacteria was decreased in irritable bowel syndrome patients compared with healthy controls using Newcastle–Ottawa Scale standards to extract high-quality literature. Future studies are warranted to further demonstrate the relationship between them.

Keywords: *Culture, intestinal microbiota, irritable bowel syndrome, meta-analysis, qPCR, systematic review*

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder. Patients with IBS often have GI symptoms such as cramping, abdominal pain or discomfort, diarrhea or constipation, bloating, and gas, all of which influence the patient's health-related quality of life (QOL). The cause of IBS remains unknown. It is generally considered a multifactorial disease, including chronic inflammation, gastrointestinal dysfunction, visceral allergies, psychological disorders, and environmental factors.[1-4] Researchers also discovered that intestinal microbiota disturbance was one of the causes of IBS.[5,6] Hence, many studies have investigated the association between intestinal microbiota and IBS.[5-7]

Although some meta-analyses of intestinal microbiota changes in IBS have been published, the results are still controversial.[8,9] In a recent meta-analysis, case–control trials stratified by results were calculated, without sensitivity analysis calculations or evaluation of the quality of included studies.[8] Some of the literature included in another meta-analysis did not meet the requirements.[9] Furthermore, as quantitative polymerase chain reaction (qPCR) and bacterial culture are the classic

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Received: **July 6, 2021** Accepted: **November 8, 2021** Available Online Date: **April 11, 2022**

DOI: **10.5152/tjg.2022.21543**

methods for microbiota analysis, we included studies based on these 2 methods.

Therefore, in this updated meta-analysis, we screened and included literature strictly for study quality. We aimed to identify intestinal microbiota characteristics in IBS patients and healthy controls (HCs) to determine whether intestinal microbiota can be used as a biomarker for IBS.
Materials and Methods

Literature Search

This study was based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for systematic review and meta-analysis. A comprehensive literature retrieval was performed using PubMed, MEDLINE, EMBASE, Web of Science, Cochrane, Scopus, Wan Fang Database, and China National Knowledge Infrastructure (CNKI), from January 2000 to December 2020. The search terms included "irritable bowel syndrome," "IBS," "microbiota," "microbiome," "microbes," "microflora," "flora," and "bacteria." Boolean operators (AND, OR, and NOT) were used to narrow or broaden the search results. Conference abstracts were manually searched to identify potentially eligible studies. A hand-search of interesting references was also performed. There were no language restrictions or any other advanced functions. Ethic approval was waived because the analysis was based on published literature.

Study Selection

Two reviewers (ZB and LP) assessed studies for inclusion independently according to inclusion and exclusion criteria. Any disagreement must be discussed before an agreement can be reached. The inclusion criteria that were required to be met in our study are as follows: (1) patients with IBS diagnosed by signs and symptoms; (2) case–control study; (3) adult participants; (4) intestinal microbiota including luminal types and mucosal types; (5) HCs recruited; (6) IBS and HCs matched in age or sex; and (7) bacterial counts results were expressed as $\log_{10}$ values per gram of feces (log 10). The exclusion criteria that required to be met were as follows: (1) publications that described non-controlled or irrelevant studies; (2) child participants; (3) secondary analysis; and (4) publications with insufficient data, including patient baseline, method of analysis, and outcome report.

Main Points

- *Our meta-analysis investigated Lactobacillus, Bacteroides, Bifidobacteria, Enterococcus, Escherichia coli, and Enterobacter in patients with irritable bowel syndrome (IBS).*
- *We found that Bifidobacteria was the only microbiota alterations of specific intestinal microbes in IBS determined by quantitative polymerase chain reaction and bacterial culture.*
- *The result highlights the necessities of supplementation of Bifidobacteria for the treatment of IBS.*

Data Extraction

To reduce error and bias of data collection, 2 reviewers extracted the relevant data independently. These data included the following items: (1) title; (2) authors; (3) publication year; (4) country; (5) study design; (6) inclusion and exclusion criteria; (7) diagnostic criteria for IBS; (9) essential characteristics (age, and sex); (9) the size of IBS groups; (10) the size of the HCs; (11) primary technique by which intestinal microbiota was analyzed, and (12) measurement of fecal bacterial which were regarded the main outcome parameters.

Quality Assessment of Included Studies

The quality of the study assessed by NOS and the standard 9-subscale was used for case–control studies. The NOS included 3 sections, including selection, comparability of baseline characteristics, and exposure.[10] The selection section contained the case definition adequate, representativeness of the cases, selection of controls, and the definition of controls. The exposure included laboratory methods, ascertainment of exposure, and attrition rate. In this study, 2 professional researchers used the more stringent NOS to reassess selected literature and extract high-quality research. The score was carried out according to every influencing factor of NOS. Options A, B, C, D, and E were rated as 4, 3, 2, 1, and 0, respectively. Options A, B, C, and D were scored as 3, 2, 1, and 0, respectively. Options A, B, and C were rated as 2, 1, and 0, respectively. Options A, B, and C were scored as 2, 1 and 0, respectively. The aggregate score was 15 points. More than or equal to 8 points were rated as high-quality studies for our meta-analysis.

Statistical Analysis

In our study, all data analyses conducted with STATA version 15.0 (Stata Corp, Texas, USA). For continuous data measurements, the SMD values were calculated with a 95% CI. I^2 and Q statistics were used to test the percentage of heterogeneity. Qualitative and quantitative analysis of heterogeneity was carried out by Q-test and

I^2 statistics. Heterogeneity qualitative analysis was performed by *P*-value qualitative analysis. When $P \geq .1$, there is no heterogeneity among the studies; otherwise, there is heterogeneity.[11] I^2 was used to analyze the heterogeneity quantitatively. There was no heterogeneity when the I^2 value approached 0%. The magnitude of the I^2 value is proportional to the quantity of heterogeneity. A high degree of heterogeneity among the studies, meta-regression analysis, subgroup analysis, and sensitivity analysis were used to explore heterogeneity. Continuous variable data were expressed by standardized weighted mean difference and corresponding 95% CI. Binary variable data were expressed by odds ratio (OR) and corresponding 95% CI. When heterogeneity was not significant ($I^2 \leqq 50\%$), the fixed-effect model was used.[12] When heterogeneity was significant ($I^2 > 50\%$), the random effect model was used.[12] I^2 was used when *P* contradicts I^2. Results

Study Selection

A total of 4380 citations were yielded initially from the literature search, of which 1454, 1576, 670, and 680 studies were from PubMed, Web of Science, Wan Fang, and CNKI databases, respectively. The full text of the 98 studies was retained after the removal of duplicates. In the screening of the 1391 on-topic articles, 1333 articles were excluded for the following reasons: 873 items were excluded from letters, reviews, case reports, and meta-analyses; 8 articles did not include the control group; 34 articles referred to animals rather than humans; 13 articles analyzed children with IBS rather than adults; 10 items were excluded because data or experimental methods were not available. Finally, 21 studies were included in our systematic review (Figure 1).

Assessment of Study Quality

We carefully assessed the primary studies based on the NOS. The quality scores were listed in Supplementary Table 1. Of the 21 studies, 6 studies were of high quality[13-18] and 15 studies were of low quality.[19-32] Therefore, the final analysis included 6 studies of high-quality.

Study Characteristics

The characteristics of the selected articles are summed up in Table 1. All the selected studies involved age-matched analyses, and all patients with IBS and HCs were adults. The fecal bacterial counts were expressed by $\log_{10}$ values per gram of feces from IBS patients and HCs. Finally, as shown in Table 1, 6 studies involving 243 IBS patients were included in our research. Of the 6 studies, 2 used qPCR to detect intestinal microbiota, 3 used culture, and 1 used both. These selected studies identified numerous intestinal microbes, such as *Lactobacillus*, *Bacteroides*, *Clostridium*, *Bifidobacterial*, *Enterococcus*, *Escherichia coli*, and *Enterobacter*. Of these articles, 4 studies were from Caucasians and 2 from Asians.

META-ANALYSIS RESULTS

A total of 6 species of bacteria from 6 articles were included in our meta-analysis. Comparing with the previous meta-analysis,[8] we mainly evaluated the alterations of *Lactobacillus*, *Bacteroides, Bifidobacterial*, *Enterococcus*, *E. coli*, and *Enterobacter* in IBS patients and HCs (Table 2). *Lactobacillus* was reported in 6 included studies.[13-18] The heterogeneity was significant ($P < .001$, $I^2 = 92.7\%$); therefore, a random effect model was applied for effect size combination. Comparing with HCs, the pooled standardized mean differences (SMDs) with 95%CI of *Lactobacillus* in IBS patients was −0.24 (−1.18, 0.70) (Figure 2a). *Bacteroides* was reported in 5 included studies.[13-15,17,18] The heterogeneity was significant too ($P < .001$, $I^2 = 82.6\%$). Therefore, a random effect model was also used for effect size combination. Comparing with HCs, the pooled SMDs with 95% CI of *Bacteroides* in IBS patients was 0.10 (−0.42, 0.61) (Figure 2b). *Bifidobacterial* was reported in 6 included studies[13-18] and the heterogeneity was evident ($P < .001$, $I^2 = 95.0\%$). Comparing with HCs, the pooled SMDs with 95%CI of *Bifidobacterial* in IBS patients was −1.01 (−2.01, −0.01) (Figure 2c). *Enterococcus* was reported in 5 included studies[13-16,18] and the heterogeneity was significant ($P < .001$ and $I^2 = 92.8\%$). Comparing with HCs, the pooled SMDs with 95%CI of *Enterococcus* in IBS patients was 0.07 (−0.69, 0.84) (Figure 2d). *E. coli* was reported in 3 included studies.[15,17] The heterogeneity was significant too ($P < .001$, $I^2 = 84.4\%$). Comparing with HCs, the pooled SMDs with 95% CI of *E. coli* in IBS patients was 0.09 (−0.46, 0.64) (Figure 2e). *Enterobacter* was reported in 3 included studies.[13,14,18] The heterogeneity was obvious because the result showed that $P < .001$ and $I^2 = 94.9\%$. Comparing with HCs, the pooled SMDs with 95% CI of *Enterobacter* in IBS patients was −0.66 (−2.25, 0.93) (Figure 2f).

CONCLUSION

Irritable bowel syndrome is a functional GIdisease. Previous studies confirmed that intestinal microbiota may be a risk factor for the development of IBS.[33-40] Increase in harmful bacteria and the decrease of beneficial bacteria in the intestine are the leading causes of IBS.[35,36,38-40] Intake of probiotics can relieve IBS symptoms, further supporting the theory of microbial imbalance.[41,42] Traditional

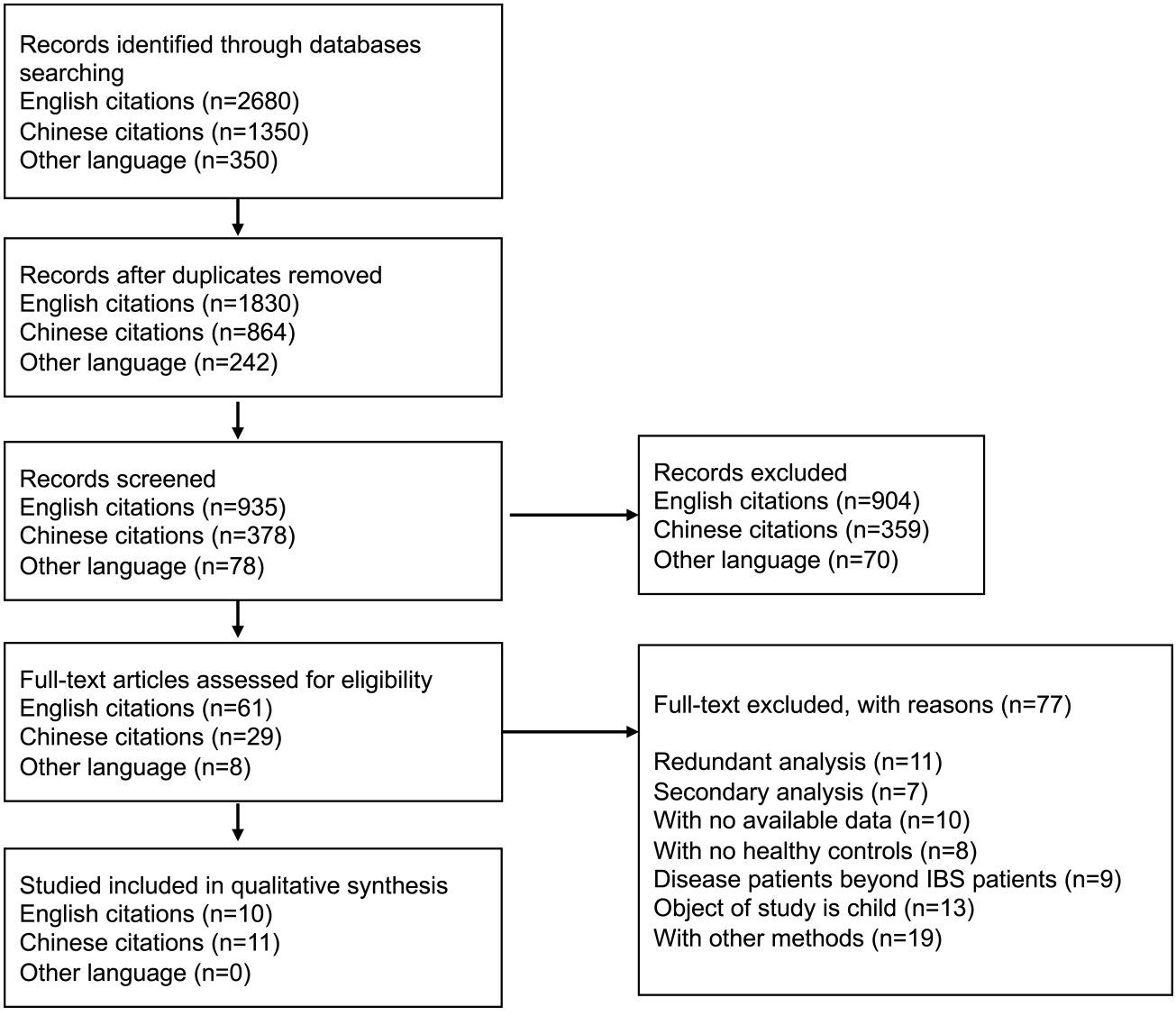

Figure 1. Flow diagram of assessment of studies identified in the meta-analysis.

method using bacterial culture and semi-quantitative methods such as denaturing gradient gel electrophoresis, Fluorescence in situ hybridization (FISH), and DNA microarray are employed to investigate on intestinal bacteria.[21,43,44] In recent years, 16S rRNA sequencing has become a routine sequencing method,[21,45] which has high sensitivity but with high price and low repeatability.[46,47] So far, however, qPCR and culture have provided the classic methods for microbiota analysis. Furthermore, qPCR and culture are often used to validate the results of 16S rRNA sequencing. In this study, we investigated the relationship between intestinal microbes and IBS from the perspective of PCR and culture. Although our research only used culture-based and qPCR methods to analyze the selective flora changes, which may not be suitable for the analysis of complex intestinal microbial ecosystems, however, compared with previously published meta-analyses, our meta-analysis has some different findings.

Several methods such as qPCR, 16S rRNA, conventional microbiological methods, analysis of intestinal fermentation mode, and detection of single pathogenic microorganism are commonly used in intestinal microbiota analysis.[21,43-47] The results showed that the bacteria associated with IBS were comprised of *Lactobacillus*, *Fusobacterium*, *Bacteroides*, *Clostridium cluster,*

Table 1. Characteristics of the Included Studies in the Meta-Analysis

First author	Year	Location	IBS, n	Control, n	IBS diagnosis	Control composition	Age, IBS (range/$x \pm$ sd)	Female (IBS), n	Age, HC (range/$x \pm$ sd)	Female (HC), n	Sample	Technique
Francavilla[13]	2019	Italy	54	55	Rome III	Healthy controls	43.3 ± 11.07	35	44.6 ± 11.25	46	Stools	qPCR
Chen[18]	2014	China	52	48	Rome III	Healthy controls	45.15 ± 11.28	23	45.92 ± 11.35	22	Stools	Culture
Carroll[17]	2012	United States	10	10	Rome III	Healthy controls	23-50	8	21-54	6	Stools	Culture
Tana[14]	2010	Japan	26	26	Rome II	Healthy controls	21.7 ± 2.0	13	21.9 ± 2.9	13	Stools	qPCR/culture
Malinen[16]	2005	Finland	27	22	Rome II	Healthy controls	20-65	20	25-63	15	Stools	qPCR
Matto[15]	2005	Finland	26	25	Rome II	Healthy controls	20-65	19	23-63	18	Stools	Culture

qPCR, quantitative polymerase chain reaction; HC, healthy control, IBS, irritable bowel syndrome.

Table 2. Alterations of Gut Microbiota in IBS Patients Versus Healthy Controls

		Number		*Enterobacter*		*Enterococcus*		*Lactobacillus*		*Bifidobacteria*		*Bacteroides*		*Escherichia coli*	
First author	Year	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Francavilla[13]	2019	54	55	5.3 ± 0.14	6.18 ± 1.22	6.71 ± 0.58	6.23 ± 0.52	4.43 ± 0.71	6.34 ± 1.11	7.11 ± 0.53	6.45 ± 0.27	4.33 ± 0.89	5.00 ± 0.80		
Chen[18]	2014	52	48	8.67 ± 1.23	8.15 ± 0.82	6.45 ± 1.12	6.82 ± 0.84	6.26 ± 1.23	7.12 ± 1.06	8.35 ± 1.05	9.03 ± 0.56	8.62 ± 0.56	8.36 ± 0.63		
Carroll[17]	2012	23	23					6.94 ± 0.44	8.94 ± 0.57	10.04 ± 0.63	9.81 ± 0.62	9.93 ± 0.63	9.54 ± 0.60	7.87 ± 0.50	9.15 ± 0.58
Tana[14]	2010	26	26	6.7 ± 0.90	6.9 ± 1.10	7.1 ± 1.10	7.1 ± 1.20	5.6 ± 1.90	4.6 ± 1.60	9.4 ± 0.90	9.7 ± 0.90	9.9 ± 0.70	9.9 ± 0.60		
Matto[15]	2005	26	25			5.4 ± 2.04	4.9 ± 1.41	5.7 ± 1.53	5.7 ± 1.06	9.1 ± 0.88	9.2 ± 1.00	9.7 ± 0.35	9.6 ± 0.45	7.1 ± 1.13	6.3 ± 1.31
Malinen[16]	2005	27	22			7.17 ± 0.38	7.37 ± 0.77	7.43 ± 0.62	7.55 ± 0.50	9.21 ± 0.51	9.35 ± 0.52			7.65 ± 0.67	7.9 ± 0.88

The results were displayed as $x \pm$ sd.

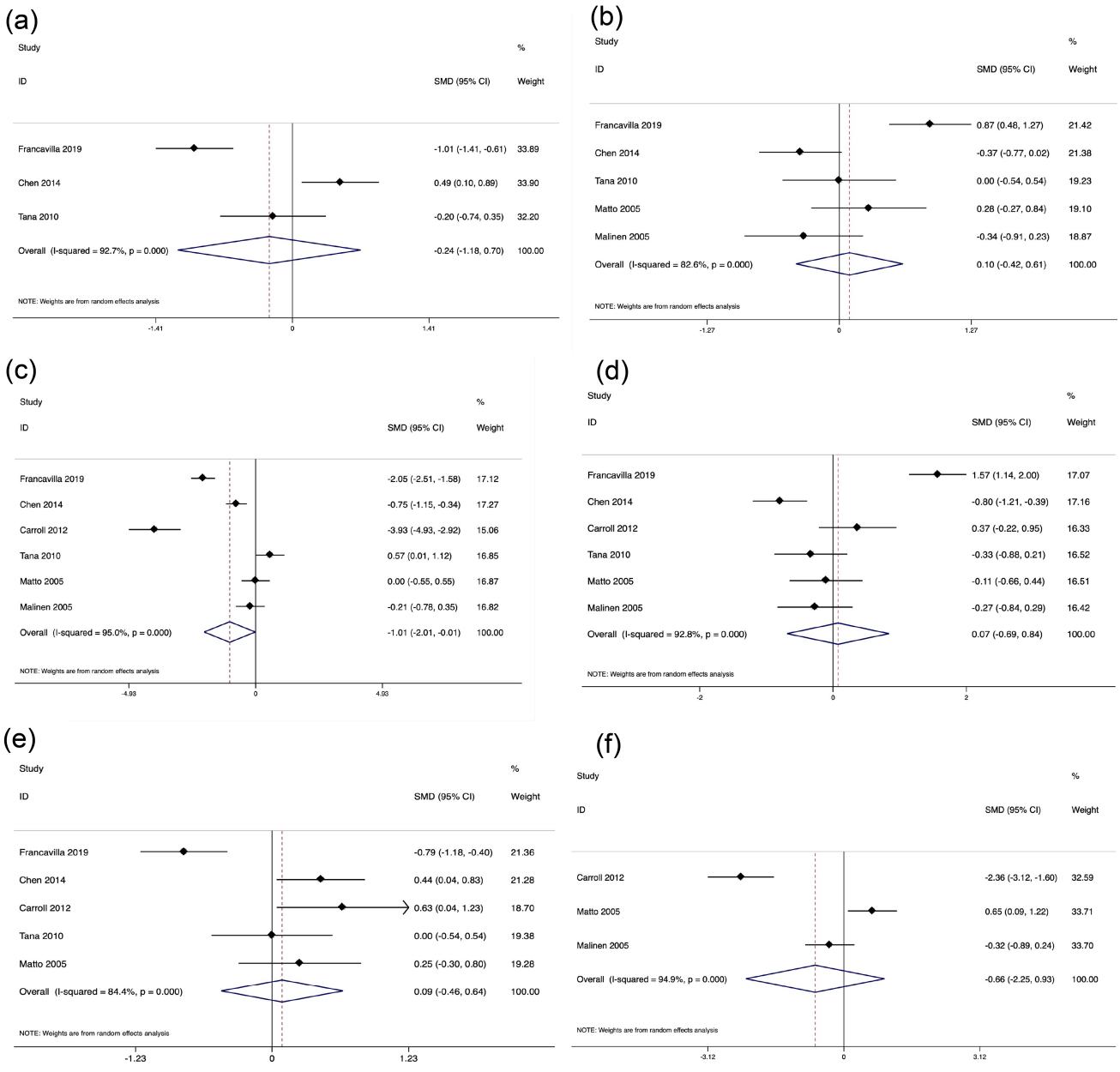

Figure 2. Forest plots of alterations of intestinal microbiota in IBS patients versus healthy controls: (a) *Lactobacillus*, (b) *Bacteroides*, (c) *Bifidobacteria*, (d) *Enterococcus*, (e) *Escherichia coli*, and (f) *Enterobacter*.

Bifidobacteria, *Faecalibact. Ererium*, *Enterococcus*, *Parabacteroides*, *E. coli*, and *Enterobacter*.[33] Based on the previously published systematic reviews and meta-analysis,[8,9,34-38] our meta-analysis has also investigated *Lactobacillus*, *Bacteroides*, *Bifidobacteria*, *Enterococcus*, *E. coli*, and *Enterobacter*. The previous literature was inconsistent in describing the relationship between the 6 strains and IBS. Most studies have shown that the number of *Lactobacillus* in IBS patients was lower than in HCs. However, the conclusions drawn from different articles were quite different among the other 5 strains. There were many similar contradictory conclusions on bacterial groups, such as *E. coli*, *Enterobacter*, and *Bacteroides*.[14-16,18,20,21,23-27,29-32] Our study suggested that these results may be unreliable, as individual studies may have relatively low credibility. Our study found

that fecal *Bifidobacteria* in IBS patients were significantly less than those in HCs. A systematic review using 16S rRNA showed that *Lactobacillus*, *Enterococcus*, and *Bacteroides* increased in IBS patients compared with HCs, while *Faecalibacterium* and *Bifidobacterium* descended.[33] Based on our result, *Bifidobacterium* might be the most significantly altered microbiota in IBS patients.

To our knowledge, there were a few meta-analyses and systematic reviews of microbiota alterations in IBS patients.[8,9,33-38] The studies of Zhuang et al[8] and Liu et al[9] did not accurately describe the quality evaluation of the included studies. We carefully reviewed the included studies of their analysis and found that some of them were of low quality. In our study, through the refinement of the detailed scored documents, it was considered that many studies were not of high quality and needed to be eliminated.

According to the 2 researchers' average score of the NOS scoring system, there was no case definition in Wang's study[32] and 15 studies did not have sufficient case definitions.[17-19,21-31,48] For example, in some studies, IBS diagnosis was provided by only 1 doctor or 1 method. "Represent activeness" in the NOS was undefined in 17 studies,[15,18-25,27-32,48] which made it impossible to tell us whether there was potential bias. Sixteen studies did not specify the source of control selection.[15,16,19-23,25,26,28,29,31,48] Most of the controls were from hospitals and did not describe the disease history in detail. Two matching methods of age and sex were used in 9 studies.[13-16,18,23,30,31] Age-matching method was applied in the most articles. More than 40% of those included studies neither described the comparability of cases and controls nor did they show age/sex characteristics.[17,19,21,22,24-27,29,32] As a result, the quality of these studies had greatly reduced. In the exposure section, we rated ascertainment of export as 5 grades and found out that there were 1-2 points in the literature. Only 1 study was rated as 3 points.[13] In terms of exposure method, the final scores were relatively consistent, which were due to the high consistency of the selection and evaluation methods of fecal specimens. Therefore, it was reasonable to believe that there were problems in the quality evaluation of previous meta-analyses[8,9,37] and the credibility of the results.

A meta-analysis is not appropriate to merge when heterogeneity exceeds 75%, and the source of heterogeneity should be explored. A sensitivity subgroup analysis can be done to explore the causes of heterogeneity and meta-regression. The result of our study indicated that the heterogeneity of the meta was too large. Therefore, it was impossible to analyze sensitivity and reduce heterogeneity by excluding one of the articles. Zhuang et al[8] also pointed out that there was great heterogeneity in his meta-analysis, but he did not explore heterogeneity and still made a mergence. Therefore, we should be cautious about the reliability of his results. After extracting high-quality articles for our meta-analysis, we found that the heterogeneity was still very significant and could not be merged. After reading each selected article carefully, we found that many cases were not representative. Therefore, it was considered that the results reported by culture and PCR methods were quite different, and it was impossible to find out the correlation among the previous articles to obtain the overall changes of intestinal microbiota in IBS patients. It was necessary to find stricter selection criteria and more effective methods to figure out the relationship between IBS and intestinal microbiota.

There are some limitations to our study. Firstly, our study did not analyze publication bias and sensitivity. Because the number of the included studies was too small, it was not suitable for publication bias, sensitivity analysis, and subgroup analysis.[49,50] However, this study was carried out in strict accordance with the PRISMA standard, and a rigorous literature quality evaluation was carried out. Therefore, we believed that our results were relatively more credible. Secondly, some selected literature had been published for a long time. The accuracy of PCR and other techniques had also been improved. However, the PCR technique itself was more rigorous and reliable than 16rRNA, so it was more accurate in analyzing limited bacteria. Thirdly, IBS patients had multiple symptoms, so it was reasonable to speculate that different microbial groups may be related to IBS subtype (diarrhea, constipation, and alternating dominance). According to the classification of IBS patients found by symptoms, it was impossible to continue discussing subtypes due to the lack of literature on each subtype. Lastly, as Rome II diagnostic criteria for IBS patients have been updated recently, we need to re-screen the studies according to the latest standards to obtain data closer to the latest version of the standard.

In conclusion, by using more stringent standards to extract high-quality literature for our meta-analysis, we found that *Bifidobacteria* was the only microbiota alterations of specific intestinal microbes in IBS determined by qPCR and bacterial culture. Further studies are warranted.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Z.B., X.Y.; Design – X.Y., L.P.; Supervision – L.P., X.Y.; Resources – B.H., Z.B.; Materials – B.H., Z.B.; Data Collection and Processing – X.Y., L.P.; Analysis and/or Interpretation – L.P., Z.B.; Literature Search – Z.B., X.Y.; Writing Manuscript – X.Y., L.P.; Critical Review – X.Y., L.P.

Acknowledgment: The authors are indebted to the authors of the primary studies.

Declaration of Interest: The authors have no conflict of interest to declare.

Funding: This study was partially supported by Medical Science Research Foundation of Health Bureau of Zhejiang Province, China (Grant number: 2014KYB001) and Natural Science Foundation of Zhejiang Province, China (Grant number: LY19H160041).

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Supplementary Table 1. Quality Assessment of Studies (NOS)

		Selection (6)			Comparability (2)			Exposure (6)			Total (15)
		Case Definition	Representativeness	Control Selection	Control Definition	Important Factor	Additional Factor	Ascertainment of Exposure	Method of Exposure	Non-Response Rate	>7
Author	Year	abc (2,1,0)	ab (1,0)	abc (2,1,0)	ab (1,0)	a (1)	b (1)	abcde (4,3,2,1,0)	ab (1,0)	abc (2,1,0)	
R Francavilla	2019	2	1	2	1	1	1	3	1	2	14*
TT Su	2018	1	0	0	1	0	0	1	1	1	5
XM Wang	2015	0	0	1	0	0	0	1	1	1	4
R Shukla	2015	1	0	0	0	1	1	1	1	1	6
ZH Cheng	2014	1	0	1	1	1	1	1	1	1	8*
LY Hu	2012	1	0	0	0	0	0	1	1	1	4
C Chassard	2012	2	0	0	0	1	0	2	1	1	7
IM Carroll	2012	1	1	2	0	0	0	1	1	1	8*
C Tana	2010	2	1	2	1	1	1	2	1	1	12*
IM Carroll	2010	1	0	1	0	0	0	1	1	1	5
SS Cui	2009	1	0	0	0	1	1	1	1	1	6
L Zhang	2009	1	0	0	0	1	1	1	1	1	6
AP kerckhoffs	2009	1	0	0	0	0	0	1	1	1	4
L Zhang	2008	1	0	0	0	1	0	1	1	1	5
YH Zhuang	2005	1	0	0	0	0	0	1	1	0	3
JL Li	2005	1	0	1	0	0	0	1	1	1	5
E Malinen	2005	2	1	0	0	1	1	2	1	1	9*
J Matto	2005	2	0	0	1	1	1	1	1	1	8*
T Rinttila	2004	1	0	0	0	0	0	1	1	1	4
CY Yu	2004	1	0	0	0	1	1	1	1	0	5
DR Zhang	1999	1	1	0	0	0	0	1	1	1	5

*High quality studies.