Systematic Review and Meta-Analysis

Medicine

OPEN

Quantitative evaluation of gut microbiota composition in pancreatic cancer

A pooled study

Dachuan Jin, MD, PhD[a,*], Shunqin Jin, MD[b], Tao Zhou, MD[c], Zhongfeng Cui, MD[a], Baoqiang Guo, MD, PhD[d], Guangming Li, MD[e], Chunming Zhang, MD[f]

Abstract

Background: Prior research has demonstrated a positive association between the composition of gut microbiota and the incidence of pancreatic cancer. Nevertheless, a thorough quantitative and systematic evaluation of the distinct properties of gut microbiota in individuals diagnosed with pancreatic cancer has yet to be conducted. The objective of this study is to examine alterations in the diversity of intestinal microbiota in individuals diagnosed with pancreatic cancer.

Methods: Search for relevant literature published before July 2023 in 4 databases: PubMed, Embase, Web of Science, and Cochrane Library, without any language restrictions.

Results: A total of 12 studies were included, including 535 patients with pancreatic cancer and 677 healthy controls. Analysis was conducted on 6 phyla, 16 genera, and 6 species. The study found significant and distinctive changes in the α-diversity of gut microbiota, as well as in the relative abundance of multiple gut bacterial groups at the phylum, genus, and species levels in pancreatic cancer patients.

Conclusion: Overall, there are certain characteristic changes in the gut microbiota of pancreatic cancer patients. However, further research is warranted to elucidate the specific mechanism of action and the potential for treatment.

Abbreviations: CI = confidence interval, df = degree of freedom, NOS score = Newcastle-Ottawa Scale score, rRNA = ribosomal ribonucleic acid, SD = standard deviation, SMD = standardized mean difference.

Keywords: α-diversity, dysbiosis, gut microbiota, pancreatic neoplasm, relative abundance

1. Introduction

The gut microbiota has received increasing attention over the past 15 years.[1] It is now believed that the microbes in the gut not only play a crucial role in human metabolism but are also associated with the development of many diseases, making them a potential source for developing disease-specific diagnostics and new therapies.[2,3] Extensive research has shown a strong link between the gut microbiota and the occurrence, progression, and prognosis of pancreatic cancer.[4] Pancreatic cancer is one of the most invasive malignant tumors and poses a major health threat worldwide.[5] This is largely due to the difficulties in early detection and its aggressive nature, resulting in poor prognosis with a 5-year survival rate of only 10% and increasing incidence.[6,7]

Although the connection between gut microbiota and pancreatic cancer has been discovered, there is still relatively limited research on the diversity of gut microbial characteristics in pancreatic cancer patients, and the findings are still inconsistent. For example, Half et al found that the Shannon index of α-diversity of gut microbiota in pancreatic cancer patients is lower than that of the normal control group, while Hashimoto et al found an increase in the Shannon index of pancreatic cancer.[8,9] Another example is Xie et al found a decrease in phylum *Bacteroidetes* in pancreatic cancer patients, while Half et al found an enrichment

DJ, SJ, and TZ contributed equally to this work.

The authors have no funding and conflicts of interest to disclose.

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

The review protocol was register on INPLASY (INPLASY202370038).

All the data in the study were retrieved from public databases, this study did not require ethical approval or patient consent.

Supplemental Digital Content is available for this article.

[a] Department of Clinical Laboratory, Sixth People's Hospital of Zhengzhou, Zhengzhou, P.R. China, [b] Department of Radiology, Hebei Medical University, Shijiazhuang, P.R. China, [c] Department of Geriatric Medicine, Key Laboratory of Cardiovascular Proteomics of Shandong University, Qilu Hospital of Shandong University, Jinan, P.R. China, [d] Faculty of Science and Engineering, Department of Life Sciences, Manchester Metropolitan University, Manchester, U.K., [e] Department of Liver Disease, Sixth People's Hospital of Zhengzhou, Zhengzhou, P.R. China, [f] Department of General Surgery, Sixth People's Hospital of Zhengzhou, Zhengzhou, P.R. China.

** Correspondence: Dachuan Jin, Clinical Laboratory, Sixth People's Hospital of Zhengzhou, Zhengzhou, 450015, P.R. China (e-mail: dachuanjin@gmail.com).*

How to cite this article: Jin D, Jin S, Zhou T, Cui Z, Guo B, Li G, Zhang C. Quantitative evaluation of gut microbiota composition in pancreatic cancer: A pooled study. Medicine 2024;103:10(e36907).

Received: 23 October 2023 / Received in final form: 15 December 2023 / Accepted: 18 December 2023

http://dx.doi.org/10.1097/MD.0000000000036907

of the *Bacteroidetes* phylum in the gut of pancreatic cancer patients.[9,10] Although there are some controversies in the current research on the characteristics of the gut microbiota in pancreatic cancer, so far, there has been no published meta-analysis on the characteristic changes of the gut microbiota in pancreatic cancer patients. In this study, we aim to comprehensively evaluate the characteristic changes of the gut microbiota in pancreatic cancer patients by reviewing relevant studies and conducting a meta-analysis.

2. Material and methods

We conducted a meta-analysis based on a registered protocol with the registration number INPLASY202370038 on the INPLASY website, following the Preferred Reporting Items for Systematic Review and Meta-Analysis statement. This review aimed to assess the differences in gut microbiota diversity and relative abundance of bacterial phyla, genera, and species between pancreatic cancer and healthy control.

2.1. Search strategy

To search for potential studies, a literature search was conducted from 4 databases (PubMed, Embase, Web of Science, and Cochrane Library) from their inception to July 1, 2023, with no language restrictions. To include relevant articles, we also reviewed the reference lists of all relevant studies and reviews to include additional eligible research. Table 1 provides an example of a search strategy using PubMed.

2.2. Inclusion and exclusion criteria

We considered and included studies that meet the population, intervention, comparator, outcome, and study design criteria: Population: adults (≥18 years old) diagnosed with pancreatic cancer meeting the guideline from National Comprehensive Cancer Network[11]; Intervention: using 16S-ribosomal ribonucleic acid sequencing technique, whole genome shotgun sequencing, or metagenomic shotgun sequencing to test the taxonomy of gut bacteria; Comparator: having a matched healthy control group; Outcomes: the research results provide α-diversity and/or microbial taxa data of gut microbiota at the phylum to species levels; Study designs: cross-sectional study, case-control study, or cohort study; no language restriction.

The following studies were excluded patients who are children or adolescents under 18 years old; studies without a control group; studies without any data on microbial diversity or taxa relative abundance; animal experimental studies; reviews, conference abstracts, case reports, and duplicated publications.

2.3. Data extraction

The research information we extracted includes the author, publication year, country or region, research design, sample size, gender, mean age, sample, results, and sequencing technology. The extracted data include the α-diversity index and relative abundance of bacteria from phylum to species. Data extraction was independently conducted by 2 researchers, S.J. and Z.C., and disagreements were resolved through discussion.

2.4. Quality assessment

The above 2 independent reviewers, S.J. and Z.C., used the Newcastle-Ottawa Scale (NOS) to assess the quality of each included study.[12] The NOS is divided into 3 domains: selection of cohort, comparability of the groups, and the outcomes' quality. Each item in the selection and outcome domains can receive a maximum of 1 point, while the comparability domain can receive a maximum of 2 points. The scale's scoring ranges from 0 to 9, with scores of 8 or 9 indicating high quality, scores of 6 or 7 indicating moderate quality, and scores of 5 or below indicating low quality. Differences were identified and resolved through discussion.

2.5. Outcomes

Our outcomes of interest include α-diversity and relative abundance of microbial taxa data of gut microbiota at the phylum to species levels.

2.6. Statistical analysis

Meta-analysis was conducted using STATA 15.1 software. Standardized mean differences (SMDs) and 95% confidence intervals (CIs) were used as effect sizes. The I^2 was used to evaluate heterogeneity between studies. It was introduced by Higgins and Thompson[13] to quantify heterogeneity in meta-analyses

Table 1

Search strategy on PubMed.

#1	"pancreatic neoplasms"(MeSH)
#2	("pancreatic neoplasms"[MeSH Terms] OR "pancreatic neoplasms"[Title/Abstract] OR "neoplasm pancreatic"[Title/Abstract] OR "pancreatic neoplasm"[Title/Abstract] OR "pancreas neoplasms"[Title/Abstract] OR "neoplasm pancreas"[Title/Abstract] OR "neoplasms pancreas"[Title/Abstract] OR "pancreas neoplasm"[Title/Abstract] OR "neoplasms pancreatic"[Title/Abstract] OR "cancer of pancreas"[Title/Abstract] OR "pancreas cancers"[Title/Abstract] OR "pancreas cancer"[Title/Abstract] OR "cancer pancreas"[Title/Abstract] OR "cancers pancreas"[Title/Abstract] OR "pancreatic cancer"[Title/Abstract] OR "cancer pancreatic"[Title/Abstract] OR "cancers pancreatic"[Title/Abstract] OR "pancreatic cancers"[Title/Abstract] OR "cancer of the pancreas"[Title/Abstract]) AND (systematicreview[Filter])
#3	#1 or #2
#4	"gastrointestinal microbiome"(MeSH)
#5	("gastrointestinal microbiome"[MeSH Terms] OR ("gastrointestinal microbiome"[Title/Abstract] OR "gastrointestinal microbiomes"[Title/Abstract] OR "microbiome gastrointestinal"[Title/Abstract] OR "gut microbiome"[Title/Abstract] OR "gut microbiomes"[Title/Abstract] OR "microbiome gut"[Title/Abstract] OR "gut microflora"[Title/Abstract] OR "microflora gut"[Title/Abstract] OR "gut microbiota"[Title/Abstract] OR "gut microbiotas"[Title/Abstract] OR "microbiota gut"[Title/Abstract] OR "gastrointestinal flora"[Title/Abstract] OR "flora gastrointestinal"[Title/Abstract] OR "gut flora"[Title/Abstract] OR "flora gut"[Title/Abstract] OR "gastrointestinal microbiota"[Title/Abstract] OR "gastrointestinal microbiotas"[Title/Abstract] OR "microbiota gastrointestinal"[Title/Abstract] OR "gastrointestinal microbial community"[Title/Abstract] OR "gastrointestinal microbial communities"[Title/Abstract] OR (("Microbiota"[MeSH Terms] OR "Microbiota"[All Fields] OR ("Microbial"[All Fields] AND "Community"[All Fields]) OR "microbial community"[All Fields]) AND "Gastrointestinal"[Title/Abstract]) OR "gastrointestinal microflora"[Title/Abstract] OR "microflora gastrointestinal"[Title/Abstract] OR "gastric microbiome"[Title/Abstract] OR "gastric microbiomes"[Title/Abstract] OR "microbiome gastric"[Title/Abstract] OR "intestinal microbiome"[Title/Abstract] OR "intestinal microbiomes"[Title/Abstract] OR "microbiome intestinal"[Title/Abstract] OR "intestinal microbiota"[Title/Abstract] OR "intestinal microbiotas"[Title/Abstract] OR "microbiota intestinal"[Title/Abstract] OR "intestinal microflora"[Title/Abstract] OR "microflora intestinal"[Title/Abstract] OR "intestinal flora"[Title/Abstract] OR "flora intestinal"[Title/Abstract] OR "enteric bacteria"[Title/Abstract] OR "bacteria enteric"[Title/Abstract])) AND (systematicreview[Filter])
#6	#4 OR #5
#7	#3 AND #6

and to help determine whether a fixed-effects or random-effects model should be applied to combine the study estimates. I^2 is calculated as $I^2 = 100\% \times (Q - df)/Q$, where Q is Cochran heterogeneity statistic and df is the degree of freedoms. The resulting value of I^2 ranges between 0% and 100%. Note that I^2 is not an acronym or abbreviation for any specific words, but it is a widely used statistical measure. In the case of high heterogeneity ($I^2 \geq 50\%$), the random-effects model was employed for calculations. For low heterogeneity ($I^2 < 50\%$), the fixed-effects model was used. Additionally, sensitivity analysis was conducted to assess the stability of the meta-analysis. Furthermore, Begg test and funnel plots were used to evaluate publication bias. The significance level for 2-sided tests is set at 0.05. This means that a $P < .05$ indicates statistical significance. We also performed sensitivity analysis to evaluate the results' stability.

3. Results

A total of 642 potentially relevant studies were identified for database retrieval, and an additional 3 studies were identified through manual searches of reference lists (Fig. 1). All records were imported into EndNote, and 384 duplicate records were removed. After evaluating the eligibility of studies based on their titles and abstracts, 90 studies were excluded. Following further exclusion of irrelevant papers, animal experiments or cytological studies, conference abstracts, or reviews, 12 studies remained with sufficient data for meta-analysis. Therefore, 12 studies met our inclusion criteria and were ultimately included[4,8–10,14–21] (Table 2).

All included studies were case-control studies, with a median NOS score of 8 (range: 6–9) (Supplementary Table S1, Supplemental Digital Content, http://links.lww.com/MD/L300). A total of 1212 patients were included, comprising 535 patients with pancreatic cancer and 677 healthy controls.

Regarding the outcomes of interest, 9 studies reported at least 1 α-diversity index,[4,8,9,14–17,19,20] and 7 studies reported the relative abundance of gut microbiota.[9,10,16–19,21] However, due to a lack of objectively mergeable data, the analysis of relative abundance at the family level had to be abandoned. The final analyzed data included 3 α-diversity indices (Chao1, Shannon, and Simpson indices), the relative abundance of 6 bacterial phyla, 16 bacterial genera, and 6 bacterial species.

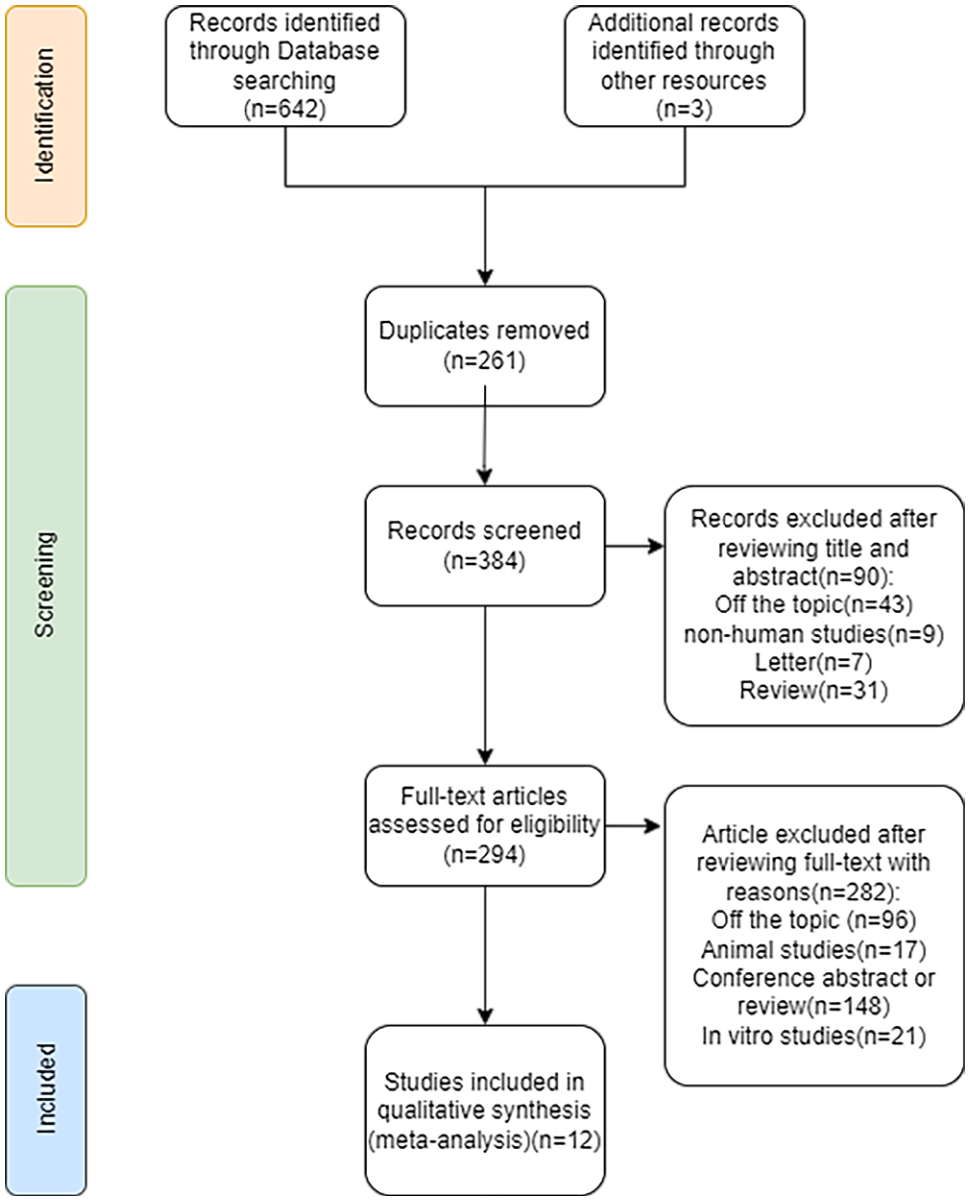

Figure 1. Study flowchart.

Table 2

Characteristics of the studies included in the meta-analysis.

Study	Nation	Study design	PC cases		Controls		Specimens	Outcomes of interest	Sequencing methods
			n/f	Age (SD)	n/f	Age (SD)			
Chen et al[17]	China	Case-control	38/19	56.58 (9.71)	39/20	56.35 (6.90)	Feces	Chao1, RA (p, g)	16s-rRNA
Hashimoto et al[8]	Japan	Case-control	5/3	75.4 (7.77)	68/39	54 (4.82)	Feces	Shannon, RA (g)	16s-rRNA
Half et al[9]	Israel	Case-control	30/14	68.9 (6.2)	13/7	59 (8.7)	Feces	Shannon, RA (p)	16s-rRNA
Ren et al[14]	China	Case-control	85/38	56 (11.25)	57/21	56 (11.25)	Feces	Chao1, Shannon	16s-rRNA
Wang et al[16]	China	Case-control	8/2	63.13 (13.11)	8/3	61.13 (12.14)	Feces	Chao1, Shannon, RA (p, s)	WGS
Zhou et al[18]	China	Case-control	32/7	59.31 (9.53)	32/6	58.63 (10.28)	Feces	Shannon, RA (p, g, s)	MGS
Kohi et al[19]	USA	Case-control	63/23	65.3 (10.83)	63/33	63.6 (9.48)	Duodenal fluid	Shannon	16s-rRNA
Chen et al[20]	China	Case-control	28/12	61.43 (9.31)	8/NA	NA	Feces	RA (g)	16s-rRNA
Xie et al[10]	China	Case-control	31/9	62.3 (4.9)	8/2	58.7 (5.3)	Feces	RA (p, g)	MGS
Zhang et al[15]	China	Case-control	14/14	64.43 (6.22)	14/14	62.50 (7.65)	Feces	Shannon, RA (p, g)	MGS
Nagata JP, 2022[14]	Japan	Case-control	43/17	57.5 (NA)	235/105	59.2 (NA)	Feces	Shannon	MGS
Nagata ES, 2022[14]	Spain	Case-control	57/21	71.5 (NA)	50/19	71.3 (NA)	Feces	Shannon	MGS
Nagata DE, 2022[14]	German	Case-control	44/15	68.7 (NA)	32/17	48 (NA)	Feces	Shannon	MGS
Kartal et al[4]	Spanish	Case-control	57/NA	NA (NA)	50/NA	NA (NA)	Feces	Shannon	16s-rRNA

MGS = metagenomic shotgun sequencing, NA = not available, n/f = total number/female number, PC = pancreatic cancer, rRNA = ribosomal ribonucleic acid, SD = standard deviation.

3.1. α-diversity indices

We conducted random-effects meta-analyses based on 9 studies[4,8,9,14,15,17,19,20,22] evaluating the Shannon index and 5 studies[4,14,15,17,19] evaluating the Simpson index, while fixed-effects model analyses were performed based on 3 studies[15,17,18] evaluating the Chao1 index (Fig. 2). Compared to the healthy control group, the Simpson index in the pancreatic cancer group was similar (SMD = 0.20, 95% CI: −0.53 to 0.94, $P = .592$, $I^2 = 94.30\%$). The Chao1 (SMD = −0.35, 95% CI: −0.61 to −0.09, $P = .009$, $I^2 = 0.00\%$) and Shannon (SMD = −0.39, 95% CI: −0.63 to −0.15, $P = .001$, $I^2 = 62.10\%$) indices in pancreatic cancer groups were significantly lower than those in the healthy control group (Supplementary Table S2, Supplemental Digital Content, http://links.lww.com/MD/L301). Subgroup analyses were conducted for Chao1, Shannon index, and Simpson index based on the sequencing method. However, the results were consistent, showing that Chao1 (Supplementary Figure S1, Supplemental Digital Content, http://links.lww.com/MD/L305) and Shannon (Supplementary Figure S2, Supplemental Digital Content, http://links.lww.com/MD/L306) were significantly lower in pancreatic cancer patients compared to the control group, while the Simpson index (Supplementary Figure S3, Supplemental Digital Content, http://links.lww.com/MD/L307) in the pancreatic cancer group did not differ significantly from the control group. Ethnicity-based subgroup analyses were also carried out for the Shannon index and Simpson index, revealing that in the Caucasian population, the Shannon index was significantly lower than in the East Asian population (SMD = −0.65, 95% CI: −0.84 to −0.46), but there was no significant difference in the Simpson index between the 2 population groups (SMD = 0.12, 95% CI: −0.57 to 0.80) (Supplementary Figure S4, Supplemental Digital Content, http://links.lww.com/MD/L308).

Begg test indicated no significant publication bias for the Shannon index ($P = .517$), Chao1 index ($P = .091$), and Simpson index ($P = .180$) (Supplementary Table S3, Supplemental Digital Content, http://links.lww.com/MD/L302). Furthermore, our sensitivity analysis (Supplementary Figure S5, Supplemental Digital Content, http://links.lww.com/MD/L309) did not identify any literature that had a substantial impact on the overall analysis results. Therefore, we believe that our results are stable and reliable. Funnel diagram showed that both sides are roughly symmetrical without significant publication bias (Supplementary Figure S6, Supplemental Digital Content, http://links.lww.com/MD/L310).

3.2. Bacterial phylum

At the phylum level of bacteria, up to 6 phyla (i.e., *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, and *Bacteroidetes*) can be analyzed because only these 6 phyla have data that can be merged. Since their $I^2 < 50\%$, we employed fixed-effects meta-analyses based on 6 studies. To assess the relative abundance of 6 phyla: *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, and *Bacteroidetes*, we performed fixed-effects meta-analyses based on 6 studies[9,10,16–19] (Fig. 3). The *Firmicutes* in the pancreatic cancer were significantly lower than in the control, showing a significant difference (SMD = −0.60, 95% CI: −0.85 to −0.35, $P = .000$, $I^2 = 0.0\%$). The composition of *Actinobacteria* was also lower than in the control, but the difference was not significant (SMD = −0.06, 95% CI: −0.35 to 0.23, $P = .673$, $I^2 = 0.0\%$). Additionally, the *Bacteroidetes* (SMD = 0.32, 95% CI: 0.06–0.57, $P = .015$, $I^2 = 49.5\%$) and *Proteobacteria* (SMD = 0.31, 95% CI: 0.06–0.57, $P = .014$, $I^2 = 0.0\%$) were significantly enriched in the pancreatic cancer group. *Fusobacteria* (SMD = 0.05, 95% CI: −0.23 to 0.33, $P = .724$, $I^2 = 0.0\%$) and *Verrucomicrobia* (SMD = 0.24, 95% CI: −0.08 to 0.56, $P = .142$, $I^2 = 0.0\%$) were also higher in the pancreatic cancer,

but the differences were not significant (Supplementary Table S4, Supplemental Digital Content, http://links.lww.com/MD/L303).

Begg test indicated no significant publication bias in the relative abundance of the phyla *Firmicutes* ($P = .583$), *Bacteroidetes* ($P = .325$), *Fusobacteria* ($P = 1.000$), *Proteobacteria* ($P = .273$), *Verrucomicrobia* ($P = .120$), and *Actinobacteria* ($P = .245$) (Supplementary Table S5, Supplemental Digital Content, http://links.lww.com/MD/L304).

3.3. Bacterial genus

At the genus level, there are a maximum of 16 genera with available data for merging the relative abundances. Therefore, we conducted an analysis of the relative abundances of these 16 genera. For the 7 genera (*Faecalibacterium*, *Eubacterium*, *Veillonella*, *Streptococcus*, *Lactobacillus*, *Prevotella*, and *Coprococcus*) with $I^2 \geq 50\%$, we used a random-effects model for analysis based on 6 studies.[8,10,16,18,19,21] For the 9 genera with $I^2 < 50\%$ (*Megamonas*, *Klebsiella*, *Bacteroides*, *Escherichia/Shigella*, *Akkermansia*, *Gemmiger*, *Alistipes*, *Bifidobacterium*, and *Clostridium*), fixed-effects analyses were performed based on 5 studies (Figs. 4 and 5).[10,16,18,19,21] The analysis indicated that the bacterial abundance of *Faecalibacterium* (SMD = −0.87, 95% CI: −1.60 to −0.13, $P = .021$, $I^2 = 83.6\%$) and *Eubacterium* (SMD = −0.77, 95% CI: −1.54 to −0.00, $P = .049$, $I^2 = 64.9\%$) in the pancreatic cancer group was significantly lower than in the healthy control group (Supplementary Table S4, Supplemental Digital Content, http://links.lww.com/MD/L303). The differences were statistically significant. The relative abundance of *Veillonella*, *Bacteroides*, *Gemmiger*, *Prevotella*, *Coprococcus*, *Bifidobacterium*, and *Clostridium* was also lower in the pancreatic cancer group, but the differences were not significant. The abundance of *Megamonas*, *Klebsiella*, *Streptococcus*, *Escherichia/Shigella*, *Lactobacillus*, *Akkermansia*, and *Alistipes* was higher in the pancreatic cancer group, but the differences were not significant, as well (Supplementary Table S4, Supplemental Digital Content, http://links.lww.com/MD/L303). Begg test indicated no significant publication bias for the relative abundances of the aforementioned *Faecalibacterium* ($P = .531$), *Eubacterium* ($P = 1.000$), and 14 other bacterial genera (Supplementary Table S5, Supplemental Digital Content, http://links.lww.com/MD/L304).

3.4. Bacterial species

At the species level, we found a maximum of 6 bacterial species that can be merged and analyzed based on 2 studies.[17,19] Therefore, a total of 6 species were analyzed. Among them, 3 had $I^2 > 50\%$ (*Escherichia coli*, *Clostridium bolteae*, and *Megamonas hypermegale*), and thus random-effects analyses were employed (Fig. 6). The other 3 (*Faecalibacterium prausnitzii*, *Prevotella stercorea*, and *Klebsiella pneumonia*) had $I^2 < 50\%$ and were analyzed using a fixed-effect model (Fig. 6). Our analysis revealed that the abundance of *F prausnitzii* (SMD = −1.22, 95% CI: −1.66 to −0.77, $P = .000$, $I^2 = 15.20\%$) was lower in pancreatic cancer patients than in the healthy control group, with a significant statistical difference. The abundance of *M hypermegale* (SMD = −0.27, 95% CI: −2.43 to 1.89, $P = .807$, $I^2 = 95.40\%$) in the pancreatic cancer group was also lower than in the healthy control group, but the difference was not significant. The abundance of *E coli* (SMD = 0.99, 95% CI: 0.03–1.95, $P = .044$, $I^2 = 77.60\%$) and *P stercorea* (SMD = 0.88, 95% CI: 0.42–1.34, $P = .000$, $I^2 = 0.00\%$) in the pancreatic cancer group was significantly higher than in the healthy control group (Supplementary Table S4, Supplemental Digital Content, http://

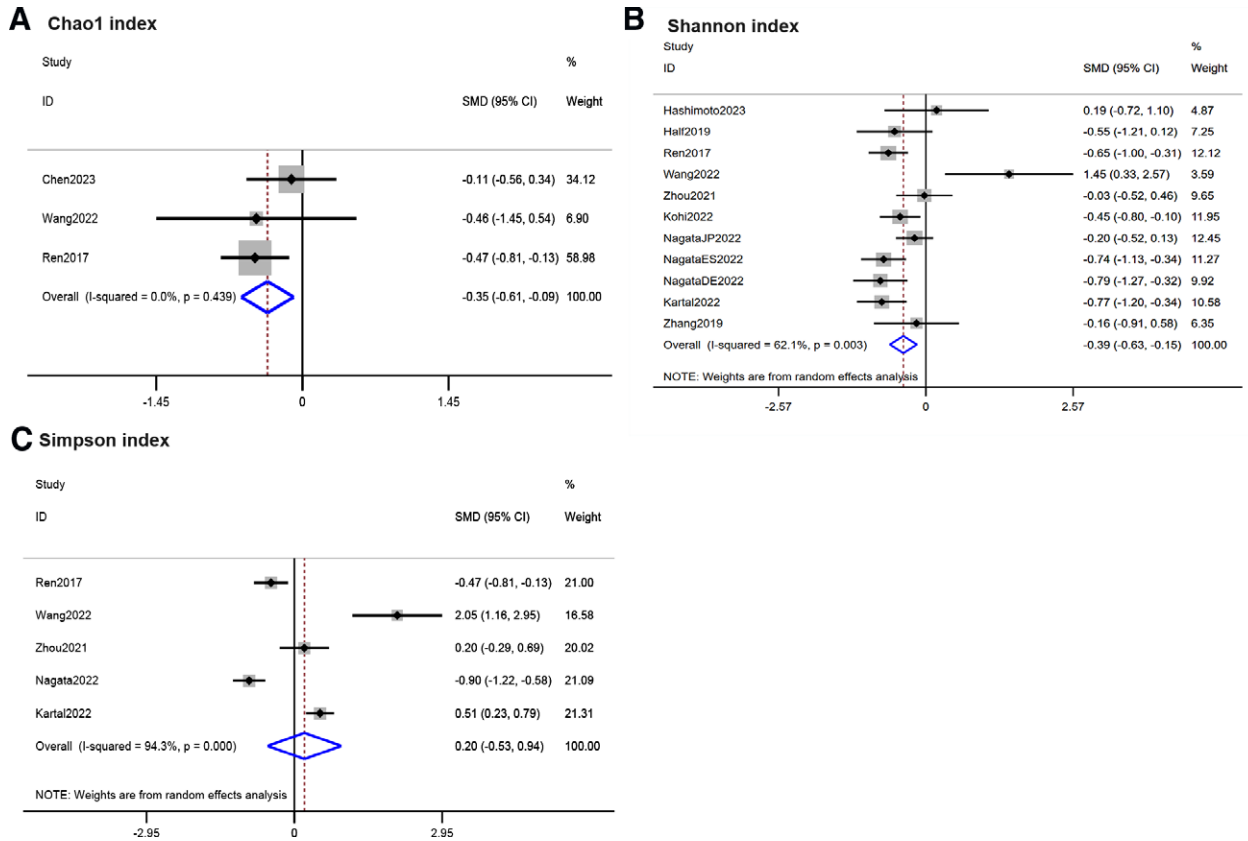

Figure 2. Forest plots for changes in α-diversity indices: (A) Chao1, (B) Shannon index, and (C) Simpson index. CI = confidence interval, SMD = standardized mean difference.

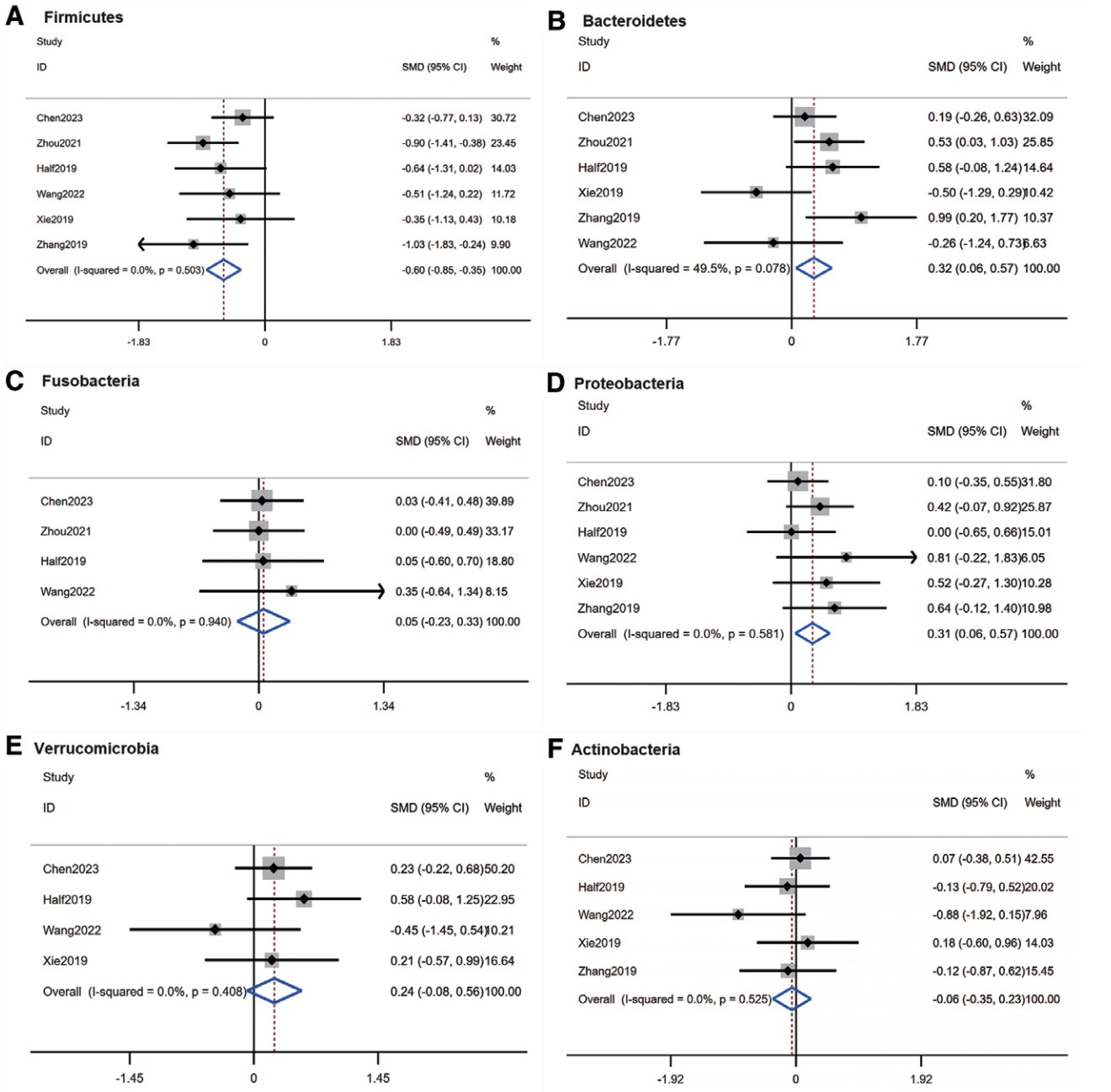

Figure 3. Forest plots for changes in relative abundance of bacterial phyla include *Firmicutes* (A), *Bacteroidetes* (B), *Fusobacteria* (C), *Proteobacteria* (D), *Verrucomicrobia* (E), *Actinobacteria* (F). CI = confidence interval, SMD = standardized mean difference.

links.lww.com/MD/L303). The differences were significant. The abundance of *C bolteae* and *K pneumonia* was also higher in the pancreatic cancer group, but the differences were not statistically significant. Begg test did not reveal significant publication bias for the relative abundance of *F prausnitzii*, *E coli*, *P stercorea*, and the other 3 species (Supplementary Table S5, Supplemental Digital Content, http://links.lww.com/MD/L304).

4. Discussion

Recently, the relationship between gut microbiota and pancreatic cancer has attracted increasing attention.[23–25] The gut microbiota has been shown to not only influence the susceptibility and progression of pancreatic cancer, but also potentially affect treatment outcomes.[26]In our meta-analysis, based on twelve case-control studies comprising a total of 1212 patients, we determined the diversity and composition changes of gut microbiota in pancreatic cancer patients. The results revealed a significant decrease in α-diversity among pancreatic cancer patients. Compared to the healthy control group, the pancreatic cancer group exhibited a lower abundance of the phylum *Firmicutes* and a higher abundance of the phylum *Bacteroidetes* and *Proteobacteria*. At the genus level, *Faecalibacterium* and *Eubacterium* exhibited reduced abundances, whereas, at the species level, there were lower abundances of *F prausnitzii*, alongside higher abundances of *E coli* and *P stercorea*. Other phyla, genera, and species did not show statistically significant differences between the pancreatic cancer group and the healthy control group in our analysis.

Some studies have suggested that the bacterial composition within the pancreas, rather than the bacterial abundance, is associated with pancreatic cancer occurrence.[27] However,

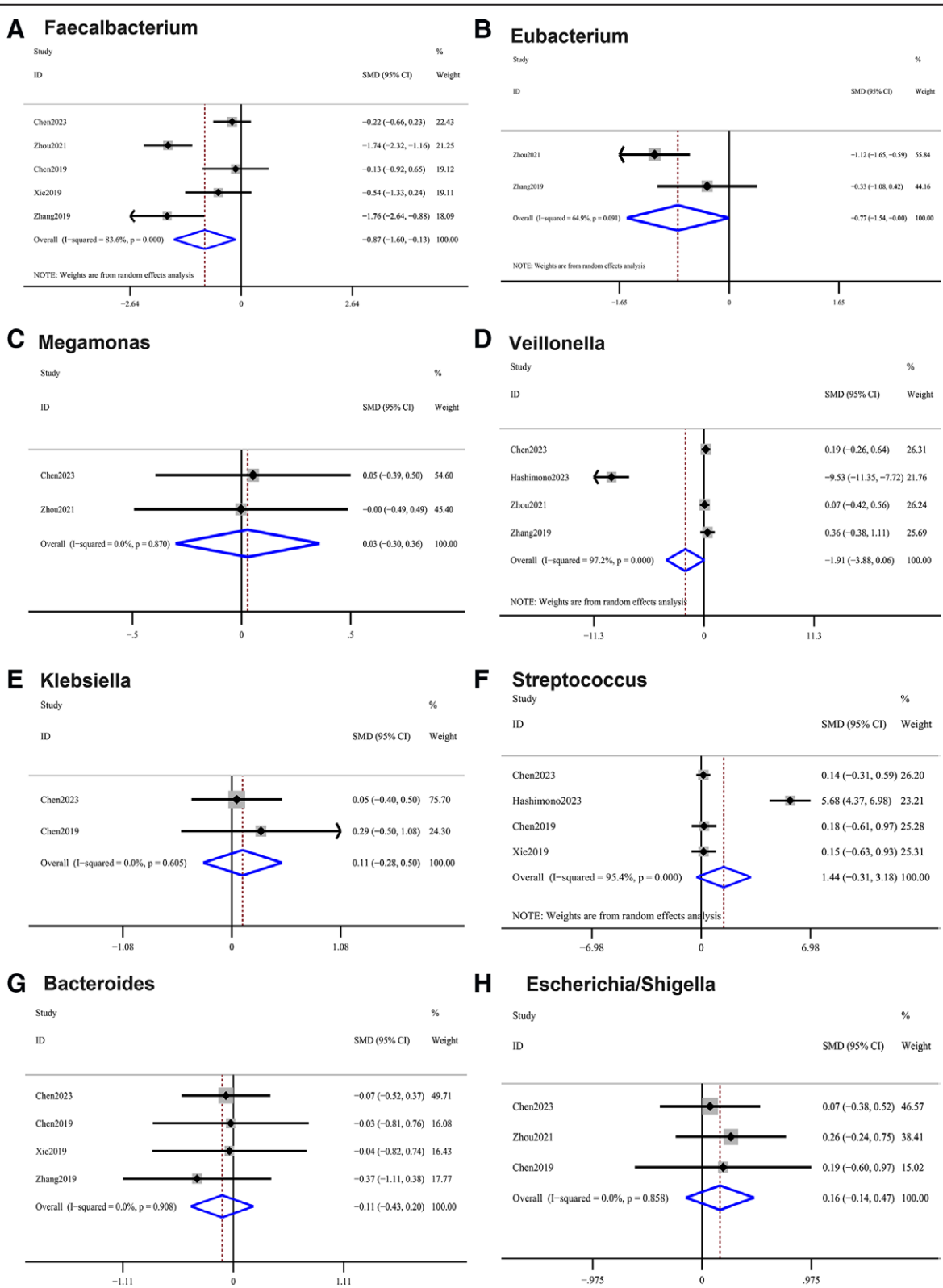

Figure 4. Forest plots for changes in relative abundance of bacterial genera include *Faecalibacterium* (A), *Eubacterium* (B), *Megamonas* (C), *Veillonella* (D), *Klebsiella* (E), *Streptococcus* (F), *Bacteroides* (G), *Escherichia/Shigella* (H). CI = confidence interval, SMD = standardized mean difference.

other studies have found a significant decrease in gut microbiota diversity among pancreatic cancer patients, particularly in the Shannon index.[28] We evaluated the differences in 3 α-diversity indices between the pancreatic cancer group and the healthy control group, and found that both Chao1 and Shannon indices were significantly decreased. Although the difference in the Simpson index between the 2 groups did not reach statistical significance, it was higher in the pancreatic cancer group. The larger the Simpson index, the lower the species diversity. Therefore, all 3 α-diversity indices support the decreased diversity of gut microbiota in pancreatic cancer patients.

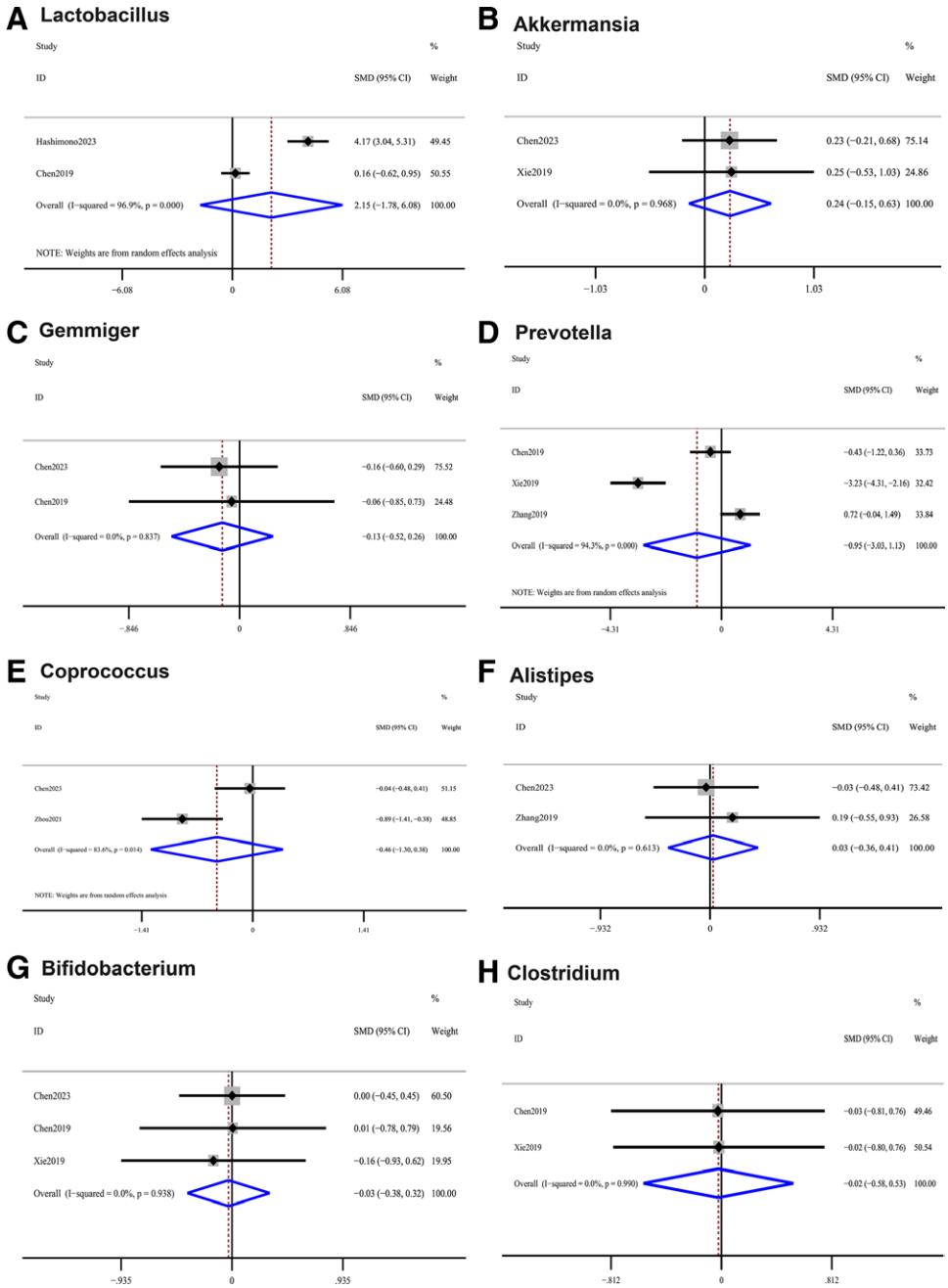

Figure 5. Forest plots for changes in relative abundance of bacterial genera include *Lactobacillus* (A), *Akkermansia* (B), *Gemmiger* (C), *Prevotella* (D), *Coprococcus* (E), *Alistipes* (F), *Bifidobacterium* (G), *Clostridium* (H). CI = confidence interval, SMD = standardized mean difference.

Prior research has yielded conflicting findings about the variations in gut microbiota composition between pancreatic cancer patients and healthy controls. For example, Chen et al[18] found that the relative abundance of *Bacteroidetes* was decreased. In contrast, Zhou et al[19] found the opposite results, indicating a higher abundance of *Bacteroidetes* in the gut of pancreatic cancer patients. In our meta-analysis, a comprehensive pooled analysis was conducted to assess the differences among studies. We found that both *Bacteroidetes* and *Proteobacteria* were enriched in the gut of pancreatic cancer patients, while *Firmicutes* showed a decrease. In the gut of healthy individuals, *Firmicutes* and *Bacteroidetes* are 2 predominant phyla.[29] *Proteobacteria* belongs to the subdominant phylum, but its relative abundance is more likely to increase during gut dysbiosis, even surpassing *Firmicutes* and *Bacteroidetes*.[30–33] This can explain the increase in *Proteobacteria* in pancreatic cancer in this study. The ratio of *Firmicutes* to *Bacteroidetes* is currently considered an important parameter reflecting gut microbiota ecology, and is associated with susceptibility to various diseases such as metabolic syndrome and Crohn disease, decreasing with weight loss.[34] As pancreatic cancer patients often experience wasting and cachexia, the causal relationship between changes in *Firmicutes* and *Bacteroidetes* and pancreatic cancer remains to be determined.

At the taxonomic level of the genus, the present study observed a notable decrease in the relative abundance of *Faecalibacterium* and *Eubacterium* among individuals diagnosed with pancreatic cancer, with statistical significance. Therefore, the observed changes in the abundance of these genera at the genus level are consistent with the significant decrease in the abundance of the phylum *Firmicutes*. Both *Faecalibacterium* and *Eubacterium* are major members of the phylum *Firmicutes* and beneficial

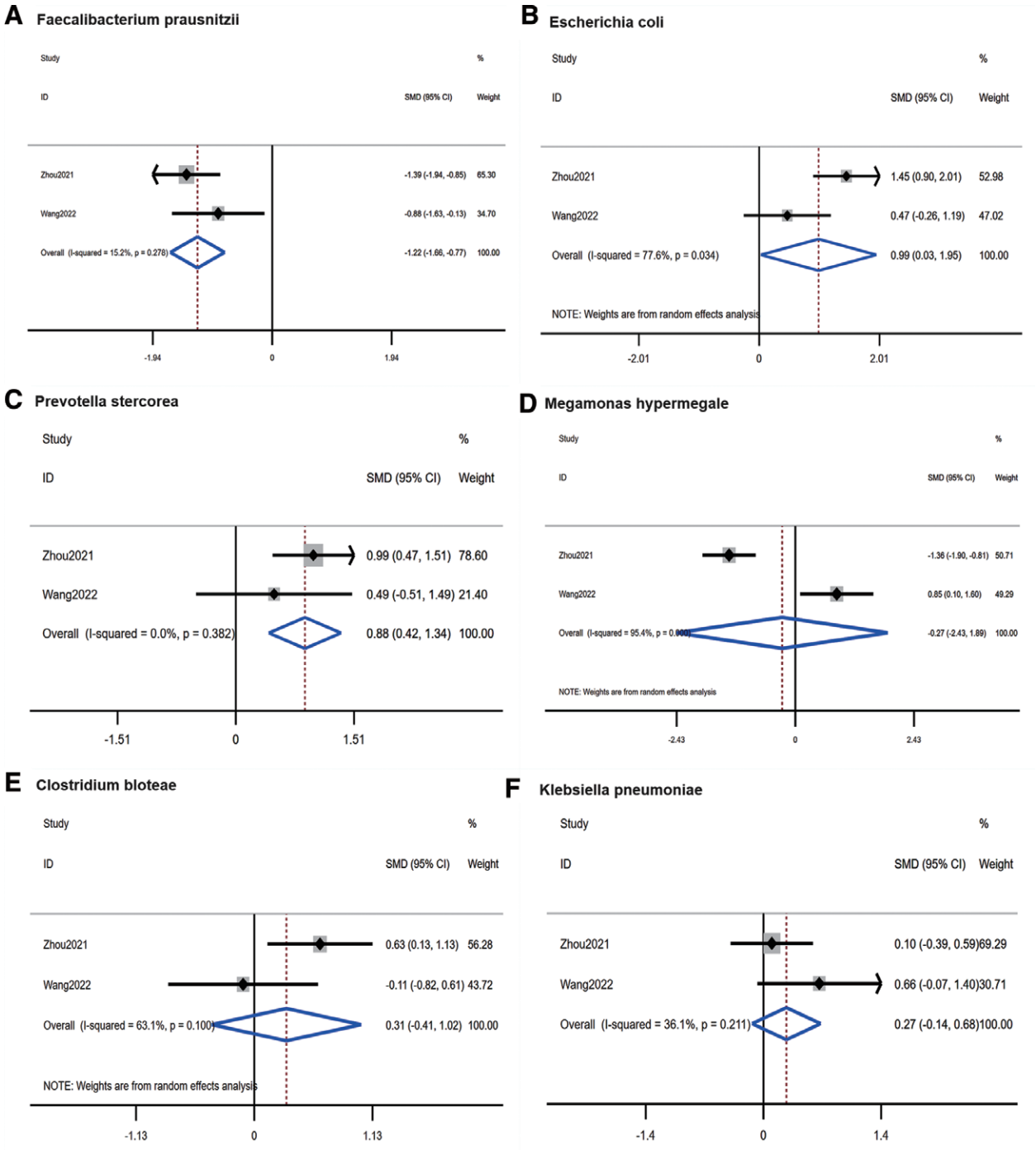

Figure 6. Forest plots for changes in relative abundance of bacterial species include *Faecalibacterium prausnitzii* (A), *Escherichia coli* (B), *Prevotella stercorea* (C), *Megamonas hypermegale* (D), *Clostridium bolteae* (E), *Klebsiella pneumoniae* (F). CI = confidence interval, SMD = standardized mean difference.

bacteria that can produce anticancer properties, such as butyrate salts.[35–37] The significant decrease in these genera may lead to an imbalance in the gut microbiota, favoring the occurrence and development of pancreatic cancer. Therefore, the alterations of *Faecalibacterium* and *Eubacterium* have the potential to become targets for the prevention and treatment of pancreatic cancer.

In comparison to the healthy control group, the present study observed a notable reduction in the prevalence of *F prausnitzii*, alongside a considerable elevation in the levels of *E coli* and *P stercorea* at the species level among individuals diagnosed with pancreatic cancer. *F prausnitzii* belongs to the genus *Faecalibacterium* of the phylum *Firmicutes*, which has been observed to be significantly decreased in pancreatic cancer patients, as previously mentioned. Therefore, the significant decrease in *F prausnitzii* in the gut of pancreatic cancer patients is consistent with the changes at the phylum and genus levels. *E coli* belongs to the phylum *Proteobacteria*, and *P stercorea* belongs to the phylum *Bacteroidetes*. The observed increase in these bacteria is also consistent with the increased abundance of the phyla *Bacteroidetes* and *Proteobacteria* in pancreatic cancer patients. Guerra et al[38] found that enhanced virulence of *E coli* in the gut of pancreatic cancer patients may induce deoxyribonucleic acid damage in pancreatic cells, leading to an

increased risk of genetic alterations and malignant transformation in infected cells. The overgrowth of *P stercorea* may promote the occurrence of pancreatic cancer through the induction of immune suppression.[39]

The present meta-analysis conducted a quantitative assessment of alterations in gut microbiota diversity and composition among individuals diagnosed with pancreatic cancer in comparison to a control group of healthy individuals. This investigation holds significant clinical implications. This study represents the initial attempt to quantitatively evaluate the dynamic alterations in gut microbiota composition among individuals diagnosed with pancreatic cancer. Moreover, it aims to identify distinct microorganisms at various taxonomic levels, including phylum, genus, and species. By doing so, this research endeavor offers valuable insights into the underlying mechanisms of pancreatic cancer development and opens up avenues for investigating potential microbial-based therapeutic interventions. Additionally, the detection of distinct microbial alterations in individuals with pancreatic cancer can contribute to the diagnostic process of pancreatic cancer. Nevertheless, it is important to acknowledge the limitations of our study.

Limitations mainly include the following aspects: excluding certain studies from quantitative analysis due to insufficient data may potentially introduce bias to our research findings; the field of pancreatic cancer microbiome analysis is in its nascent stage, resulting in a scarcity of available studies on the topic, with overall limited research quality; the majority of included studies (92%) used matched healthy controls, but a few studies (8.57%) used benign pancreatic diseases as controls. However, considering that Begg test and sensitivity analysis did not identify significant biases and did not impact the overall studies, those studies using benign pancreatic diseases as controls were not excluded. Among the included studies, there were 7 Chinese studies, accounting for 44% of the total population. We conducted subgroup analysis based on ethnicity and found a significant lower Shannon index in the Caucasian population but no significant difference in the Simpson index. This suggests that there may be certain differences in gut microbiota composition among different racial populations. With more future relevant research, it is necessary to update these findings at an appropriate time in the future. As mentioned by other researchers, meta-analysis of observational studies is different from meta-analysis of randomized controlled trials, and heterogeneity is a well-known and unavoidable issue. Differences in participant sample sizes, races, seasons, diets, ages, genders, physical exercise, and other factors can all have an impact. Even with the use of 16S-ribosomal ribonucleic acid gene sequencing, inconsistencies can arise due to factors such as sample collection techniques, deoxyribonucleic acid extraction methods, primer sets, sequencing platforms, and sequencing depths. However, in many cases, only observational study data are available. According to the opinions of the Meta-Analysis of Observational Studies in Epidemiology group, despite these challenges, meta-analysis of observational studies remains an effective method of assessment. It can provide important supplementary information in situations where ideal conditions cannot be realized.

5. Conclusion

Our meta-analysis revealed disparities in the diversity and composition of the gut microbiota between pancreatic cancer patients and healthy individuals. In pancreatic cancer, there is a characteristic dysbiosis of the gastrointestinal microbiota characterized by a significant decrease in α-diversity and alterations in specific microbial taxa. However, further research is required to determine the function of these microbial imbalances in the occurrence and progression of pancreatic cancer.

Author contributions

Conceptualization: Dachuan Jin.
Writing – original draft: Dachuan Jin.
Data curation: Shunqin Jin, Tao Zhou, Zhongfeng Cui.
Investigation: Shunqin Jin, Zhongfeng Cui.
Resources: Shunqin Jin, Zhongfeng Cui.
Formal analysis: Tao Zhou.
Methodology: Baoqiang Guo.
Software: Baoqiang Guo.
Supervision: Guangming Li, Chunming Zhang.
Validation: Guangming Li, Chunming Zhang.
Writing – review & editing: Chunming Zhang.

References

[1] Cani PD. Human gut microbiome: hopes, threats and promises. Gut. 2018;67:1716–25.
[2] Ohkusa T, Nishikawa Y, Sato N. Gastrointestinal disorders and intestinal bacteria: advances in research and applications in therapy. Front Med. 2022;9:935676.
[3] Sethi V, Kurtom S, Tarique M, et al. Abstract 5127: Eradication of the gut microbiota reduces cancer burden in multiple models by modulating the immune system. Cancer Res (Chicago, Ill). 2018;78(13 Supplement):5127.
[4] Kartal E, Schmidt TSB, Molina-Montes E, et al. MAGIC Study Investigators. A faecal microbiota signature with high specificity for pancreatic cancer. Gut. 2022;71:1359–72.
[5] Memba R, Duggan SN, Ni Chonchubhair HM, et al. The potential role of gut microbiota in pancreatic disease: a systematic review. Pancreatology. 2017;17:867–74.
[6] Hessmann E, Schneider G; 1(st) Virtual Göttingen-Munich-Marburg Pancreatic Cancer Meeting. New insights into pancreatic cancer: notes from a virtual meeting. Gastroenterology. 2021;161:785–91.
[7] Paiji C, Lennon AM, Afghani E. Pancreatic cancer screening: target populations, methods, and protocols for clinical practice. In: Dominguez-Munoz E, ed. Clinical pancreatology for practising gastroenterologists and surgeons. 2nd ed. Chichester, UK: John Wiley & Sons Ltd; 2021:157–65..
[8] Hashimoto S, Tochio T, Funasaka K, et al. Changes in intestinal bacteria and imbalances of metabolites induced in the intestines of pancreatic ductal adenocarcinoma patients in a Japanese population: a preliminary result. Scand J Gastroenterol. 2023;58:193–8.
[9] Half E, Keren N, Reshef L, et al. Fecal microbiome signatures of pancreatic cancer patients. Sci Rep. 2019;9:16801.
[10] Xie G, Zhang Y, Guo X, et al. Study on diversity of gut microbiome in pancreatic cancer patients with damp-heat syndrome based on high-throughput sequencing technology. Shanghai J Tradit Chin Med. 2019;53:9–16.
[11] Tempero MA, Malafa MP, Al-Hawary M, et al. Pancreatic adenocarcinoma, version 22021, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2021;19:439–57.
[12] Cook DA, Reed DA. Appraising the quality of medical education research methods: the Medical Education Research Study Quality Instrument and the Newcastle-Ottawa Scale-Education. Acad Med. 2015;90:1067–76.
[13] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in medicine. 2002;21:1539–58.
[14] Nagata N, Nishijima S, Kojima Y, et al. Metagenomic identification of microbial signatures predicting pancreatic cancer from a multinational study. Gastroenterology. 2022;163:222–38.
[15] Ren ZG, Jiang JW, Xie HY, et al. Gut microbial profile analysis by MiSeq sequencing of pancreatic carcinoma patients in China. Oncotarget. 2017;8:95176–91.
[16] Zhang D, Zhou W, Wang L, et al. Changes in intestinal microbiota in female patients with pancreatic cancer. Acad J Chin PLA Med Sch. 2019;40:1087–93.
[17] Wang XY, Sun ZX, Makale EC, et al. Gut microbial profile in patients with pancreatic cancer. Jundishapur J Microbiol. 2022;15:e122386.
[18] Chen T, Li X, Li G, et al. Alterations of commensal microbiota are associated with pancreatic cancer. Int J Biol Markers. 2023;38:89–98.
[19] Zhou W, Zhang D, Li Z, et al. The fecal microbiota of patients with pancreatic ductal adenocarcinoma and autoimmune pancreatitis characterized by metagenomic sequencing. J Transl Med. 2021;19:215.
[20] Kohi S, Macgregor-Das A, Dbouk M, et al. Alterations in the duodenal fluid microbiome of patients with pancreatic cancer. Clin Gastroenterol Hepatol. 2022;20:e196–227.

[21] Chen Z, Qian X, Zhang A, et al. Correlation between different TCM syndrome types of pancreatic cancer and their prognosis based on structural characteristics of intestinal flora. Zhejiang J Integr Tradit Chin West Med. 2019;29:965–70.
[22] Zhang Q, Yang J, Lou Y, et al. Alteration of gut microbiome and its influence in tumor microenvironment during the initiation and progression of pancreatic cancer. Pancreas. 2019;48:1560.
[23] Schepis T, De Lucia SS, Nista EC, et al. Microbiota in pancreatic diseases: a review of the literature. J Clin Med. 2021;10:5920.
[24] Thomas RM, Jobin C. Microbiota in pancreatic health and disease: the next frontier in microbiome research. Nat Rev Gastroenterol Hepatol. 2020;17:53–64.
[25] Ertz-Archambault N, Keim P, Von Hoff D. Microbiome and pancreatic cancer: a comprehensive topic review of literature. World J Gastroenterol. 2017;23:1899–908.
[26] Pan LL, Li BB, Pan XH, et al. Gut microbiota in pancreatic diseases: possible new therapeutic strategies. Acta Pharmacol Sin. 2021;42:1027–39.
[27] Li QX, Jin M, Liu YH, et al. Gut microbiota: its potential roles in pancreatic cancer. Front Cell Infect Microbiol. 2020;10:572492.
[28] Xie Y, Cao L, Wang X, et al. The composition spectrum of intestinal bacterial diversity in Chinese pancreatic cancer patients: a systematic review. Chin J Evid-Based Med. 2023;23:186–90.
[29] Koliada A, Syzenko G, Moseiko V, et al. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. BMC Microbiol. 2017;17:120.
[30] Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33:496–503.
[31] Rizzatti G, Lopetuso LR, Gibiino G, et al. Proteobacteria: a common factor in human diseases. Biomed Res Int. 2017;2017:9351507.
[32] Litvak Y, Byndloss MX, Tsolis RM, et al. Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction. Curr Opin Microbiol. 2017;39:1–6.
[33] Riquelme E, Zhang Y, Zhang L, et al. Tumor microbiome diversity and composition influence pancreatic cancer outcomes. Cell. 2019;178:795–806.e12.
[34] Johnson EL, Heaver SL, Walters WA, et al. Microbiome and metabolic disease: revisiting the bacterial phylum Bacteroidetes. J Mol Med (Berl). 2017;95:1–8.
[35] Martín R, Miquel S, Benevides L, et al. Functional characterization of novel Faecalibacterium prausnitzii strains isolated from healthy volunteers: a step forward in the use of F prausnitzii as a next-generation probiotic. Front Microbiol. 2017;8:1226.
[36] Wang R, Yang X, Liu J, et al. Gut microbiota regulates acute myeloid leukaemia via alteration of intestinal barrier function mediated by butyrate. Nat Commun. 2022;13:2522.
[37] Mukherjee A, Lordan C, Ross RP, et al. Gut microbes from the phylogenetically diverse genus Eubacterium and their various contributions to gut health. Gut Microbes. 2020;12:1802866.
[38] Guerra L, Guidi R, Frisan T. Do bacterial genotoxins contribute to chronic inflammation, genomic instability and tumor progression? FEBS J. 2011;278:4577–88.
[39] Pushalkar S, Hundeyin M, Daley D, et al. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. Cancer Discov. 2018;8:403–16.