Gut microbiome diversity measures for metabolic conditions: A systematic scoping review

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Abstract

Background Evidence on the association between the gut microbiome and metabolic conditions has been increasing during the past decades. Unlike the straightforward identification of beneficial non-pathogenic bacteria as a potential probiotic for clinical use, the analysis of gut microbiome diversity is more complex. It requires a better understanding of various measures.

Aim To summarize an elaborated list of gut microbiome diversity measures and analyze each measure's benefits and drawbacks.

Design and setting Systematic scoping review.

Method Systematic search was conducted in three databases: PubMed, Embase, and Cochrane Central Register of Clinical Trials for the relationship between gut microbiota and metabolic diseases published in 2019.

Results Of 5929 potential studies, 47 were included in the systematic review (14632 patients). Of the 13 alpha diversity measures, the Shannon index was the most commonly used in 37 studies (78.7%), followed by the Chao1 index (19 studies) and Operational Taxonomic Unit (OTU) richness (15 studies). The advantages of Shannon diversity are simplicity and appropriateness for the community dominant by two or three species. The UniFrac was the most commonly used of the two beta diversity measures in 24 studies (17 Unweighted and 16 Weighted studies), followed by Bray-Curtis dissimilarity (16 studies). There is no predilection for beta diversity.

Conclusion Various measurements of gut microbiome diversity have been used in the literature. They have unique characteristics, advantages, and disadvantages, leading to different usage frequencies. The measures were chosen considering cost, simplicity, and types of research.

J Physiol Biomed Sci. 2022; 33(2): 11-19

Keywords: *Gut microbiome; alpha diversity; beta diversity; metabolic condition; systematic scoping review*

Introduction

Human microbiota are microorganisms that live in several areas of the body, including the oral cavity, genital organs, respiratory tract, skin, and gastrointestinal system.[1] The number of human microbiota, including bacteria, fungi, and viruses, is approximately 10^{13}-10^{14} microbial cells, with the ratio of microbial cells to human cells being 1:1.[1,2] The dominant bacterial phyla in the human gastrointestinal tract are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria.[3] Current research has found associations between microbiota and systemic diseases, particularly type I and type II diabetes, obesity, and metabolic syndrome, which are related with immune response processes.[4]

Nowadays, the identification of dominant microbial communities is increasing with the invention of high-throughput sequencing technology. The most important and widely used diversities are alpha-diversity and beta-diversity.[5] Alpha diversity, including Shannon index diversity, chao1 diversity, etc., is the average species diversity within a habitat type at a local scale.[6] Numerous alpha-diversity indices exist, such as Shannon index diversity and chao1 diversity, each representing a unique aspect of community diversity. The key distinctions include how the indices evaluate variation in rare species, whether they focus only on presence/absence or also on abundance, and how they interpret shared absence.[6] Conversely, beta-diversity, such as Bray-Curtis dissimilarity and Unifrac, indicates the differentiation between microbial communities from different environments.[7] Beta diversity is an essential measure for several widely used statistical techniques in ecology, such as ordination-based methods. It is often used to investigate the relationship between environmental factors and microbial composition.[6] Both diversities consider two aspects of a community: the number of different organisms in a

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Received 20 May 2022 / Revised 24 August 2022 / Accepted 25 November 2022

Available online at *https://li01.tci-thaijo.org/index.php/j-pbs/index* or *www.j-pbs.org*

sample and the range of abundance for each one.[8]

Many researchers have found the relationship between gut microbiota and metabolic diseases by diversity analysis.[9-11] However, no systematic study focused on the most widely used method for diversity measurement of the association between gut microbiota and metabolic diseases. Additionally, there was no systematic review examining the benefits and drawbacks of each measure of gut microbiome diversity. This systematic scoping review aimed to discuss and compare the measurement methods of microbiome diversity that are widely used in current research.

Materials and Methods

Registration of protocols

This study was conducted following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Review (PRISMA-ScR) statement. We registered the systematic review with OSF, The Open Science Framework (registration: osf.io/ux2fs).

Data sources and searches

We used PubMed, Embase, and Cochrane Central Register of Clinical Trials to search for articles published in 2019 in the English language. We excluded articles before 2019 because they would contain many articles to extract. We conducted a systematic review of a single year and hypothesized that the previous year's tool utilization followed a similar pattern. The terms "gastrointestinal microbiome", "gut microbiome", "microbiota", and "microflora" were used in combination with "diversity", "richness", "evenness", and "dissimilarity" as the keywords for literature search along with their synonyms. The search strategy is presented in detail in Supplementary Appendix 1. Additionally, the reference lists of included articles and related citations from other journals via Google Scholar were searched.

Study selection

For this systematic scoping review, we worked with an information specialist to design an appropriate search strategy to identify original peer-reviewed articles of randomized controlled trials, quasi-experimental, and observational studies evaluating gut microbiome diversity in patients with a diagnosis of metabolic disease, including metabolic syndrome, diabetes mellitus, hypertension, dyslipidemia, obesity, and nonalcoholic fatty liver disease (NAFLD). Two independent reviewers (CS and TN) screened the articles for eligible studies. Discrepancies between the two reviewers were resolved by consensus.

Data extraction

Data extraction was done by two independent reviewers (CS and TN) using the published summary gut microbiome diversity index. Discrepancies between the two reviewers were resolved by consensus. We extracted the following data: (1) study characteristics (authors, study type, journal name, contact information, country, and funding), (2) patient characteristics (sample size, type of metabolic disease, and mean age), (3) outcomes (measurement methods of alpha and beta diversity of gut microbiome) as well as any other relevant information. All relevant text, tables, and figures were examined for data extraction. We contacted the authors of the study with incompletely reported data. If the study authors did not respond within 14 days, we analyzed only the available data.

Data synthesis and analysis

The primary outcome was measurement methods of alpha and beta diversity of the gut microbiome. We synthesized the overall usage of the gut microbiome diversity index, whether alpha and beta diversities had been measured in the included studies and which index had been used. We then provided subgroup analyses based on study design, type of metabolic disease, geographical location, and country income.

Patient and public involvement

There were no patient or public involvement in the design and conduct of this systematic scoping review. Nonetheless, the gut diversity measures summarized in this review could be helpful for provider-patient education and treatment decision processes.

Results

Study selection

The database search identified 5,929 potential records. After removing duplicates, 4,111 titles passed the initial screen, and 527 theme-related abstracts were selected for further full-text articles assessed for eligibility (Figure 1). A total of 480 were excluded as the following: 338 were non-peer-reviewed, 65 did not report microbiome diversity, 25 were protocol, 22 had the wrong publication year, 15 were in vitro, 6 were review articles, 4 were letters to the editor, three non-English, and two editorials. Forty-seven studies were eligible for data synthesis.

Study characteristics

Of the 47 included studies, there were 33 observational studies, nine randomized controlled trials, and five quasi-experimental studies (Table 1). The number of patients per study ranged from 12 to 6,627, totaling 14,632 patients. The mean age of patients varied from the day of life 3 to 69 years old (Figure 2). There were 28 studies focused on obesity, 12 on type II diabetes mellitus, four on hypertension, four on NAFLD, three on metabolic syndrome, three on gestational diabetes mellitus, two on dyslipidemia, and a study on type I diabetes mellitus. The characteristics of included studies were shown in Supplementary Table 1.

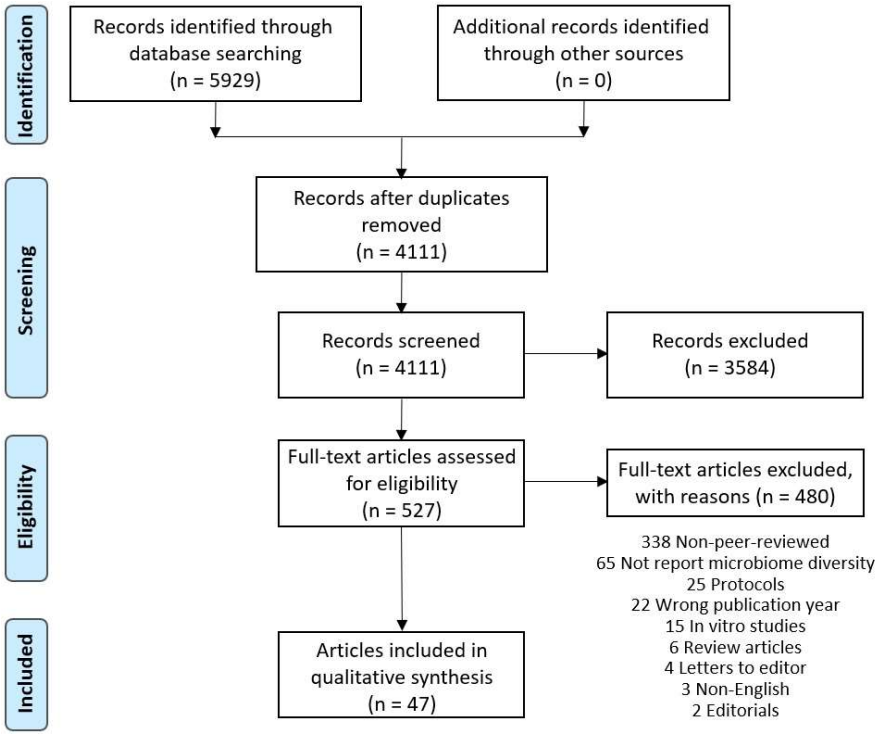

Figure 1 Flow chart diagram presenting the study selection with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.

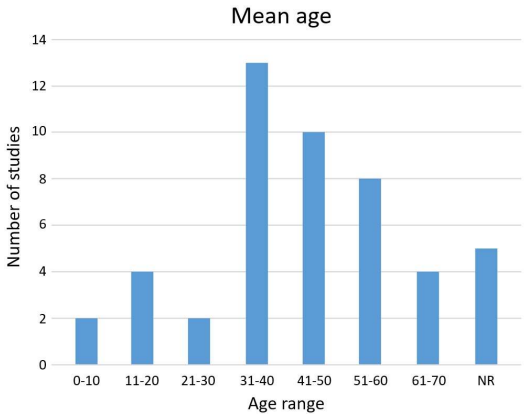

Figure 2 The mean age of patients in the included articles stratified by age range.

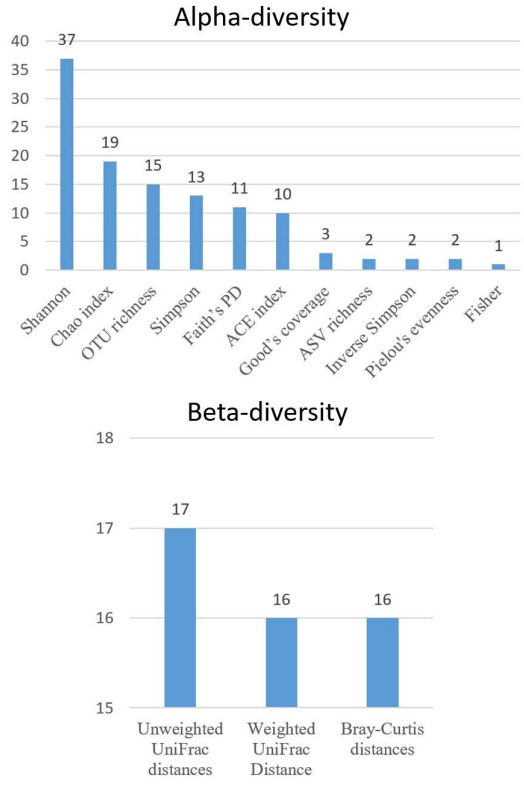

Figure 3 The number of Alpha-diversity and Beta-diversity used in the included articles.

According to the WHO region, there were 21 studies conducted in the Western Pacific Region, 14 in the European Region, 10 in the Regions of the Americas, and two in the Eastern Mediterranean Region. According to the World Bank[12] there were 27 studies conducted in high-income countries, 19 in upper-middle-income countries, and one in low-middle-income countries.

Gut microbiome diversity measures

Of the 13 alpha diversity measures, the Shannon index was the most commonly used in 37 studies (78.7%), followed by the Chao1 index (19 studies), Operational Taxonomic Unit (OTU) richness (15 studies), Simpson

Table 1 Gut microbiome diversity index usage in metabolic disease articles.

	No.of Studies	Alpha-Diversity										Beta-Diversity		
		OUT richness	Shan-non	ACE index	Chao index	Fisher	Pielou's evenness	ASV richness	Faith's PD	Good's coverage	Inverse Simpson	Unweighted Unifrac distances	Weighted Unifrac distances	Bray-Curtis distances
Total	47	15 (31.9)	37 (78.7)	10 (21.3)	19 (40.4)	1 (2.1)	2 (4.3)	2 (4.3)	11 (23.4)	3 (6.4)	2 (4.3)	17 (36.2)	16 (34.0)	16 (34.0)
Study design														
Observational	33	12 (36.4)	28 (84.8)	8 (24.2)	15 (45.5)	1 (3.0)	2 (6.1)	1 (3.0)	7 (21.2)	3 (9.1)	2 (6.1)	15 (45.5)	12 (36.4)	10 (30.1)
Quasi-exp	5	1 (20.0)	2 (40.0)	2 (40.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (60.0)	0 (0.0)	0 (0.0)	1 (20.0)	2 (40.0)	1 (20.0)
RCT	9	2 (22.2)	7 (77.8)	0 (0.0)	3 (33.3)	0 (0.0)	0 (0.0)	1 (11.1)	1 (11.1)	0 (0.0)	0 (0.0)	1 (11.1)	2 (22.2)	5 (55.6)
Metabolic diseases														
DLP	2	0 (0.0)	1 (50.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
GDM	3	0 (0.0)	3 (100.0)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	2 (66.7)
HT	4	2 (50.0)	3 (75.0)	1 (25.0)	2 (50.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (25.0)	2 (50.0)	1 (25.0)
Metabolic syndrome	3	0 (0.0)	2 (66.7)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)
NAFLD	4	1 (25.0)	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (25.0)	0 (0.0)	0 (0.0)	2 (50.0)	1 (25.0)	2 (50.0)
Obesity	28	11 (39.3)	20 (71.4)	6 (21.4)	10 (35.7)	1 (3.6)	1 (3.6)	1 (3.6)	7 25.0)	2 (7.1)	1 (3.6)	10 (35.7)	11 (39.3)	8 (28.6)
T1DM	1	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
T2DM	12	3 (25.0)	10 (83.3)	2 (16.7)	4 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (25.0)	0 (0.0)	0 (0.0)	4 (33.3)	3 (25.0)	5 (41.7)
WHO region														
Americas	10	4 (40.0)	8 (80.0)	0 (0.0)	1 (10.0)	1 (10.0)	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)	1 (10.0)	6 (60.0)	4 (40.0)	2 (20.0)
Eastern Mediterranean	2	2 (100.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	1 (50.0)
European	14	3 (21.4)	9 (64.3)	2 (14.3)	6 (42.9)	0 (0.0)	1 (7.1)	1 (7.1)	4 (28.6)	0 (0.0)	0 (0.0)	3 (21.4)	4 (28.6)	5 (35.7)
Western Pacific	21	6 (28.6)	19 (90.5)	8 (38.1)	12 (57.1)	0 (0.0)	1 (4.8)	1 (4.8)	5 (23.8)	3 (14.3)	1 (4.8)	7 (33.3)	7 (33.3)	8 (38.1)
Country income														
HICs	27	7 (25.9)	19 (70.4)	2 (7.4)	8 (29.6)	1 (3.7)	1 (3.7)	2 (7.4)	7 (25.9)	0 (0.0)	2 (7.4)	8 (29.6)	8 (29.6)	10 (37.0)
LMICs	1	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)
UMICs	19	7 (36.8)	17 (89.5)	10 (21.3)	11 (57.9)	0 (0.0)	1 (5.3)	0 (0.0)	4 (21.1)	3 (15.8)	0 (0.0)	8 (42.1)	7 (36.8)	6 (31.6)

Numbers in parentheses are percentages of total studies in each row. ACE, abundance-based coverage estimators; ASV, amplicon sequence variant; DLP, dyslipidemia; GDM, gestational diabetes mellitus; HICs, high-income countries; HT, hypertension; LMICs, low-middle-income countries; NAFLD, nonalcoholic fatty liver disease; OTU, operational taxonomic unit; PD, phylogenetic diversity; Quasi-exp, quasi-experimental; RCT, randomized controlled trial; T1DM, type I diabetes mellitus; T2DM, type II diabetes mellitus; UMICs, upper-middle-income countries; WHO, World Health Organization.

index (13 studies), Faith's Phylogenetic diversity (11 studies), Abundance-based Coverage Estimators (ACE) index (10 studies), Good's coverage (3 studies), Pielou's evenness index (2 studies), Amplicon Sequence Variant (ASV) richness (2 studies), inversed Simpson index (2 studies), and Fisher alpha index (1 study). The alpha-diversity and beta-diversity of included studies are shown in Figure 3.

Of the two beta diversity measures, the UniFrac was the most commonly used in 24 studies, including Unweighted UniFrac (17 studies) and Weighted UniFrac (16 studies), followed by Bray-Curtis dissimilarity (16 studies). The summary result is shown in Table 1, and the result for each included study was provided in the online Supplementary Appendix 2.

Type of metabolic diseases and gut microbiome diversity measures

The most common microbiome alpha diversity for almost all metabolic diseases was Shannon diversity. The most common microbiome beta-diversity measures for obesity and type II diabetes mellitus were Weighted Unifrac (39.3%) and Bray-Curtis dissimilarity (33.3%), respectively.[13-16] The type of metabolic diseases and gut microbiome diversity measures are shown in Figures 4A and 4B.

Country income and gut microbiome diversity measures

The most common microbiome alpha diversity for all types of country income was Shannon diversity. Amplicon Sequence Variant (ASV) richness (2 studies),[17,18] and inversed Simpson index (2 studies) were both used in only HICs.[19,20] Good's coverage (3 studies) were used only in UMICs.[21-23]

There was a variation of the most common microbiome beta-diversity measures among different country incomes. For HICs, Bray-Curtis dissimilarity was the most common beta diversity measure (37%), Unweighted Unifrac for UMICs (42.1%), and both Weighted and Unweighted Unifrac for LMIC (100%).[24]

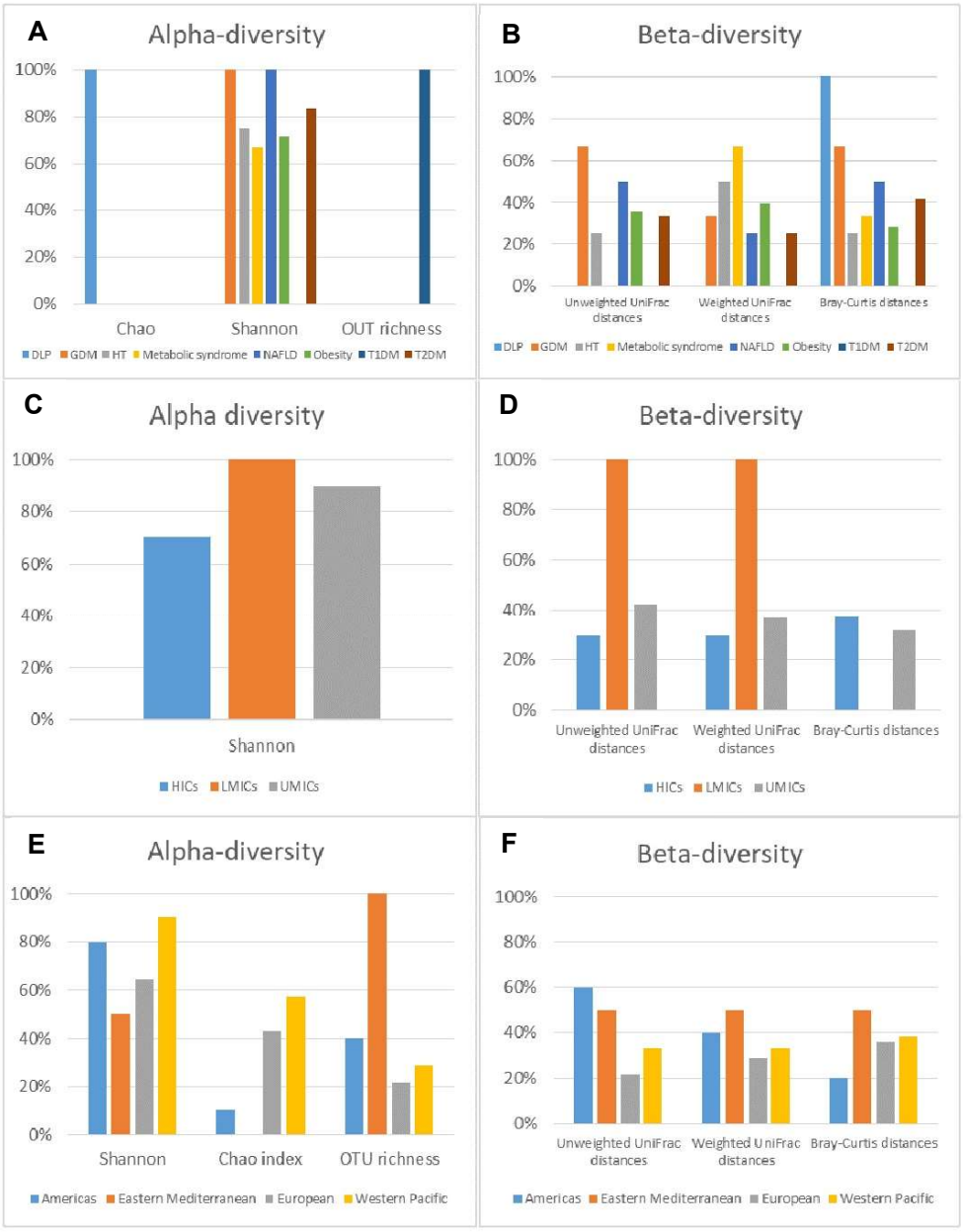

Figure 4 Alpha-diversity and beta-diversity of the included articles categorized by type of metabolic diseases, country income, and geographical location. **A,** Alpha-diversity categorized by metabolic diseases; **B,** beta-diversity categorized by metabolic diseases; **C,** alpha-diversity categorized by country income; **D,** beta-diversity categorized by country income; **E,** alpha-diversity categorized by geographical location; **F,** beta-diversity categorized by geographical location.

Country outcome and gut microbiome diversity measures are shown in Figures 4C and 4D.

Geographical location and gut microbiome diversity measures

The most common microbiome alpha-diversity for studies in Western Pacific, European, and America Regions was Shannon diversity. The most common microbiome alpha-diversity in Eastern Mediterranean Region was OTU richness. Good's coverage (3 studies) was used only in Western Pacific Region.[21-23]

The most common microbiome beta-diversity measures for Western Pacific, European, and America were Bray-Curtis dissimilarity (38.1%), Bray-Curtis dissimilarity (35.7%),[13,15-17,25] and Unweighted Unifrac (60%) respectively.[26-31] For microbiome beta-diversity measures for Eastern Mediterranean, all beta-diversity were equal in number (1 study, 50%). Geographical location and gut microbiome diversity measures are shown in Figures 4E and 4F.

Discussion

This systematic review reports the choice of gut microbiome diversity measurements in patients with metabolic conditions. The systematic review identified 47 articles that met the inclusion and exclusion criteria. A meta-analysis was not performed because this study aimed to determine the usage of gut microbiome diversity measurement in metabolic disease studies. The results suggested variations in measures of gut microbiome diversity in the metabolic disease literature. For alpha diversity, 13 different measurement methods were used to analyze gut microbiota. The Shannon index was the most commonly used, which was presented in 37 studies (78.7%).

In contrast, other methods, including the Chao1 index, Operational Taxonomic Unit (OTU) richness, Simpson index, Faith's Phylogenetic diversity, and Abundance-based Coverage Estimators (ACE) index, were used only 20-40%. For beta-diversity, the UniFrac was the most commonly used assessed in 24 studies (Unweighted 17 studies and Weighted 16 studies), followed by Bray-Curtis dissimilarity (16 studies). All beta-diversity measures were used in a similar quantity. Therefore, our findings have shown that Shannon diversity is the most widely used alpha diversity, while there is no predilection for beta diversity.

The strength of our systematic review is that this is the first systematic review reporting the choice of gut microbiome diversity measurements in patients with metabolic conditions. Moreover, we reported the distinct characteristics, advantages, and disadvantages of each microbiome diversity measurement method, leading to various usage frequencies in metabolic condition studies. However, there were several limitations in this systematic scoping review. First, this systematic scoping review aims to provide evidence on the usage of gut microbiome diversity measurement in metabolic disease studies; thus, meta-analysis was not planned to perform. Second, this study only focused on the gut microbiome diversity measures in patients with metabolic conditions. The generalizability should be considered when applying the results in studies on other diseases.

No study conducted a systematic review reporting the choice of gut microbiome diversity measurements in patients with metabolic conditions. A microbiome diversity index is an essential tool for diagnosing metabolic disorders. Metabolic diseases reached epidemic proportions and burdened world health related to the gut microbiome.[32] More than 500 individual research on the relationship between the human microbiome and metabolic health have been published.[32] However, no study has indicated which microbiome diversity tools should be used to obtain these essential results. Therefore, we conducted numerous literature reviews to conclude these microbiome diversity tools in our systematic review and connect the information with our findings.

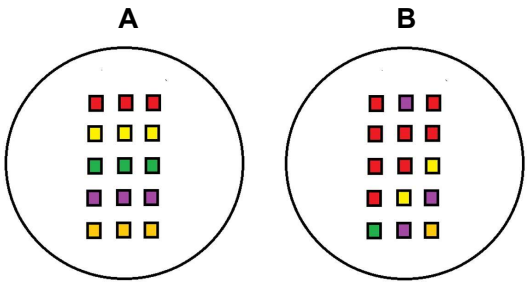

Figure 5 The illustration depicting the definition of species richness and evenness by showing the community "**A**" and community "**B**." Communities were defined as circles. The squares contained within each circle represented species of organism, with each color representing a different species. Community "**A**" and community "**B**" have the ***same species richness***, five species each. The organisms in community "**A**" are more evenly distributed than in community "**B**" (community "**A**" have ***greater evenness*** than community "**B**").

Alpha diversity is used to identify the richness (number of taxonomic groups), evenness (distribution of abundances of the groups), or both. There are three subtypes of alpha diversity for estimation: richness estimators, richness and evenness estimators, and phylogenetic estimators. The difference between these estimators is shown in online Supplementary Table 2. Species richness refers to the number of species present in a community, while evenness compares the uniformity of the species' population (Figure 5).

Shannon diversity was the most common alpha diversity measurement of all included studies. Shannon diversity is an example of the richness and evenness estimators in which diversity's value increases both when the number of species increases and when evenness grows. The concept behind this measure is that the more species seen, the more evenly distributed their abundances are.[33] It is a measurement of entropy and the uncertainty of the sampling outcome. Shannon diversity also contemplates the relative abundances of different species.[34] The advantages of Shannon diversity are simplicity and appropriateness for the community dominant by two or three species. However, Shannon diversity weighs more on species richness which causes measures of the character of the species abundance distribution (evenness) to be less sensitive.

Another richness and evenness estimator is Simpson's diversity. The advantage of Simpson's diversity is simplicity, while the drawback is the insensitivity of the species richness measurement. Due to simplicity and ability to measure both richness and evenness, Shannon's and Simpson's diversity are widely used in all studies.[35]

Another type of estimator is the richness estimator, which includes OTU richness, Chao1 index, and ACE index. OTU richness is defined as the count of different species represented in a

community. Similar to Shannon and Simpson's diversity, the advantage of OTU richness is simplicity which makes OTU richness in the top three of alpha diversity measurement usage. However, the disadvantage of OTU richness is the sensitivity to sample size. In our study, the Chao1 index was used in 19 studies (40.4%) and the ACE index in 10 studies (21.3%). Richness estimators evaluate the total richness of a community.[36] Chao1 and ACE have been developed to estimate richness from abundance data. They are indicators of species richness that are sensitive to rare OTUs. Chao1 is based on the theory that rare species provide the most information about the number of missing species, which is helpful for rare species and performs accurately if the sample size is reasonably large. Therefore, Chao1 index is handy for low-abundance species, while underestimating rich and highly heterogeneous species is the drawback.

Finally, phylogenetic diversity (PD) is defined as connecting all organisms in a phylogenetic tree, which estimates diversity across a tree and provides a phylogenetic analog of taxonomic diversity.[37] PD provides a convenient, evolutionary measure of diversity that does not depend on the ability to identify species count, which ultimately leads to a complex and relatively stable community of microorganisms.[38] PD was a more accurate predictor of ecosystem function than species richness or the number of functional groups, and it supported functional diversity in explaining species richness ecosystem function connections. Suppose the intricacies are taken into account in future studies. In that case, the impacts of Phylogenetic diversity have the potential to conceptually unite the disparate domains of community ecology, evolutionary ecology, conservation biology, and ecosystem ecology.[39]

Beta-diversity in our research, including Unifrac distance and Bray-Curtis dissimilarity, are similar in usage quantities. "UniFrac" considers the phylo-genetic relationships between the microbes found in two samples (Similar to phylogenetic diversity), which provides a convenient measure of diversity that does not depend on the ability to identify species count.[40] It estimates differences between samples or groups based on phylogenetic distance. Unifrac distance is divided into Unweighted and Weighted Unifrac. Unweighted UniFrac is the fraction of branch length between all microbes in both samples that are different between the samples.[40,41] Weighted UniFrac is similar to Unweighted UniFrac but takes the abundance of microbes in the samples into account. The abundances of microbes broadly impact weighted UniFrac, while Unweighted UniFrac does not take abundance into account. Unweighted Unifrac and Weighted Unifrac are used to determine whether communities differed significantly. Unweighted Unifrac is sensitive to detecting microbial richness changes in rare species, while Weighted Unifrac can incorporate abundance information and reduce the rare species' contribution.

Bray-Curtis dissimilarity shows the microbes' abundances which are shared between two samples. Bray-Curtis dissimilarity quantifies the dissimilarity between two samples or groups ranging from 0 to 1 which is not a true distance. For example, if both samples have the same number of microbes at the same abundance, their dissimilarity will equal zero. On the other hand, the dissimilarity will equal one if two samples have no shared microbes. The benefit of Bray-Curtis dissimilarity is that it gives more weight to common species. Moreover, Bray-Curtis dissimilarity is simple and does not make assumptions about genetic relationships.[42]

The study of the microbiome in metabolic conditions has become increasingly attractive in recent years and has been conducted worldwide. Additionally, there were emerging tools for assessing the microbiome's diversity in clinical studies. The primary consideration was that we should use the appropriate diversity in the relevant research and a manner consistent with the context of the resources. In our study, we discussed each method's distinct characteristics, advantages, and disadvantages for measuring microbiome diversity. The complexity and efficacy of various tools for microbiome analysis were distinctive. In addition, the cost to analyze varied between these measurements. Further researchers studying metabolic conditions with microbiome diversity measurements will have objective evidence regarding the measurement methods that are most rational in terms of simplicity, cost, and efficacy for their studies.

How this fits in

Alpha- and beta-diversity are the two most diverse measures of gut microbiota diversity, with no consensus on which measurement methods should be used in a metabolic condition study. This is the first systematic scoping review that demonstrated distinct characteristics, advantages, and disadvantages of each microbiome diversity measurement method leading to various usage frequencies in metabolic condition studies. Further, physician-scientists interested in metabolic conditions with microbiome diversity measurement will have impartial evidence on which measurement methods are most rationally appropriate for their studies regarding simplicity, cost, and efficacy.

Acknowledgments

The authors are thankful to the Prince Mahidol Award Youth Program and Professor Nijasri C. Suwanwela for their research and academic assistance, as well as Miss Pim Sermsaksasithorn for providing mental support.

Conflict of Interest

None to declare.

References

1. Kho Z, Lal S. The human gut microbiome - A potential controller of wellness and disease. Front Microbiol. 2018; 9: 1835.
2. Kim BS, Jeon YS, Chun J. Current status and future promise of the human microbiome. Pediatr Gastroenterol Hepatol Nutr. 2013; 16: 71-9.
3. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano G, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms. 2019; 7: 14.
4. Pang SQ, Luo ZT, Wang CC, Hong XP, Zhou J, Chen F, et al. Effects of Dioscorea polystachya 'yam gruel' on the cognitive function of diabetic rats with focal cerebral ischemia-reperfusion injury via the gut-brain axis. J Integr Neurosci. 2020; 19: 273-83.
5. Wagner B, Grunwald G, Zerbe G, Mikulich-Gilbertson S, Robertson C, Zemanick E, et al. On the use of diversity measures in longitudinal sequencing studies of microbial communities. Front Microbiol. 2018; 9: 1037.
6. Palme K. Key terms in microbiome projects 2019 [Available from: https://biomcare.com/info/key-terms-in-microbiome-projects/#:~:text=While%20alpha%20diversity%20is%20a%20measure%20of%20microbiome,exist%20each%20reflecting%20different%20aspects%20of%20community%20heterogeneity].
7. Walters K, Martiny J. Alpha-, beta-, and gamma-diversity of bacteria varies across habitats. PLoS One. 2020; 15: e0233872.
8. Youssef NH, Ashlock-Savage KN, Elshahed MS. Phylogenetic diversities and community structure of members of the extremely halophilic Archaea (order Halobacteriales) in multiple saline sediment habitats. Appl Environ Microbiol. 2012; 78: 1332-44.
9. Shen G, Wu J, Ye B, Qi N. Gut microbiota-derived metabolites in the development of diseases. Can J Infect Dis Med Microbiol. 2021; 2021: 6658674.
10. Shi Q, Dai L, Zhao Q, Zhang X. A review on the effect of gut microbiota on metabolic diseases. Arch Microbiol. 2022; 204: 192.
11. Wu J, Wang K, Wang X, Pang Y, Jiang C. The role of the gut microbiome and its metabolites in metabolic diseases. Protein Cell. 2021; 12: 360-73.
12. World Bank Country and Lending Groups 2021 [Available from: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups].
13. Horvath A, Leber B, Feldbacher N, Tripolt N, Rainer F, Blesl A, et al. Effects of a multispecies synbiotic on glucose metabolism, lipid marker, gut microbiome composition, gut permeability, and quality of life in diabesity: a randomized, double-blind, placebo-controlled pilot study. Eur J Nutr. 2020; 59: 2969-83.
14. Tao S, Li L, Li L, Liu Y, Ren Q, Shi M, et al. Understanding the gut-kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition. Acta Diabetol. 2019; 56: 581-92.
15. Frost F, Storck L, Kacprowski T, Gärtner S, Rühlemann M, Bang C, et al. A structured weight loss program increases gut microbiota phylogenetic diversity and reduces levels of Collinsella in obese type 2 diabetics: A pilot study. PLoS One. 2019; 14: e0219489.
16. van Bommel E, Herrema H, Davids M, Kramer M, Nieuwdorp M, van Raalte D. Effects of 12-week treatment with dapagliflozin and gliclazide on faecal microbiome: Results of a double-blind randomized trial in patients with type 2 diabetes. Diabetes Metab. 2020; 46: 164-8.
17. Kern T, Blond M, Hansen T, Rosenkilde M, Quist J, Gram A, et al. Structured exercise alters the gut microbiota in humans with overweight and obesity-A randomized controlled trial. Int J Obes (Lond). 2020; 44: 125-35.
18. Kim H, Joo E, Cheong H, Kim Y, Kim H, Shin H, et al. Gut microbiota and risk of persistent nonalcoholic fatty liver diseases. J Clin Med. 2019; 8: 1089.
19. Stanislawski M, Dabelea D, Lange L, Wagner B, Lozupone C. Gut microbiota phenotypes of obesity. NPJ Biofilms Microbiomes. 2019; 5: 18.
20. Ponzo V, Ferrocino I, Zarovska A, Amenta M, Leone F, Monzeglio C, et al. The microbiota composition of the offspring of patients with gestational diabetes mellitus (GDM). PLoS One. 2019; 14: e0226545.
21. Lv Y, Qin X, Jia H, Chen S, Sun W, Wang X. The association between gut microbiota composition and BMI in Chinese male college students, as analysed by next-generation sequencing. Br J Nutr. 2019; 122: 986-95.
22. Mushtaq N, Hussain S, Zhang S, Yuan L, Li H, Ullah S, et al. Molecular characterization of alterations in the intestinal microbiota of patients with grade 3 hypertension. Int J Mol Med. 2019; 44: 513-22.
23. Wang F, Bai R, Yan W, Yan M, Dong L, Song M. Differential composition of gut microbiota among healthy volunteers, morbidly obese patients and post-bariatric surgery patients. Exp Ther Med. 2019; 17: 2268-78.
24. Salah M, Azab M, Ramadan A, Hanora A. New insights on obesity and diabetes from gut microbiome alterations in egyptian adults. Omics. 2019; 23: 477-85.
25. Conterno L, Martinelli F, Tamburini M, Fava F, Mancini A, Sordo M, et al. Measuring the impact of olive pomace enriched biscuits on the gut microbiota and its metabolic activity in mildly hypercholesterolaemic subjects. Eur J Nutr. 2019; 58: 63-81.
26. de la Cuesta-Zuluaga J, Mueller N, Álvarez-Quintero R, Velásquez-Mejía E, Sierra J, Corrales-Agudelo V, et al. Higher fecal short-chain fatty acid levels are associated with gut microbiome dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. Nutrients. 2018; 11: 51.
27. Medina-Vera I, Sanchez-Tapia M, Noriega-López L, Granados-Portillo O, Guevara-Cruz M, Flores-

López A, et al. A dietary intervention with functional foods reduces metabolic endotoxaemia and attenuates biochemical abnormalities by modifying faecal microbiota in people with type 2 diabetes. Diabetes Metab. 2019; 45: 122-31.
28. Shen N, Caixàs A, Ahlers M, Patel K, Gao Z, Dutia R, et al. Longitudinal changes of microbiome composition and microbial metabolomics after surgical weight loss in individuals with obesity. Surg Obes Relat Dis. 2019; 15: 1367-73.
29. Maskarinec G, Hullar M, Monroe K, Shepherd J, Hunt J, Randolph T, et al. Fecal microbial diversity and structure are associated with diet quality in the multiethnic cohort adiposity phenotype study. J Nutr. 2019; 149: 1575-84.
30. Faucher M, Greathouse K, Hastings-Tolsma M, Padgett R, Sakovich K, Choudhury A, et al. Exploration of the vaginal and gut microbiome in african american women by body mass index, class of obesity, and gestational weight gain: A pilot study. Am J Perinatol. 2020; 37: 1160-72.
31. Fei N, Bernabé B, Lie L, Baghdan D, Bedu-Addo K, Plange-Rhule J, et al. The human microbiota is associated with cardiometabolic risk across the epidemiologic transition. PLoS One. 2019; 14: e0215262.
32. Michels N, Zouiouich S, Vanderbauwhede B, Vanacker J, Indave Ruiz B, Huybrechts I. Human microbiome and metabolic health: An overview of systematic reviews. Obes Rev. 2022; 23: e13409.
33. Lynch D. Alpha Diversity: An overview of common alpha diversity metrics for assessing within-sample community diversity. 2021 [Available from: https://docs.onecodex.com/en/articles/4136553-alpha-diversity].
34. Lemos L, Fulthorpe R, Triplett E, Roesch L. Rethinking microbial diversity analysis in the high throughput sequencing era. J Microbiol Methods. 2011; 86: 42-51.
35. Kim B, Shin J, Guevarra R, Lee J, Kim D, Seol K, et al. Deciphering Diversity Indices for a Better Understanding of Microbial Communities. J Microbiol Biotechnol. 2017; 27: 2089-93.
36. Shen T-J, Chao A, Lin C-F. Predicting the number of new species in further taxonomic sampling. Ecol. 2003; 84: 798-804.
37. Faith DP. Conservation evaluation and phylogenetic diversity. Biol Conserv. 1992; 61: 1-10.
38. Garson J, Plutynski A, Sarkar S, eds. The Routledge Handbook of Philosophy of Biodiversity. London: Taylor & Francis; 2016.
39. Srivastava D, Cadotte M, MacDonald A, Marushia R, Mirotchnick N. Phylogenetic diversity and the functioning of ecosystems. Ecol Lett. 2012; 15: 637-48.
40. Lozupone C, Hamady M, Knight R. UniFrac -- an online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics. 2006; 7: 371.
41. Chen J, Bittinger K, Charlson E, Hoffmann C, Lewis J, Wu G, et al. Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatics. 2012; 28: 2106-13.
42. Morgan X, Huttenhower C. Chapter 12: Human microbiome analysis. PLoS Comput Biol. 2012; 8: e1002808.