APPENDIX A

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REVIEW
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Gut Microbiota Composition in Prediabetes and Newly Diagnosed Type 2 Diabetes: A Systematic Review of Observational Studies

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Evidence of gut microbiota involvement in regulating glucose metabolism and type 2 diabetes mellitus (T2DM) progression is accumulating. The understanding of microbial dysbiosis and specific alterations of gut microbiota composition that occur during the early stages of glucose intolerance, unperturbed by anti-diabetic medications, is especially essential. Hence, this systematic review was conducted to summarise the existing evidence related to microbiota composition and diversity in individuals with prediabetes (preDM) and individuals newly diagnosed with T2DM (newDM) in comparison to individuals with normal glucose tolerance (nonDM). A systematic search of the PubMed, MEDLINE and CINAHL databases were conducted from inception to February 2021 supplemented with manual searches of the list of references. The primary keywords of "type 2 diabetes", "prediabetes", "newly-diagnosed" and "gut microbiota" were used. Observational studies that conducted analysis of the gut microbiota of respondents with preDM and newDM were included. The quality of thestudies was assessed using the modified Newcastle-Ottawa scale by independent reviewers. A total of 18 studies (5,489 participants) were included. Low gut microbialdiversity was generally observed in preDM and newDM when compared to nonDM. Differences in gut microbiota composition between the disease groups and nonDM were inconsistent across the included studies. Four out of the 18 studies found increased abundance of phylum *Firmicutes* along with decreased abundance of *Bacteroidetes* in newDM. At the genus/species levels, decreased abundance of *Faecalibacterium prausnitzii*, *Roseburia*, *Dialister*, *Flavonifractor*, *Alistipes*, *Haemophilus* and *Akkermansia muciniphila* and increased abundance of *Lactobacillus*, *Streptococcus*, *Escherichia*, *Veillonella* and *Collinsella* were observed in the disease groups in at least two studies.

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APPENDIX A

Lactobacillus was also found to positively correlate with fasting plasma glucose (FPG), HbA1c and/or homeostatic assessment of insulin resistance (HOMA-IR) in four studies. This renders a need for further investigations on the species/strain-specific role of endogenously present *Lactobacillus* in glucose regulation mechanism and T2DM disease progression. Differences in dietary intake caused significant variation in specific bacterial abundances. More studies are needed to establish more consistent associations, between clinical biomarkers or dietary intake and specific gut bacterial composition in prediabetes and early T2DM.

Keywords: gut microbiota, type 2 diabetes, prediabetes, 16S rRNA sequencing, systematic review

INTRODUCTION

It is expected that by 2030, 578 million people worldwide will have diabetes, with type 2 diabetes mellitus (T2DM) accounting for about 90% of this staggering figure (Saeedi et al., 2019). With an estimated world population of 8,548 million by 2030, this would predict approximately 6% of the world's population having T2DM by 2030 (Worldometers, 2021). In addition, approximately 8% of the world adult population is projected to have prediabetes by 2030, thereby also being at risk of developing full-blown T2DM (International Diabetes Federation (IDF), 2019). T2DM is a non-communicable disease characterized by an elevated blood glucose level or hyperglycaemia, defined by a fasting plasma glucose (FPG) level of ≥ 7.0mmol/l or 2-hour postprandial glucose level of ≥ 11.1mmol/l following oral glucose tolerance test (OGTT) (Alberti and Zimmet, 1998). Intermediate hyperglycaemia or prediabetes state, is defined by an impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), after OGTT (Alberti and Zimmet, 1998). It was estimated that up to 21% of individuals with prediabetes will develop T2DM within three years (Eades et al., 2014).

Individuals with T2DM have an increased risk of developing complications such as kidney failure, retinopathy, neuropathy, cardiovascular disease and limb amputations (Centers for Disease Control Prevention, 2014). Recent evidence suggests a strong association between alterations in the gut microbiota composition and several metabolic disorders, including diabetes (Han and Lin, 2014). Several potential microbial molecular mechanisms have been suggested to contribute towards the onset and progression of T2DM (Gurung et al, 2020). On the other hand, use of metformin was found to be associated with modification ingut microbiota composition that contributed towards thetherapeutic effects as well as known intestinal adverse effects of this most commonly used antidiabetic drug (Forslund et al., 2015). This emphasizes the need to detach observations of gut microbial alterations occurring in disease alone, free from the effects of drugs. As such, these specific changes in gut microbiota composition of prediabetic individuals and/or newly diagnosed diabetic individuals who have not begun pharmacotherapy, may serve as a predictive tool for identifying individuals at high-risk for developing T2DM. This would also enable future studies focusing on the specific microbial species to distinguish their role in disease as either cause or effect or both. This systematic review therefore aims to evaluate and summarise the existing evidence related to microbiota composition and diversity in individuals with prediabetes (preDM) and individuals newly diagnosed with T2DM (newDM) in comparison to individuals with normal glucose tolerance (nonDM). Findings on the association between the gut microbiota composition and clinical ordietary factors are also summarised.

METHODS

A systematic review of observational studies was performed according to a protocol published in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42020160458, 10/7/2020) (Pathmanathan et al., 2020) and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

Search Strategy and Eligibility Criteria

A systematic search of published literature from inception to February 2021 was conducted using electronic databases including PubMed Central by the National Center for Biotechnology Information (PMC-NCBI), MEDLINE Complete and CINAHL (EBSCO Host). Briefly, main keywords included in the search were "type 2 diabetes", "prediabetes", "newly-diagnosed" and "gut microbiota". The complete search strategy is provided in Table 1. Additional eligible studies were identified by hand-searching the reference lists of included studies. The search was limited to only studies published in English.

Studies that met the following criteria were included: (1) observational studies including case-control, cohort and cross-sectional study design; (2) studies on adult participants with newDM or preDM (3) studies that included a nonDM control group and (4) studies in which faecal samples were collected for gut microbial analysis. We excluded studies in which the participants were receiving treatment or dietary interventionprior to the investigation, studies in which participants provided microbiota samples from other body sites, animal studies and studies in which participants had other types of

APPENDIX A

TABLE 1 | Search terms and search strategy.

Search terms and search strategy		
type II diabetes/ pre-diabetic	1	((("type 2 diabet*") OR "type II diabet*")) OR type 2 diabetes[MeSH Terms]
	2	(((((prediabetes) OR prediabetics) OR prediabetic) OR pre-diabetes) OR pre-diabetics) OR pre-diabetic
	3	(("treatment naive") OR "newly diagnosed") OR "new diagnosis"
	4	("impaired glucose tolerance") OR "impaired fasting glucose"
	5	2 OR 3 OR 4 (((((((("treatment naive") OR "newly diagnosed") OR "new diagnosis")) OR ((((((prediabetes) OR prediabetics) OR prediabetic) OR pre-diabetes) OR pre-diabetics) OR pre-diabetic)))) OR (("impaired glucose tolerance") OR "impaired fasting glucose")
	6	1 AND 5 ((((((("type 2 diabet*") OR "type II diabet*")) OR type 2 diabetes[MeSH Terms])))) AND (((((("treatment naive") OR "newly diagnosed") OR "new diagnosis")) OR ((((((prediabetes) OR prediabetics) OR prediabetic) OR pre-diabetes) OR pre-diabetics) OR pre-diabetic))
gut microbiota composition	7	(((microbiome) OR microbiota) OR microflora) OR "gut bacteria"
	8	6 AND 7 Search ((((((((("treatment naive") OR "newly diagnosed") OR "new diagnosis")) OR ((((((prediabetes) OR prediabetics) OR prediabetic) OR pre-diabetes) OR pre-diabetics) OR pre-diabetic)))) OR (("impaired glucose tolerance") OR "impaired fasting glucose"))) AND (((((microbiome) OR microbiota) OR microflora) OR "gut bacteria"))
	9	Remove duplicates from 8

diabetes besides T2DM. Review articles, unpublished data, and articles in other languages were also excluded.

Selection of Studies and Data Extraction

All search results were exported to a reference manager software (Endnote X9.3.1). Two authors (GL and SGP) selected the articles based on their titles and abstracts. Any disagreements between the reviewers were resolved through discussion with the third reviewer (NA). The full texts of eligible studies were assessed, and studies deemed irrelevant were excluded. A standard form was used to extract the data of included studies (Supplementary Excel 1).

Data recorded were general study characteristics (author name, year of publication, journal name, study design, country, sample size, gender distribution, mean age) and characteristics of methodology used by selected studies (microbiota quantification methods and diversity indices used) and the outcome measured. The primary outcomes were gut microbial abundance and differences between study groups at the phylum, class, order, family and genus taxonomic ranks. Species were grouped according to their respective genus. The secondary outcomes included clinical biomarkers, dietary intake and other parameters measured along with their correlation with the gut microbial composition.

Quality Assessment

The study quality was assessed using the modified Newcastle Ottawa scale (Wells et al., 2014; Bjerre et al., 2017). The scale involves a maximum rating of nine stars divided into three categories: (1) samples selection, (2) comparability (comparison of the baseline parameters) and (3) exposure (defined measure of exposure and response rate between cases and controls). The studies were categorised based on their quality which were very good (score of 9); good (score of 7 to 8); fair (score of 5 to 6); and poor (score less than 5). Two researchers independently assessed the studies (GL and NB) and any discrepancies were resolved by another researcher (NA).

RESULTS

Study Characteristics

Of 3,994 articles identified, 18 studies were included in this systematic review (See Figure 1 for PRISMA flow diagram). The characteristics of the study population are summarised in Table 2. The 18 observational studies had been conducted between 2013 and 2021. They included twelve case-control(Zhang et al., 2013; Lambeth et al., 2015; Bhute et al., 2017; Allin et al., 2018; Chen et al., 2019; Nuli et al., 2019; Zhao et al.,2019; Zhong et al., 2019; Gaike et al., 2020; Ghaemi et al., 2020; Liet al., 2020; Wang et al., 2021), four cross-sectional studies (Diener et al., 2021; Egshatyan et al., 2016; Chávez-Carbajal et al., 2020; Wu et al., 2020) and two cohort studies (Karlsson

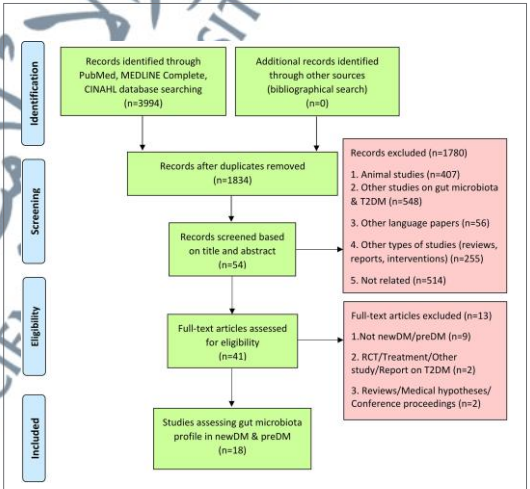

FIGURE 1 | Flow chart of process undertaken to identify eligible studies, according to the PRISMA guidelines.

APPENDIX A

TABLE 2 | Summary of study characteristics.

No.	Study Reference	Type of Study	Country	Sample Size, n	Age group (average)	Ethnicity	No. of Subjects, n (female/male)				
							preDM			newDM	Controls
							IGT	IFG	CGI		
1	(Allin et al., 2018)	Case-control	Denmark	268	55-68	Danish		134 (53/81)			134 (53/81)
2	(Bhute et al., 2017)	Case-control	India	49	40-60	Indian				14	19
3	(Chávez-Carbajal et al., 2020)	Cross-sectional	Mexico	217	40-63	Mexicans		54 (36/18)			76 (50/26)
4	(Chen et al., 2019)	Case-control	Taiwan	100	20-80	N/A				50 (14/36)	50 (22/28)
5	(Diener et al., 2021)	Cross-sectional	Mexico	430	24-66	Mexicans	42 (29/13)	52 (29/23)	57 (39/18)	48 (31/17)	214 (165/49)
6	(Egshatyan et al., 2016)	Cross-sectioal	Russia	97	25-75	Caucasian	25 (18/7)			23 (13/10)	49 (38/11)
7	(Ericson et al., 2020)	Cohort	Sweden	1726	>18	N/A		260 (137/123)			1466 (800/666)
8	(Gaike et al., 2020)	Case-control	India	102	30-60	Indian		17 (11/6)		11 (2/9)	35 (18/17)
9	(Ghaemi et al., 2020)	Case-control	Iran	90	40-60	Iranian	30				30
10	(Karlsson et al., 2013)	Cohort	Sweden	145 (all women)	70	European	49				43
11	(Lambeth et al., 2015)	Case-control	USA	49	55-62	Caucasian white, Hispanics, Native Americans		20 (14/6)			15 (10/5)
12	(Li et al., 2020)	Case-control	China	60	40 -50	N/A				30 (26/4)	30 (26/4)
13	(Nuli et al., 2019)	Case-control	China	60	30-70	Chinese (Uyghur)	20 (8/12)			20 (9/11)	20 (8/12)
14	(Wang et al., 2021)	Case-control	China	126	40-70	Chinese	33 (22/11)				63 (40/23)
15	(Wu et al., 2020)	Cross-sectional	Sweden	1495	50-64	Swedish	DC (178) [98/80] VC (132) [74/58]	DC (189) [98/91]	DC (75)[28/47] VC (88)[39/49]	DC (46) [17/29] VC (58) [27/32]	DC:523 [lrNGT: 226 (127/99); hrNGT: 297: (200/97)] VC:206 (100/106)
16	(Zhang et al., 2013)	Case-control	China	121	50-55	N/A	64			13	44
17	(Zhao et al., 2019)	Case-control	China	100	40-60	Chinese				16 (9/7)	35 (17/18)
18	(Zhong et al., 2019)	Case-control	China	254	49-75	Chinese	80 (39/41)			77 (44/33)	97 (65/32)

DC, discovery cohort; VC, validation cohort; lrNGT, low-risk NGT; hrNGT, high-risk NGT.

et al., 2013; Ericson et al., 2020). Six studies were conducted in Asia [India (Bhute et al., 2017; Gaike et al., 2020), Iran (Ghaemi et al., 2020), Taiwan (Chen et al., 2019) and China (Zhang et al., 2013; Nuli et al., 2019; Zhao et al., 2019; Zhong et al., 2019; Li et al., 2020; Wang et al., 2021)], *fi*ve in Europe [Denmark (Allin et al., 2018), Russia (Egshatyan et al., 2016) and Sweden (Karlsson et al., 2013; Wu et al., 2020; Ericson et al., 2020)] and three in North America [USA (Lambeth et al., 2015) and Mexico (Diener et al., 2021; Chávez-Carbajal et al., 2020)]. These studies had compared the gut microbial pro*fi*les of either preDM alone (Allin et al., 2018; Ericson et al., 2020), newDM alone (Chen et al., 2019; Li et al., 2020), preDM and newDM (Zhang et al., 2013; Egshatyan et al., 2016; Nuli et al., 2019; Zhong et al., 2019; Wu et al., 2020), preDM and knownDM (Karlsson et al., 2013; Lambeth et al., 2015; Ghaemi et al., 2020; Chávez-Carbajal et al., 2020; Wang et al., 2021), newDM and knownDM (Bhute et al., 2017; Zhao et al., 2019) or all three preDM, newDM and knownDM (Diener et al., 2021; Gaike et al., 2020) in comparison to gut microbial pro*fi*le of nonDM. The *fi*ndings on knownDM were not analysed in this review.

The 18 studies consisted of 5,489 participants. In 15 of the 18 studies, 43% of the participants were male (n=2, 252) and 57%

UNIVERSITI SAINS ISLAM MALAYSIA ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

APPENDIX A

were female (n= 2,978). Three studies (Zhang et al., 2013; Bhute et al., 2017; Ghaemi et al., 2020) did not specify the participants' gender. The mean age of participants was 50 ± 7.82 years. There was a total of 3,149 participants in the control or nonDM group. The remaining 2,340 participants with varying glucose levels included 1,599 preDM, 406 newDM and 335 knownDM. PreDM was diagnosed using either IFG (Lambeth et al., 2015; Allin et al., 2018; Gaike et al., 2020; Chávez-Carbajal et al., 2020; Ericson et al., 2020), IGT (Karlsson et al., 2013), combined glucose intolerance (CGI) (Wu et al., 2020), IFG and/or IGT (Diener et al., 2021; Zhang et al., 2013; Egshatyan et al., 2016; Zhong et al., 2019; Ghaemi et al., 2020; Wu et al., 2020; Wang et al., 2021). NewDM was diagnosed using OGTT (Diener et al., 2021; Zhang et al., 2013; Egshatyan et al., 2016; Bhute et al., 2017; Nuli et al., 2019; Zhao et al., 2019; Zhong et al., 2019; Li et al., 2020; Wu et al., 2020), HbA1c (Egshatyan et al., 2016; Zhao et al., 2019; Gaike et al., 2020) and fasting plasma glucose (FPG) (Chen et al., 2019). In 10 out of 11 studies that had participants with newDM, no information was provided regarding period between newDM diagnosis to sample collection while one study reported to only have included participants with newDM who had disease duration of <12 months and a HbA1c range of 6.5 – 9.0% (Egshatyan et al., 2016).

Quality Assessment for Risk of Bias

Table S1 summarises the quality assessment of the studies included. The mean score for the studies included was 8 (range of 6-9) out of a possible total of 9. All studies received either very good or good scores. One study received three out of five maximum scores in the section on study selection as it did not describe sampling strategy or justify the sample size (Chávez-Carbajal et al., 2020), which are criteria to be fulfilled for cross sectional studies, based on the decision rule in the modified Newcastle Ottawa scale used in this review. In the comparability section, five studies (Zhang et al., 2013; Bhute et al., 2017; Chen et al., 2019; Gaike et al., 2020; Ghaemi et al., 2020) stated that cases and controls were matched based on diabetes status alone and did not take into account other confounding factors such as gender or age. Meanwhile, in the exposure section, 11 studies (Zhang et al., 2013; Lambeth et al., 2015; Bhute et al., 2017; Allin et al., 2018; Chen et al., 2019; Nuli et al., 2019; Zhao et al., 2019; Zhong et al., 2019; Gaike et al., 2020; Ghaemi et al., 2020; Li et al., 2020) did not mention the number of study participants who dropped out, if any.

Microbiome Analysis Methods

Table 3 summarises the methodologies adapted by the studies to analyse the microbiome data. These studies used varying DNA kits for DNA extraction and different microbiome sequencing platforms including Illumina Miseq (Diener et al., 2021; Lambeth et al., 2015; Egshatyan et al., 2016; Allin et al., 2018; Nuli et al., 2019) or Hi-Seq (Karlsson et al., 2013; Gaike et al., 2020; Wu et al., 2020; Ericson et al., 2020; Wang et al., 2021) sequencing platforms, the 454 GS FLX Titanium pyro-sequencer (Zhang et al., 2013), PGM sequencing (Ion Touch 2) (Bhute et al., 2017; Chávez-Carbajal et al., 2020), Ion S5 sequencer with Ion Torrent Technology (Zhao et al., 2019), shotgun metagenomics sequencing using the BGISEQ-500 platform (Zhong et al., 2019) as well as quantitative polymerase chain reaction (qPCR)(Chen et al., 2019; Ghaemi et al., 2020). When the 16S rDNA was targeted for sequencing, the hypervariable regions targeted included V4 (Diener et al., 2021; Lambeth et al., 2015; Allin et al., 2018; Gaike et al., 2020), V3-V4 (Egshatyan et al., 2016; Nuli et al., 2019; Zhao et al., 2019), V3 (Bhute et al., 2017; Chávez-Carbajal et al., 2020), V1-V3 (Ericson et al., 2020) or V3-V5 (Zhang et al., 2013) regions, while five studies did not specify the target regions (Karlsson et al., 2013; Zhong et al., 2019; Li et al., 2020; Wu et al., 2020; Wang et al., 2021). The two studies using qPCR to assess the microbiota had used ten and six pairs of specific bacterial 16S rRNA primers, respectively, to target *Atopobium cluster, Bacteroides fragilis, Bifidobacterium, Clostridium coccoides, Clostridium leptum, Clostridium perfringens, Enterobacteriaceae, Enterococcus, Lactobacillus* and *Prevotella* (Chen et al., 2019) and *Akkermansia, Bacteroides, Bifidobacterium, E.coli, Faecalibacterium and Lactobacillus* (Ghaemi et al., 2020).

Following sequencing, the microorganisms were classified and reported as operational taxonomic units (OTUs) (Zhang et al., 2013; Lambeth et al., 2015; Egshatyan et al., 2016; Bhute et al., 2017; Allin et al., 2018; Nuli et al., 2019; Zhao et al., 2019; Gaike et al., 2020; Li et al., 2020; Chávez-Carbajal et al., 2020; Ericson et al., 2020), metagenomic clusters (MGCs) (Karlsson et al., 2013; Wu et al., 2020), microbiota (log10 cell/g) (Chenet al., 2019; Ghaemi et al., 2020), metagenome (Wang et al., 2021), metagenomic linkage group (MLGs) (Zhong et al., 2019) or amplicon sequence variants (ASVs) (Diener et al., 2021).

Diversity in Gut Microbiota

The α-diversity or the average microbial diversity within a single sample was reported by 13 of the 18 studies and the key measures used were Shannon, Simpson, observed species richness, abundance-based coverage estimator (ACE), Chao1 and phylogenetic diversity indexes (Table 3).

Six of the studies found a statistically significant lower α-diversity in the disease groups namely preDM (Allin et al., 2018; Chávez-Carbajal et al., 2020), newDM (Zhang et al., 2013; Bhute et al., 2017; Gaike et al., 2020) and in both preDM and newDM (Nuli et al., 2019) in comparison to nonDM. Two other studies reported a non-significant reduction in α-diversity in preDM (Lambeth et al., 2015) and newDM (Zhao et al., 2019). On the other hand, another two studies, (Zhong et al., 2019) and (Wang et al., 2021) found no significant difference in α-diversity among preDM and newDM in comparison to nonDM. Diener et al. (2021) found that genera *Ruminococcaceae* was the most positively correlated with α-diversity while *Fusobacterium*, *Flavonifractor*, and *Parasutterella* were the most negatively correlated with α-diversity (Diener et al., 2021). Allin et al. (2018) went on to demonstrate that α-diversity in the total group of subjects was negatively correlated with T2DM biomarkers particularly plasma triacylglycerol and high sensitivity C-reactive protein (hsCRP), as well as body mass index (BMI), hip circumference (HC), fasting blood glucose (FBG), fasting plasma insulin (FINS), plasma C-peptide and HOMA-IR (Allin et al., 2018). Ericson et al. (2020) examined the

APPENDIX A

TABLE 3 | Characteristics of the methodology used by the 18 selected articles for gut microbiota composition and diversity assessments.

No.	Study Reference	DNA extraction kit/method	Gut microbiota amplification region and sequencing platform used	Taxonomical classification	Gut microbiota diversity assessment measures	
					a-diversity index	b-diversity index
1	(Allin et al., 2018)	NucleoSpin Soil Mini Kit, Macharey-Nagel	16S rRNA V4 region - Illumina Miseq	OTU	Observed OTUs and Phylogenetic Diversity	Unweighted UniFrac and PCoA
2	(Bhute et al., 2017)	QIAmp DNA Stool Mini Kit, Qiagen	Eubacterial 16S rRNA, Archaeal 16S, Eukaryotic 18S and fungal ITS genes- Ion Torrent PGM	OTU	Observed OTUs and Chao1	Weighted, Unweighted UniFrac and PCoA
3	(Chávez-Carbajal et al., 2020)	MoBio PowerSoil DNA Isolation Kit, Mo Bio Laboratories	16S rRNA V4 region – Ion Torrent PGM	OTU	Observed OTUs, Chao1, Shannon and Simpson	Unweighted UniFrac and PCoA
4	(Chen et al., 2019)	QIAamp Fast DNA Stool Mini Kit, Qiagen	specific bacterial 16S rRNA primers -quantitative polymerase chain reaction (qPCR)	Microbiota (log10 cell/g)	Not stated	Not stated
5	(Diener et al., 2021)	MoBio PowerSoil DNA Isolation Kit, Mo Bio Laboratories	16S rRNA V4 region - Illumina Miseq	ASV	Shannon	Not stated
6	(Egshatyan et al., 2016)	Chemical-based method	16S rRNA V3-V4 region – Illumina Miseq	OTU	Not stated	UniFrac and Multidimensional Scaling (MDS) plot
7	(Ericson et al., 2020)	QIAmp DNA Stool Mini Kit, Qiagen	16S rRNA V1-V3 region – Illumina HiSeq	OTU	Shannon	Not stated
8	(Gaike et al., 2020)	QIAmp DNA Stool Mini Kit, Qiagen	16S rRNA V4 region - Illumina HiSeq	OTU	Observed OTUs and Simpson	Weighted and Unweighted UniFrac
9	(Ghaemi et al., 2020)	QIAmp DNA Stool Mini Kit, Qiagen	specific bacterial 16S rRNA primers -qPCR	Log10 CFU/g stool	Not stated	Not stated
10	(Karlsson et al., 2013)	QIAmp DNA Stool Mini Kit, Qiagen	Illumina HiSeq 2000	Metagenomic Clusters (MGC)	Not stated	Not stated
11	(Lambeth et al., 2015)	QIAmp DNA Stool Mini Kit, Qiagen	16S rRNA V4 region - Illumina MiSeq	OTU	Shannon	Bray-Curtis, Unweighted and Weighted UniFrac
12	(Li et al., 2020	MoBio PowerSoil DNA Isolation Kit, Mo Bio Laboratories	16S rRNA full length - Illumina Nova	OTU	ACE, Chao1, Shannon and Simpson	Not stated
13	(Nuli et al., 2019)	QIAmp DNA Stool Mini Kit, Qiagen	16S rRNA V3-V4 region - Illumina Miseq	OTU	ACE, Chao1, Shannon, Simpson and Sobs	Not stated
14	(Wang et al., 2021)	Sodium dodecyl sulfate (SDS) method	Illumina HiSeq	Metagenome	Shannon	Not stated
15	(Wu et al., 2020)	Repeated bead beating method	Illumina Hiseq	MGC	Not stated	Bray-Curtis and PCoA
16	(Zhang et al., 2013)	Commercial kit, iNtRON Biotechnology	16S rRNA V3-V5 region - 454 GS FLX Titanium pyro-sequencer	OTU	Chao1 and Shannon	Principal component analysis (PCA)
17	(Zhao et al., 2019)	FastDNA Spin Kit, MP Biomedicals	16S rRNA V3-V4 region - Ion S5 sequencer	OTU	Chao1, Shannon and Simpson	Unweighted UniFrac and PCoA
18	(Zhong et al., 2019)	Chemical-based method	Shotgun metagenomic sequencing - BGISEQ-500	Metagenomic Linkage Groups (MLG)	Shannon	Bray-Curtis

correlation between the food patterns and a-diversity in preDM and nonDM, but discovered no statistically significant association (Ericson et al., 2020).

The b-diversity or the measure of how gut microbial composition vary between study groups was reported by 11 studies using principal coordinate analysis (PCoA), principal component analysis (PCA) of either weighted or unweighted UniFrac or Bray Curtis dissimilarities distance matrices (Table 3).

Six studies found a significant difference in b-diversity among preDM (Chávez-Carbajal et al., 2020), newDM (Bhute et al., 2017; Zhao et al., 2019; Li et al., 2020) and in both preDM and newDM (Nuli et al., 2019; Wu et al., 2020) in comparison to nonDM. Four studies found no difference in the b-diversity between disease groups and nonDM groups (Zhang et al., 2013; Allin et al., 2018; Zhong et al., 2019; Wang et al., 2021). Gaike et al. (2020) observed using Principal Coordinate Analysis (PCoA), that bacterial diversity of newDM was distinct from that of nonDM, whereas preDM formed an overlapping cluster with nonDM indicating similarity in bacterial diversity (Gaike et al., 2020).

Gut Microbiota Composition

All eighteen studies provided information on microbial abundance by genus/species ranks. Eleven studies (Zhang et al., 2013; Karlsson et al., 2013; Lambeth et al., 2015; Egshatyan et al., 2016; Bhute et al., 2017; Nuli et al., 2019; Zhao et al., 2019; Zhong et al., 2019; Gaike et al., 2020; Li et al., 2020; Chávez-Carbajal et al., 2020) reported the taxonomic rank of microbial abundance by phylum level. Nine studies (Zhang et al., 2013; Karlsson et al., 2013; Lambeth et al., 2015; Bhute et al., 2017; Allin et al., 2018;

UNIVERSITI SAINS ISLAM MALAYSIA

APPENDIX A

Nuli et al., 2019; Zhao et al., 2019; Li et al., 2020; Chávez-Carbajal et al., 2020) reported by ranks of class, order and family.

Eight studies reported that *Bacteroidetes* and *Firmicutes* were the predominant phyla in all groups studied, i.e., preDM and/or newDM and nonDM (Zhang et al., 2013; Karlsson et al., 2013; Lambeth et al., 2015; Egshatyan et al., 2016; Nuli et al., 2019; Zhao et al., 2019; Zhong et al., 2019; Li et al., 2020). *Proteobacteria* was reported as the next predominant phyla in five out of these eight studies (Karlsson et al., 2013; Lambeth et al., 2015; Nuli et al., 2019; Zhao et al., 2019; Zhong et al., 2019). All eighteen studies reported significant differences in gut microbiota composition by microbial taxa in the disease groups i.e. preDM and/or newDM when compared to the nonDM control group (Table 4 and Table S2). In four studies, a significant increase in the phylum *Firmicutes* along with a significant decrease in phylum *Bacteroidetes* were observed in the newDM group (Bhute et al., 2017; Nuli et al., 2019; Zhao et al., 2019; Gaike et al., 2020). Two of these four studies each found a significant increase (Zhao et al., 2019; Gaike et al., 2020) or significant decrease (Bhute et al., 2017; Nuli et al., 2019) in *Proteobacteria* respectively. Meanwhile, two other studies reported a significant decrease in phylum *Verrucomicrobia* in preDM group (Zhang et al., 2013; Egshatyan et al., 2016). Two studies reported increased *Firmicutes/Bacteroidetes* ratio (*F/B* ratio) among newDM (Zhao et al., 2019; Li et al., 2020) and one study reported increased *F/B* ratio among both preDM and newDM (Gaike et al., 2020).

Figure 2 depicts findings from all 18 studies on the significantly differing genera/species belonging to the six predominant gut bacterial phyla in a heatmap-like format. When focusing exclusively on changes reported by two or more studies, the composition of a particular genera/species demonstrated distinct changes in disease groups (Table 4 and Table S2). The number of *Streptococcus* (Karlsson et al., 2013; Allin et al., 2018; Zhong et al., 2019), *Escherichia* (Diener et al., 2021; Zhong et al., 2019; Ghaemi et al., 2020) and *Veillonella* (Diener et al., 2021; Nuli et al., 2019) in preDM were increased. Similarly, *Lactobacillus* (Bhute et al., 2017; Chen et al., 2019; Gaike et al., 2020) and *Collinsella* (Zhang et al., 2013; Zhong et al., 2019), were increased in newDM. On the other hand, *Faecalibacterium prausnitzii* (Karlsson et al., 2013; Allin et al., 2018; Zhong et al., 2019; Ghaemi et al., 2020; Wu et al., 2020), *Akkermansia* (Zhang et al., 2013; Allin et al., 2018), *Alistipes* (Karlsson et al., 2013; Wu et al., 2020), *Flavonifractor* (Nuli et al., 2019; Wu et al., 2020) and *Roseburia* (Karlsson et al., 2013; Zhong et al., 2019) were decreased in preDM while *Akkermansia* (Zhong et al., 2019; Gaike et al., 2020), *Dialister* (Zhong et al., 2019; Li et al., 2020), *Haemophilus* (Zhang et al., 2013; Zhong et al., 2019), *Roseburia* (Zhang et al., 2013; Zhong et al., 2019) and *Faecalibacterium* (Zhang et al., 2013; Bhute et al., 2017; Wu et al., 2020) were decreased in newDM. *Bacteroides* (Zhang et al., 2013; Karlsson et al., 2013; Allin et al., 2018; Zhao et al., 2019; Ghaemi et al., 2020; Li et al., 2020) and *Prevotella* (Zhang et al., 2013; Egshatyan et al., 2016; Bhute et al., 2017; Zhao et al., 2019) of the phylum *Bacteroidetes*; *Blautia* (Diener et al., 2021; Egshatyan et al., 2016; Allin et al., 2018; Zhao et al., 2019; Gaike et al., 2020), *Eubacterium* (Zhang et al., 2013; Karlsson et al., 2013; Zhao et al., 2019; Ericson et al., 2020), *Clostridium* (Karlsson et al., 2013; Allin et al., 2018; Chen et al., 2019; Zhong et al., 2019; Wu et al., 2020) and *Coprococcus* (Allin et al., 2018; Zhao et al., 2019; Zhong et al., 2019; Wu et al., 2020) of the phylum *Firmicutes* all exhibited changes in both directions.

Correlation of Gut Microbiota Composition With Other Parameters

Fifteen studies assessed clinical indices and dietary habits in their study groups and correlated them with individual gut microbial taxa abundance (Diener et al., 2021; Zhang et al., 2013; Karlsson et al., 2013; Lambeth et al., 2015; Egshatyan et al., 2016; Bhute et al., 2017; Allin et al., 2018; Chen et al., 2019; Nuli et al., 2019; Zhao et al., 2019; Gaike et al., 2020; Li et al., 2020; Chávez-Carbajal et al., 2020; Ericson et al., 2020; Wang et al., 2021). These are depicted in Tables S3, S4 respectively (Supplementary Material).

Nine studies reported that preDM (Zhang et al., 2013; Egshatyan et al., 2016; Allin et al., 2018; Wu et al., 2020; Ericson et al., 2020) and newDM (Zhang et al., 2013; Chen et al., 2019; Zhao et al., 2019; Wu et al., 2020) had significantly increased BMI. Five studies found a positive correlation between the increase of BMI with genera *Blautia*, *Eubacterium*, *Roseburia* (Ericson et al., 2020), *Streptococcus*, *Veillonella*, *Prevotella* (Zhao et al., 2019) or negative correlation with genera *Prevotella* (Nuli et al., 2019) and *Clostridium* (Allin et al., 2018), and specifically *Clostridium coccoides* (Chen et al., 2019). Meanwhile, the increase in FPG was inversely correlated to *Bacteroides uniformis* (Li et al., 2020) and *Prevotella copri* (Bhute et al., 2017), but positively correlated to the genus *Escherichia* (Gaike et al., 2020) and *Coprococcus comes* (Allin et al., 2018). Additionally, it was noted that FPG and/or HOMA-IR were positively associated with the genus *Lactobacillus* (Diener et al., 2021; Karlsson et al., 2013; Chen et al., 2019), *Blautia wexlerae* (Allin et al., 2018), *Clostridium* (Allin et al., 2018) and specifically *Clostridium leptum* and *Clostridium coccoides* (Chen et al., 2019). Similarly, both FPG and HbA1c were observed to either correlate positively (Zhao et al., 2019) or negatively (Gaike et al., 2020) with genus *Akkermansia*, negatively with genus *Clostridium* (Karlsson et al., 2013) and positively with genus *Lactobacillus* (Chen et al., 2019; Gaike et al., 2020) and specifically *Lactobacillus gasseri* (Karlsson et al., 2013). Besides that, it was discovered that inflammatory marker C-reactive protein was positively associated with the genus *Veillonella* (Diener et al., 2021) and negatively associated with the genus *Clostridium* (Allin et al., 2018). Likewise, interleukin-6 (IL-6) inversely correlated with the genus *Blautia* (Diener et al., 2021) whereas adiponectin was positively correlated with genus *Clostridium* (Lambeth et al., 2015).

Regarding dietary intake, five of the 15 studies assessed dietary intake using various measures (Egshatyan et al., 2016; Chen et al., 2019; Nuli et al., 2019; Chávez-Carbajal et al., 2020; Ericson et al., 2020). These included a 24-hour dietary recall (Chávez-Carbajal et al., 2020), a 3-day food record of at least 1 weekend or 1 weekday (Chen et al., 2019), a 4-day web-based food record, developed by the Swedish National Food Institute

UNIVERSITI SAINS ISLAM MALAYSIA ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

APPENDIX A

TABLE 4 | The changes (increase or decrease) noted in gut microbiota of preDM and newDM in comparison to nonDM by microbial taxa and number of reporting studies. All findings are significant ($p < 0.050$).

Taxa level	Increased in ≥ 3 papers		Increased in 2 papers		Increased in 1 paper		Decreased in 1 paper		Decreased in 2 papers		Decreased in ≥ 3 papers	
	preDM	newDM	preDM	newDM	preDM	newDM	preDM	newDM	preDM	newDM	preDM	newDM
Phylum		*Firmicutes* (Bhute et al., 2017; Gaike et al., 2020; Nuli et al., 2019; Zhao et al., 2019)		*Proteobacteria* (Gaike et al., 2020; Zhao et al., 2019)	*Actinobacteria* (Nuli et al., 2019) *Bacteroidetes* (Lamberth et al., 2015) *Firmicutes* (Allin et al., 2018; Nuli et al., 2019) *Saccharibacteria* (Nuli et al., 2019)	*Actinobacteria* (Nuli et al., 2019) *Saccharibacteria* (Nuli et al., 2019)	*Bacteroidetes* (Nuli et al., 2019) *Firmicutes* (Lamberth et al., 2015) *Proteobacteria* (Nuli et al., 2019)	*Verrucomicrobia* (Gaike et al., 2020)	*Verrucomicrobia* (Egshatyan et al., 2016; Zhang et al., 2013)	*Proteobacteria* (Bhute et al., 2017; Nuli et al., 2019)		*Bacteroidetes* (Bhute et al., 2017; Gaike et al., 2020; Nuli et al., 2019; Zhao et al., 2019)
Class					*Betaproteobacteria* (Zhang et al., 2013) *Deferribacteres* (Nuli et al., 2019)	*Betaproteobacteria* (Zhang et al., 2013) *Clostridia* (Zhang et al., 2013) *Deferribacteres* (Nuli et al., 2019)	*Betaproteobacteria* (Chávez-Carbajal et al., 2020)	*Bacteroidia* (Li et al., 2020) *Betaproteobacteria* (Chávez-Carbajal et al., 2020)				
Order					*Clostridiales* (Karlsson et al., 2013)	*Clostridiales* (Zhang et al., 2013) *Selenomonadales* (Li et al., 2020) *Vallonellales* (Li et al., 2020)	*Burkholderiales* (Chávez-Carbajal et al., 2020)	*Acidaminococcales* (Li et al., 2020) *Burkholderiales* (Chávez-Carbajal et al., 2020)		*Bacteroidales* (Li et al., 2020; Nuli et al., 2019)	*Clostridiales* (Allin et al., 2018; Karlsson et al., 2013; Nuli et al., 2019)	
Family				*Lachnospiraceae* (Nuli et al., 2019; Zhang et al., 2013)	*Comamonadaceae* (Chávez-Carbajal et al., 2020) *Pasteurellaceae* (Nuli et al., 2019) *Pseudonocardiaceae* (Lamberth et al., 2015)	*Ruminococcaceae* (Nuli et al., 2019) *Selenomonadaceae* (Li et al., 2020)	*Alcaligenaceae* (Chávez-Carbajal et al., 2020) *Clostridiaceae* (Allin et al., 2018) *Christensenellaceae* (Allin et al., 2018) *Porphyromonadaceae* (Nuli et al., 2019) *Rikenellaceae* (Allin et al., 2018)	*Acidaminococcaceae* (Li et al., 2020) *Alcaligenaceae* (Chávez-Carbajal et al., 2020) *Lachnospiraceae* (Bhute et al., 2017) *Porphyromonadaceae* (Nuli et al., 2019) *Prevotellaceae* (Bhute et al., 2017) *Ruminococcaceae* (Bhute et al., 2017)	*Coriobacteriaceae* (Karlsson et al., 2013; Nuli et al., 2019) *Lachnospiraceae* (Allin et al., 2018; Karlsson et al., 2013) *Ruminococcaceae* (Allin et al., 2018; Nuli et al., 2019)			
Genus/ Species	*Escherichia* (Diener et al., 2021) *Escherichia coli* (Ghaemi et al., 2020; Zhong et al., 2019) *Streptococcus* (Allin et al., 2018) *Streptococcus mutans* (Karlsson	*Lactobacillus* (Bhute et al., 2017; Chen et al., 2019; Gaike et al., 2020) *Lactobacillus ruminis* (Bhute et al., 2017)	*Bacteroides fragilis* (Ghaemi et al., 2020) *Bacteroides uniformis* (Allin et al., 2018) *Blautia* (Allin et al., 2018; Egshatyan et al., 2016) *Blautia wexlerae* (Allin et al., 2018) *Clostridium*	*Blautia* (Egshatyan et al., 2016; Zhao et al., 2019) *Collinsella* (Zhang et al., 2013) *Collinsella intestinalis* (Zhong et al., 2019) *Coprococcus 1* (Zhao et al., 2019) *Coprococcus*	*Chloracidobacteria* (Lamberth et al., 2015) *Coprococcus comes* (Allin et al., 2018) *Dorea* (Allin et al., 2018) *Dorea longicatena* (Allin et al., 2018) *Eggerthella sp* (Zhong et al., 2019) *Faecalibacterium prausnitzii* (Allin et al., 2018)	*Abiotrophia* (Zhang et al., 2013) *Bacteroides caccae* (Zhong et al., 2019) *Bacteroides finegoldii* (Zhong et al., 2019) *Catobacterium* (Nuli et al., 2019) *Clostridium bolteae* (Wu et al., 2020) *Clostridium clostridioforme* (Wu et al., 2020)	*Anaerostipes* (Diener et al., 2021) *Anaerotruncus* (Ericson et al., 2020) *Barnesiella* (Nuli et al., 2019) *Bilophila* (Allin et al., 2018) *Clostridiales bacterium* (Wu et al., 2020) *Dialister invisus* (Zhong et al., 2019) *Dialma* (Nuli et al., 2019)	*Anaerostipes* (Diener et al., 2021) *Alistipes sp* (Wu et al., 2020) *Barnesiella* (Nuli et al., 2019) *Coprococcus sp.* (Zhong et al., 2019) *Dialma* (Nuli et al., 2019) *Megamonas* (Zhang et al., 2013) *Oscillibacter sp.* (Wu et al., 2020)	*Akkermansia muciniphila* (Allin et al., 2018; Zhang et al., 2013) *Alistipes* (Karlsson et al., 2013) *Alistipes obesi* (Karlsson et al., 2013) *Alistipes sp* (Wu et al., 2020) *Bacteroides* (Allin et al., 2018) *Bacteroides*	*Akkermansia* (Gaike et al., 2020) *Akkermansia muciniphila* (Zhong et al., 2019) *Bacteroides* (Zhang et al., 2013; Zhao et al., 2019) *Bacteroides uniformis* (Li et al., 2020) *Bacteroides stercoris* (Li et al., 2020)	*Clostridium* (Allin et al., 2018; Karlsson et al., 2013) *Clostridium botulinum* (Karlsson et al., 2013) *Clostridium baijiranicid* (Karlsson et al., 2013) *Clostridium hathewayi*	

(Continued)

APPENDIX A

TABLE 4 | Continued

Taxa level	Increased in ≥ 3 papers		Increased in 2 papers		Increased in 1 paper		Decreased in 1 paper		Decreased in 2 papers		Decreased in ≥ 3 papers	
	preDM	newDM	preDM	newDM	preDM	newDM	preDM	newDM	preDM	newDM	preDM	newDM
	et al., 2013) *Streptococcus salivarius* (Zhong et al., 2019) *Streptococcus thermophilus* (Allin et al., 2018)		*boltae* (Wu et al., 2020) *Clostridium clostridioforme* (Karlsson et al., 2013; Wu et al., 2020) *Prevotella* (Egshatyan et al., 2016; Zhang et al., 2013) *Veillonella* (Diener et al., 2021; Nuli et al., 2019)	*eutactus* (Zhong et al., 2019) *Eubacterium* (Zhang et al., 2013) *Eubacterium halii* (Zhao et al., 2019) *Prevotella* (Egshatyan et al., 2016; Zhang et al., 2013)	*Haemophilus* (Nuli et al., 2019) *Lachnospira* (Ericson et al., 2020) *Lactobacillus* (Karlsson et al., 2013) *Lactobacillus gasseri* (Karlsson et al., 2013) *Lactobacillus salivarius* (Karlsson et al., 2013) *Megamonas* (Nuli et al., 2019) *Megasphaera elsdenii* (Zhong et al., 2019) *Roseburia* (Ericson et al., 2020) *Ruminococcus* (Allin et al., 2018) *Ruminococcus gnavus* (Allin et al., 2018) *Ruminococcus torques* (Allin et al., 2018) *Serratia* (Egshatyan et al., 2016) *Sutterella* (Allin et al., 2018)	*Dorea* (Zhang et al., 2013) *Escherichia* (Diener et al., 2021) *Faecalibacterium prausnitzii* (Zhong et al., 2019) *Lachnospira* (Nuli et al., 2019) *Megamonas* (Li et al., 2020) *Megamonas funiformis* (Li et al., 2020) *Megapshaera elsdenii* (Zhong et al., 2019) *Mucispirillum* (Nuli et al., 2019) *Peptostreptococcus* (Zhang et al., 2013) *Proteiniphilum* (Nuli et al., 2019) *Ruminococcus* (Zhang et al., 2013) *Serratia* (Egshatyan et al., 2016) *Sporobacter* (Zhang et al., 2013) *Streptococcus* (Bhute et al., 2017) *Subdoligranulum* (Zhang et al., 2013) *Sutterella* (Chávez Carbajal et al., 2020) *Tyzzerella* (Nuli et al., 2019) *Veillonella* (Diener et al., 2021)	*Dorea longicatena* (Karlsson et al., 2013) *Desulfurispirillum indicum* (Karlsson et al., 2013) *Haemophilus parainfluenza* (Zhong et al., 2019) *Intestinimonas butyriciproducens* (Wu et al., 2020) *Klebsiella oxytoca* (Wang et al., 2021) *Lachnospira* (Allin et al., 2018) *Oscillibacter spp.* (Wu et al., 2020) *Pseudoflavonifractor spp.* (Wu et al., 2020) *Pyramidobacter piscolens* (Karlsson et al., 2013) *Ruminococcus* (Nuli et al., 2019) *Ruminclostridium* (Nuli et al., 2019) *Sutterella* (Nuli et al., 2019) *Streptococcus* (Zhang et al., 2013)	*Phascolarctobacterium faecium* (Li et al., 2020) *Pseudoflavonifractor sp.* (Wu et al., 2020) *Ruminococcus* (Gaike et al., 2020) *Ruminoclostridium* (Nuli et al., 2019) *Streptococcus* (Zhang et al., 2013)	*intestinalis* (Karlsson et al., 2013) *Blautia* (Allin et al., 2018; Diener et al., 2021) *Coprococcus* (Allin et al., 2018) *Coprococcus eutactus* (Wu et al., 2020) *Coprococcus sp.* (Zhong et al., 2019) *Eubacterium* (Ericson et al., 2020) *Eubacterium eligens* (Karlsson et al., 2013) *Flavonifractor* (Nuli et al., 2019) *Flavonifractor plautii* (Wu et al., 2020) *Roseburia* (Karlsson et al., 2013) *Roseburia hominis* (Zhong et al., 2019)	*Blautia* (Diener et al., 2021; Gaike et al., 2020) *Clostridium coccoides* (Chen et al., 2019) *Clostridium bartletti* (Zhong et al., 2019) *Clostridium hathewayi* (Zhong et al., 2019) *Clostridium leptum* (Chen et al., 2019) *Clostridium sp* (Wu et al., 2020) *Dialister invisus* (Zhong et al., 2019) *Dialister succinatiphilus* (Li et al., 2020) *Haemophilus* (Zhang et al., 2013) *Haemophilus parainfluenza* (Zhang et al., 2013; Zhong et al., 2019) *Faecalibacterium prausnitzii* (Bhute et al., 2017; Zhang et al., 2013) *Faecalibacterium sp* (Wu et al., 2020) *Roseburia* (Zhang et al., 2013) *Roseburia hominis* (Zhong et al., 2019) *Prevotella* (Zhao et al., 2019) *Prevotella copri* (Bhute et al., 2017)	(Zhong et al., 2019) *Clostridium sp* (Karlsson et al., 2013; Wu et al., 2020) *Clostridium thermocellum* (Karlsson et al., 2013) *Faecalibacterium prausnitzii* (Allin et al., 2018; Ghaemi et al., 2020; Karlsson et al., 2013; Zhong et al., 2019) *Faecalibacterium sp.* (Wu et al., 2020)	

All findings are significant ($p < 0.05$).

UNIVERSITI SAINS ISLAM MALAYSIA
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

APPENDIX A

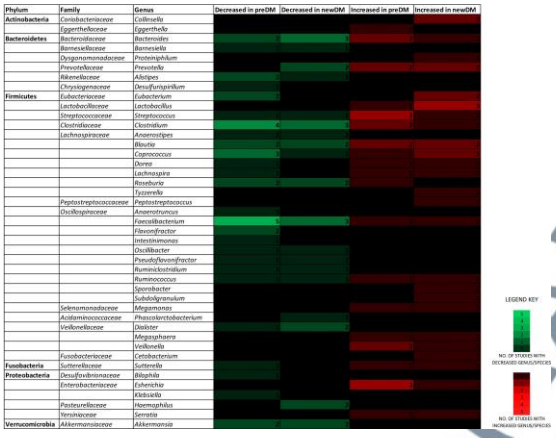

FIGURE 2 | A heatmap-like view depicting the genera/species of the six predominant gut microbial phyla, found to increase or decrease in the preDM and newDM groups, by number of reporting studies.

(Ericson et al., 2020), a quantitative evaluation of food intake based on a computer program 'Analysis of Human Nutrition' (Egshatyan et al., 2016) and a 12-month dietary recall that recorded frequency of 84 food items consumed daily, weekly, monthly, annually or never (Nuli et al., 2019).

Chen et al. (2019) reported significantly lower daily fibre intake and significantly higher fat intake in newDM when compared to the healthy controls. In newDM, they observed a positive correlation between cholesterol intake and *Clostridium coccoides* and *Clostridium leptum* while both carbohydrate and cholesterol intake were positively associated with *Bacteroides fragilis*. Fibre intake was also found to be positively associated with genus *Bifidobacterium*. On the other hand, the study noted negative correlation between carbohydrate and fat intake with family *Enterobacteriaceae* as well as between fat intake and genus *Enterococcus* (Chen et al., 2019).

Meanwhile, Nuli et al. (2019) found that daily intake of cereals, meat, salt and oil were excessive while vegetables, fish, shrimp and dairy products were insufficient in newDM when compared to nonDM. They reported the following positive correlations: energy and protein intake with genus *Prevotella*, carbohydrate intake with genus *Dialister*, cholesterol intake with genus *Megasphaera* and fibre intake with phylum *Spirochaetae* as well as negative correlation between fat intake and phylum *Actinobacteria* in both preDM and newDM (Zhang et al., 2013).

Chávez-Carbajal et al. (2020) noted increased daily energy and macronutrients intake among preDM when compared to nonDM. On the other hand, Egshatyan et al. (2016) noted higher levels of daily energy and carbohydrate consumption in newDM when compared to preDM. They also found a negative correlation between energy and cholesterol intake with *Bifidobacterium*, starch intake with *Blautia* and sugar intake with *Catenibacterium*. Meanwhile, a positive correlation was noted between starch intake with *Bifidobacterium* and carbohydrate intake with *Prevotella*. In this study, the study groups were stratified by dietary intake before analysing the association of gut microbiota with glucose intolerance. Among participants with glucose intolerance, the abundance of *Blautia* was increased even in those consuming lower carbohydrate or fat while high abundance of *Serratia* was increased among participants who consumed equal amounts of energy and carbohydrate. Regardless of energy intake per day, the glucose intolerance group had a significantly decreased abundance of the phylum *Verrucomicrobia* (Egshatyan et al., 2016).

Ericson et al. (2020) observed that a diet consisted of what they defined as a 'health-conscious food pattern' was associated with a lower prevalence of prediabetes. This association, according to their findings, is linked to BMI and gut microbiota, especially a higher abundance of *Roseburia*. They also found that in preDM, fibre intake had a positive association with genera *Roseburia* and *Lachnospira* and a negative association with *Eubacterium* (Karlsson et al., 2013).

DISCUSSION

This systematic review aimed to provide an overview of changes reported in the gut microbiota of subjects with preDM and newDM, unperturbed by pharmacotherapy, when compared to individuals with normal glucose tolerance. The comprehensive search of the databases and references list resulted in inclusion of a majority of high-quality studies that contributed to the review's strength. We discuss herein the consistent differences in the composition of gut microbiota and their correlation with various parameters.

Bacteria in the gut predominantly belong to six phyla i.e., *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*,

UNIVERSITI SAINS ISLAM MALAYSIA ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

APPENDIX A

Fusobacteria, and *Verrucomicrobia*, with the two phyla *Firmicutes* and *Bacteroidetes* accounting for 90% of the total gut microbial composition (Rinninella et al., 2019). The changesin the abundance of specific *Firmicutes and Bacteroidetes* species and the overall increase or decrease in *F/B* ratio are often associated with several diseases. Studies in obesity and known T2DM have found that the F/B ratio increases (Silva et al., 2020),decreases (Levy et al., 2016; d'Hennezel et al., 2017; Parada Venegas et al., 2019) or even remains unchanged (Stojanov et al., 2020; Anhêet al., 2021). In the present review, a significant increase in *Firmicutes* along with a significant decrease in *Bacteroidetes* among newDM were observed (Bhute et al., 2017; Nuli et al., 2019; Zhao et al., 2019; Gaike et al., 2020). However only three (Zhao et al., 2019; Gaike et al., 2020; Li et al., 2020) out of the 18 studies reported on the F/B ratio, even so with contradictory findings. In past studies, an increase in *Firmicutes* or a higher *F/B* ratio has been linked with development of obesity, as *Firmicutes* are more efficient than *Bacteroidetes* in harvesting energy from food, thus contributing to the extra calories (Magne et al., 2020). While obesity is a major risk factor for T2DM, the disease is also characterized by a state of low-grade inflammation that precedes the onset of glucose intolerance. The pro-inflammatory cytokines are said to impair insulin signalling, increase permeability and inflammation in the intestinal epithelium, eventually leading to development of insulin resistance (Tamanai-Shacoori et al., 2017). While the notion remains that this continuous low-grade inflammatorystate is caused by lipopolysachharide (LPS) produced by the Gram-negative gut microbiota (Cani et al., 2007), it is now evident that *Bacteroidetes*, the most abundant group of Gram- negative bacteria in the gut produces distinct subtypes of LPS with immunoinhibitory functions that prevents inflammation (d'Hennezel et al., 2017). Logically, a decrease in either of the two dominant *Firmicutes* or *Bacteroidetes* phyla, could possibly increase the relative abundance of other Gram-negative bacteria such as those belonging to the phylum *Proteobacteria*, hence inducing production of more pro-inflammatory LPS subtypes (Magne et al., 2020; Anhêet al., 2021). An increase in other phyla, however, may not necessarily further affect the F/B ratio (Stojanov et al., 2020). Moreover, the F/B ratio does not consider compositional changes that may be occurring in the larger variety of family, genus and species taxonomic levels of each phylum. The relevance of the *F/B* ratio to serve as a disease marker for metabolic diseases is therefore inconclusive. Another important microbiota-associated factor in health and disease is the production of short-chain fatty acids (SCFAs). SCFAs, namely acetate, propionate and butyrate are metabolic products of fibre fermentation by bacteria in the gut. They are shown to exert many beneficial effects on human metabolism and immune system (Parada Venegas et al., 2019; Silva et al., 2020). *Firmicutes* are the primary producers of butyrate while *Bacteroidetes* mainly produce acetate and propionate (Levy et al., 2016; Parada Venegas et al., 2019). Gut microbiota dysbiosis has been shown to alter SCFA production, thereby affecting the epigenetic regulation of genes modulating insulin resistanceand inflammatory reactions seen in T2DM (Remely et al., 2014). In relation to this, although an increase in phyla *Firmicutes* was noted, several members of this phyla were constantly found to be decreased in the disease groups. They were *Faecalibacterium prausnitzii*, *Roseburia*, *Dialister* and *Flavonifractor*. The role of these organisms as biomarkers of health is well established. Their beneficial effects are mainly attributed to their ability to produce SCFAs, especially butyrate, that play a major role in maintaining intestinal barrier integrity, energy homeostasis, attenuating inflammation and modulating glycaemic response (Tamanai-Shacoori et al., 2017; Martínet al., 2018; Mukherjee et al., 2020). Previous studies also found *F. prausnitzii* (Graessler et al., 2013; Remely et al., 2014; Candela et al., 2016), *Dialister* (Almugadam et al., 2020) and *Roseburia* (Forslund et al., 2015) to be reduced among known T2DM patients on medication. Taken together, these findings indicate that these bacteria are depleted in the gut microbiome prior to the onset of diabetes and may remain so even after treatment is initiated.

Other bacteria noted to be decreased in the disease groups in this review were *Alistipes* of phylum *Bacteroidetes*, *Akkermansia muciniphila*, of phylum *Verrucomicrobia* and *Haemophilus*, of phylum *Proteobacteria*. *Alistipes*, however was found to be increased in abundance among known T2DM patients (Wu et al., 2010; Qin et al., 2012). Indeed this bacterium is known to have protective effects against certain diseases including cardiovascular disease, while also being pathogenic in others due to its inflammatory potential (Parker et al., 2020). Previous studies showed that the mucin-degrading and gut barrier protecting *A. muciniphila* improve glucose tolerance in high fat diet-induced diabetic mice upon metformin treatment (Shin et al., 2014; de la Cuesta-Zuluaga et al., 2017). On the other hand, a decrease in abundance of this bacterium increases gut permeability, a known characteristic in T2DM progression (Cani et al., 2008). The decrease in *Haemophilus* is in agreement with a study investigating the gut microbiota pattern in women with active vs sedentary lifestyle that noted an increase in this genus, along with other health-promoting bacterial species *Faecalibacterium prausnitzii*, *Roseburia hominis* and *Akkermansia muciniphila* in active women (Bressa et al., 2017). This is unexpected given that the genus *Haemophilus* is a mucosal pathogen and its abundance has been associated with varying pathogenicity in infections (Nørskov-Lauritsen, 2014), multiple sclerosis (Chen et al., 2016) and colorectal carcinoma (Liu et al., 2020).

On the other hand, the bacteria found to have increased in abundance among the disease groups included *Firmicutes*: *Streptococcus*, *Veillonella* and *Lactobacillus*, specifically *L. ruminis*, *L. gasseri* and *L.salivarius as well as Escherichi*a (a *Proteobacteria*) and *Collinsella* (an *Actinobacteria*). *Streptococcus*, *Escherichia* and *Collinsella* are all known gut inhabitants whose abundance are associated with several inflammatory diseases including T2DM (Qin et al., 2012; Candela et al., 2016). Increased abundance of *Escherichia coli* is also linked with increased microbial infections in diabetic patients (Wiwanitkit, 2011). All three genera are positively associated with animal-based diet consumption and studies have reported that these bacteria can be successfully reduced

UNIVERSITI SULTAN ZAINAL ABIDIN ISLAMIC SCIENTIFIC UNIVERSITY OF MALAYSIA

APPENDIX A

through fibre-rich and plant-based dietary interventions (Candela et al., 2016; van Soest et al., 2020). Bacteria belonging to genus *Veillonella* are Gram-negative bacteria (unlike most *Firmicutes*) (Megrian et al., 2020), however there is little evidence for their role in health and disease to date. The immunomodulatory and probiotic properties of *Lactobacillus* are well established. However, along with its association seen herein with preDM and newDM, the present review also found noteworthy positive association between the genus *Lactobacillus* and glycaemic markers including FPG, HbA1c and the associated HOMA-IR index. The abundance of *Lactobacillus* has also been linked to chronic inflammation seen in known diabetic subjects (Zeuthen et al., 2006; Larsen et al., 2010; Lê et al., 2013). Although probiotic strains of *Lactobacillus* have been found to have beneficial anti-diabetic effects in mouse models (Yun et al., 2009; Park et al., 2015; Lee et al., 2018), it is likely that the effects of endogenous *Lactobacillus* species towards health and disease is strain dependent and further studies are required to investigate their direct effect on T2DM.

Overall, the increased levels of glycaemic and pro-inflammatory markers along with low diversity of gut bacteria among the preDM and newDM generally observed in this review suggest the possibility of gut bacteria- associated inflammation-induced environment preceding the development of T2DM. Although the studies included in this review found significant association between clinical biomarkers and the abundance of specific bacterial groups, no consistent findings were observed between studies, with the exception of the correlation between glycaemic markers and *Lactobacillus* abundance. Similarly, no consistent findings between studies were noted in correlation between dietary intake and specific gut bacterial composition. However, the studies were able to conclude that a less healthy food pattern including increased carbohydrate, fat or energy intake or a reduced fibre intake correlated with prevalence of preDM or newDM and differences in dietary intake caused significant variation in specific bacterial abundance.

Limitations

One of the limitations of the present review is that it only summarises studies investigating gut microbiota based on composition found in faecal samples. There is a need for more studies to look into microbiota of mucosal biopsies in addition to faeces, in order to differentiate mucosa-associated bacteria from the total composition present in faeces. This is because mucosa-associated bacteria may have a more pertinent role in the pathogenesis of disease. It is also important to emphasise that findings from observational studies selected herein do not conclusively establish whether changes seen in microbiota composition were a cause or effect of glucose intolerance. Hence, bacteria found to be decreased or increased in the disease groups could not be conclusively termed as being 'protective' or 'pro-diabetic', respectively.

Studies involving taxonomic analyses of microbiota have several biases. One of it is that compositional analyses of microbiota requires that the microbial proportions to be summed to a 100% total, thus reporting the relative abundance and not the absolute composition of the microorganisms present in the faecal samples of participants. Thus, if one bacterial group appears to be reduced, the others will naturally appear to be increased and vice versa. Differences in sampling fractions may also tend to introduce false positives and false negatives (Lin and Peddada, 2020).

Another challenge in analysing gut microbiome data across studies is the biases caused by the heterogenous methodology adapted. This limits the comparability of studies and leads to ambiguous results across similar studies. A meta-analysis was also not workable for the same reason. Although all studies targeted the 16S rRNA gene, factors that differed across the studies i.e. the choice of sequencing region, pipelines and databases used for bioinformatics analysis influenced the results produced. Moreover, the usage of different diversityindexes and statistical analysis affects the comparability of microbial diversity between the studies. The choice of the diversity index affects the interpretation of the microbiome data and leads to a lack of generalizable results across the studies. Besides methodological limitations, the use of alpha diversity as a biomarker in health and disease may be confounded by colonic transit time (Roager et al., 2016) and stool consistency (Falony et al., 2016).

More importantly, the taxonomic diversity exhibited by these analyses does not take into account, the functional redundancy among members of the microbiota. Taxonomically distinct bacterial species are able to perform similar metabolic functions and as such, taxonomic variation does not reflect functional variation (Louca et al., 2019). Therefore, profiling of the microbial metabolic function would be of more significance to assess impact of the microbiota on the human host in health and disease.

CONCLUSION

The 18 studies included herein were found to have heterogeneity in methodology and inconsistencies in the findings on gut microbial changes observed among preDM and newDM when compared to nonDM. By focusing on changes that were similarly reported in two or more studies, it was evident that certain bacteria were found to be increased (*Lactobacillus, Streptococcus, Escherichia, Veillonella* and *Collinsella*) and/or decreased(*Faecalibacterium prausnitzii* , *Roseburia* , *Dialister* , *Flavonifractor, Alistipes, Haemophilus* and *Akkermansia muciniphila)* in preDM and newDM. These alterations were however not consistent across all studies included, hence emphasising the uncertainty that lies in this field of study. The increased presence of *Lactobacillus* in preDM and newDM along with its positive correlation with glycaemic markers were also inconsistent observations. This renders a need for more investigation on the species/strain-specific role of this genus in T2DM disease progression and glucose regulation mechanism. Healthier food intake inversely correlated with prevalence of preDM and newDM, while differences in dietary intake caused significant variation in specific bacterial abundances. More studies should investigate the correlation of clinical biomarkers and dietary intake with gut bacterial composition in prediabetes and early T2DM to establish more consistent associations.

UNIVERSITI SAINS ISLAM MALAYSIA
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

APPENDIX A

AUTHOR CONTRIBUTIONS

SP and NA conceived the review protocol. GL and SP carried the systematic searches and extracted the data. GL, NB and NA performed the quality assessment of the selected papers. MM, NB, MO, FM, FA, NM, ZI and SP critically checked the extracted data. GL wrote the first draft of the manuscript. SP reviewed and edited the entire manuscript. MM, NA and BL critically reviewed the final manuscript. All authors contributed to writing and revising the manuscript.

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SUPPLEMENTARY MATERIAL

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APPENDIX A

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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APPENDIX B

PER - PAT 301

KEMENTERIAN KESIHATAN MALAYSIA
PERKHIDMATAN PATOLOGI

UNTUK KEGUNAAN MAKMAL
LAB No.

1. Nama:		2. No. Pendaftaran:	
3. No. K/P:		4. Jantina: ☐ Lelaki ☐ Perempuan	
5. Umur:	6. Keturunan:	7. Wad/Klinik:	
8. Tarikh Masuk Wad:	9. Pekerjaan:	10. Taraf Perkahwinan:	11. ☐ Bayaran ☐ Percuma

12. No. Laporan Dahulu:

14. Ringkasan Klinikal, Penemuan Pembedahan dan Riwayat Keluarga:

15. Diagnosis:

13. Butiran Penting

	Ya	Tidak
Jaundice	☐	☐
Lymphadenopathy	☐	☐
Hepatomegaly	☐	☐
Splenomegaly	☐	☐
Bleeding Tendency	☐	☐
H/O Transfusion	☐	☐

Haematinics .....

.....

.....

Drug/Chemical History

.....

.....

Data Makmal Terdahulu

Hb .....

Platelet .....

TWDC .....

16. Kategori Permohonan/Jenis Ujian:

Patologi Kimia ☐	Klinikal ☐	Hematologi ☐	Histo/Salitologi	Mikro/Immunologi	
B. Sugar ☐	Bld. Count ☐	FBP ☐	Spesimen	Spesimen	Ujian
B. Urea ☐	ESR ☐	BM ASP. ☐			
S. Elec ☐	BFMP ☐	Hb. Analysis ☐			
B. Gases ☐	U. Sugar ☐	Coagulation ☐			
S. Billirubin ☐	U. Alb. ☐	PT ☐			
LFT ☐	U. ME ☐	PTT ☐			
Sc. Creatinine ☐	Stool ME ☐	BTCT ☐			

Lain-lain .....

.....

.....

17. Pengambilan Spesimen: Tarikh: ☐☐☐☐☐☐ Masa: ☐☐☐☐

18. Nama Doktor: .....

19. Tarikh: .....

.....
Tandatangan dan cop Doktor

LAPORAN "Sila Lihat Sebelah"

UNI

APPENDIX C

UNIVERSITI SAINS ISLAM MALAYSIA
جامعة العلوم الإسلامية الماليزية
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

RISALAH MAKLUMAT PESERTA DAN
BORANG PERSETUJUAN atau KEIZINAN PESERTA / *PARTICIPANT INFORMATION SHEET AND INFORMED CONSENT FORM*
*(*untuk subjek dewasa dan penyelidikan intervensi*) / (for adult subjects and interventional studies)*

1. **Tajuk penyelidikan / *Title of study***: Peranan mikrobiota usus mengikut kaum di dalam patogenesis penyakit diabetes mellitus jenis 2 / *Ethnicity-related gut microbiota involvement in the pathogenesis of type 2 diabetes mellitus via alterations in metabolic regulators*

2. **Pengenalan / *Introduction*:**
Anda telah dijemput untuk menyertai penyelidikan ini kerana sama ada anda didapati menghidap penyakit kencing manis (diabetes) atau berada dalam fasa pra-diabetik atau disahkan tidak menghidapi diabetes. Penyelidikan ini telah mendapat kelulusan Jawatankuasa Etika dan Penyelidikan Perubatan, Kementerian Kesihatan Malaysia.
You are invited to participate in a research study because you have been either newly diagnosed for having diabetes, are in the pre-diabetic phase or confirmed to be non-diabetic. The details of the study are described in this document. This study has been approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia.

3. **Apakah tujuan penyelidikan ini dilakukan? / *What is the purpose of the study?***
Tujuan penyelidikan ini dilakukan adalah bagi menentukan komposisi microbiota usus dan pengambilan makanan individu pra-diabetik dan diabetik berbanding dengan individu yang tidak diabetik. Penyelidikan ini diperlukan kerana strategi terapi yang sedia ada bagi merawat atau mengelak diabetes adalah tidak mencukupi. Kajian antarabangsa baru baru ini telah membuktikan bahawa microbiota usus memainkan peranan penting dalam pengawalan kadar gula dan diabetes. Maka ada harapan besar bagi memanipulasi (atau membaikpulih) microbiota usus bagi mengelak atau merawat diabetes. Tetapi data adalah masih tidak mencukupi, lebih lebih lagi di kalangan rakyat Malaysia terutamanya di kalangan 3 kaum utama Malaysia. Sejumlah 135 orang termasuk anda, akan mengambil bahagian di dalam kajian ini. Penyelidikan ini akan berlangsung selama 3 tahun dan sumbangan masa anda lebih kurang 1 jam diperlukan bagi penyelidik menerangkan perjalanan kajian ini. Kemudian penyelidik akan mendapatkan 1 sampel najis dan sampel darah daripada anda.
The purpose of this study is to determine the gut microbial composition and dietary intake of newly diagnosed diabetic patients as compared to non-diabetic individuals. This research is necessary because the currently available strategies for therapy and prevention of diabetes is insufficient. Recent research around the world shows that gut microbiota play an important role in glycaemic control and diabetes. There is a possibility of manipulating gut microbiota to prevent or treat the onset of diabetes. However there is lack of data to demonstrate gut microbiota relatedness to diabetes among the Malaysian population specifically the three major ethnic groups. A total of 135 subjects like you will be participating in this study. The whole study will last about 3 years and your participation will be about one hour for the investigators to explain the experimental details, a one-time collection of faeces and blood sample.

1. **Siapakah yang membiayai penyelidikan ini? / *Who is funding the research?***
Kajian ini ditaja sepenuhnya oleh Kementerian Pendidikan Malaysia yang akan membayar semua peralatan penyelidikan dan prosedur yang berkaitan. Mana-mana prosedur dan rawatan lain yang tidak diperlukan dalam penyelidikan ini tetapi merupakan sebahagian daripada rawatan harian anda, adalah tanggungan anda sendiri ataupun pihak insurans anda. Anda akan menerima sedikit cenderahati dan wang tunai RM20 sebagai perbelanjaan perjalanan untuk penyelidikan ini.
This study is sponsored by the Ministry of Education, Malaysia, who will pay for all study materials and procedures. All other drugs and procedures that are not required by the study but are part of your routine medical care will have to be paid by you or your insurance. You will be given a goody bag and reimbursed RM20 for your travel expenses and time spent for participating in this study.

2. **Adakah maklumat perubatan saya akan dirahsiakan? / *Will my medical information be kept private?***
Segala maklumat anda yang diperolehi dalam penyelidikan ini akan disimpan dan dikendalikan secara sulit, bersesuaian dengan peraturan-peraturan dan/ atau undang-undang yang berkenaan. Sekiranya hasil penyelidikan ini diterbitkan atau dibentangkan kepada orang ramai, identiti anda tidak akan didedahkan tanpa kebenaran anda terlebih dahulu. Hasil kajian ini akan diterbitkan di dalam makalah saintifik di akhir kajian tetapi identiti anda tidak akan didedahkan sama sekali pada bila-bila masa. Hasil kajian akan membezakan antara data pesakit diabetik dan bukan diabetik secara umum.
All your information obtained in this study will be kept and handled in a confidential manner, in accordance with applicable laws and/or regulations. When publishing or presenting the study results, your identity will not be revealed without your expressed consent. Data from the study will be published in a scientific journal at the end of the study, but your identity will not be revealed at any time. Results will be discussed as obtained from diabetic and non-diabetic patients generally.

3. **Siapakah yang perlu saya hubungi sekiranya saya mempunyai sebarang pertanyaan?**
Anda boleh menghubungi doktor penyelidikan ini, Dr Siva Gowri Pathmanathan (011 2691 9894), Dr Marlini Muhamad (013 304 3414), Dr Nizam Baharom (012 251 2960), Dr Fathima Begum (019 278 2611) atau Dr Rahman Omar (016 770 4707) sekiranya anda mempunyai sebarang pertanyaan mengenai penyelidikan ini atau jika anda mengesyaki anda mengalami kecederaan yang terhasil daripada penyelidikan ini dan anda mahukan maklumat tentang rawatannya. Jika anda mempunyai sebarang pertanyaan berkaitan dengan hak-hak anda sebagai peserta dalam penyelidikan ini, sila hubungi:
Please contact the study doctors: Dr Siva Gowri Pathmanathan (011 2691 9894), Dr Marlini Muhamad (013 304 3414), Dr Nizam Baharom (012 251 2960), Dr Fathima Begum (019 278 2611) or Dr Rahman Omar (016 770 4707), if you have any questions about the study or if you think you have a study related injury and you want information about treatment. If you have any questions about your rights as a participant in this study, please contact:

JAWATANKUASA ETIKA & PENYELIDIKAN PERUBATAN
(MEDICAL RESEARCH & ETHICS COMMITTEE)
KEMENTERIAN KESIHATAN MALAYSIA
Kompleks Institut Kesihatan Negara (NIH),
No.1, Jalan Setia Murni U13/52,
Seksyen U13 Bandar Setia Alam,
40170 Shah Alam, Selangor.
No.Tel: 03-33628407/ 33628205/ 33628888

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APPENDIX C

BORANG PERSETUJUAN/ KEIZINAN PESERTA

Tajuk Penyelidikan: Ethnicity-related gut microbiota involvement in the pathogenesis of type 2 diabetes mellitus via alterations in metabolic regulators (Peranan mikrobiota usus mengikut kaum di dalam patogenesis penyakit kencing manis)

Dengan menandatangani di bawah, saya mengesahkan bahawa :

- Saya telah diberi maklumat tentang penyelidikan di atas secara lisan dan bertulis dan saya telah membaca dan memahami segala maklumat yang diberikan dalam risalah ini.
- Saya telah diberikan masa yang secukupnya untuk mempertimbangkan penyertaan saya dalam penyelidikan ini dan telah diberi peluang untuk bertanyakan soalan dan semua persoalan saya telah dijawab dengan sempurna dan memuaskan.
- Saya juga faham bahawa penyertaan saya adalah secara sukarela dan pada bila-bila masa saya bebas menarik diri daripada penyelidikan ini tanpa harus memberi sebarang alasan dan ianya sama sekali tidak akan menjejaskan rawatan perubatan saya pada masa akan datang. Saya tidak mengambil bahagian dalam mana-mana penyelidikan lain pada masa ini. Saya juga memahami tentang risiko dan manfaat penyelidikan ini dan saya secara sukarela memberi persetujuan untuk menyertai penyelidikan ini di bawah syarat-syarat yang telah dinyatakan di atas. Saya faham saya harus mematuhi nasihat dan arahan yang berkaitan dengan penyertaan saya dalam penyelidikan ini daripada doktor penyelidikan (penyelidik) .
- Saya faham bahawa kakitangan penyelidikan, pemantau dan juruaudit terlatih , pihak penaja atau gabungannya, dan pihak berkuasa kerajaan atau undang-undang, mempunyai akses langsung dan boleh menyemak laporan perubatan saya bagi memastikan penyelidikan ini dijalankan dengan betul dan data direkodkan dengan betul. Segala maklumat dan data peribadi akan dianggap sebagai SULIT.
- Saya telah menerima satu salinan 'Risalah Maklumat Peserta' untuk dibawa pulang.

*(*Potong mana yang tidak berkenaan)*

Subjek :

Tandatangan:	Nombor K/P:
Nama:	Tarikh :

Penyelidik yang mengendalikan proses menandatangani borang keizinan:

Tandatangan:	Nombor K/P:
Nama:	Tarikh :

Saksi tidak-berpihak/adil: *(Diperlukan; jika subjek adalah buta huruf dan kandungan risalah maklumat peserta disampaikan secara lisan kepada subjek)*

Tandatangan:	Nombor K/P:
Nama:	Tarikh :

INFORMED CONSENT FORM

Title of Study: Ethnicity-related gut microbiota involvement in the pathogenesis of type 2 diabetes mellitus via alterations in metabolic regulators

By signing below I confirm the following:

- I have been given oral and written information for the above study and have read and understood the information given.
- I have had sufficient time to consider participation in the study and have had the opportunity to ask questions and all my questions have been answered satisfactorily.
- I understand that my participation is voluntary and I can at any time withdraw from the study without giving a reason and this will in no way affect my future treatment. I am not taking part in any other research study at this time. I understand the risks and benefits, and I freely give my informed consent to participate under the conditions stated. I understand that I must follow the study doctor's (investigator's) instructions related to my participation in the study.
- I understand that study staff, qualified monitors and auditors, the sponsor or its affiliates, and governmental or regulatory authorities, have direct access to my medical record in order to make sure that the study is conducted correctly and the data are recorded correctly. All personal details will be treated as STRICTLY CONFIDENTIAL
- I will receive a copy of this study information to bring home.

*(*delete which is not applicable)*

Subject:

Signature:	I/C number:
Name:	Date:

Investigator conducting informed consent:

Signature:	I/C number:
Name:	Date:

Impartial witness: *(Required if subject is illiterate and contents of participant information sheet is orally communicated to subject)*

Signature:	I/C number:
Name:	Date:

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APPENDIX D

UNIVERSITI SAINS ISLAM MALAYSIA
جامعة العلوم الإسلامية الماليزية
ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

Fakulti Perubatan dan Sains Kesihatan
Faculty of Medicine and Health Sciences
Jabatan Sains Perubatan Asas II
Department of Basic Medical Sciences II

USIM/FRGS/FPSK/055002/50418
SKIM GERAN KAJIAN ASAS, KPM
FUNDAMENTAL RESEARCH GRANT SCHEME, MOE

SULIT
CONFIDENTIAL

Soal Selidik
Questionnaire

Mikrobiota di dalam usus pada pelbagai kaum dan penglibatannya dengan Diabetis Melitus jenis 2 melalui perubahan pada pengatur metabolik
Ethnicity-related gut microbiota involvement in the pathogenesis of type 2 Diabetes Mellitus via alterations in metabolic regulators.

Penyelidik Utama / *Principal Investigator* : Siva Gowri Pathmanathan

Semua maklumat yang diberikan di sini adalah dirahsiakan dan hanya digunakan untuk tujuan akademik sahaja. Kejayaan kajian ini amat bergantung kepada kerjasama pihak tuan/puan dalam menjawab kesemua soalan yang dikemukakan. Segala kerjasam yang tuan/puan berikan saya didahului dengan ribuan terima kasih.

Your personal information given in this questionnaire is for research purpose only. It will be kept strictly confidential. I would be grateful if you could help me by completing this questionnaire.

Seksyen A / *Section A:* Sosiodemografi / *Sociodemography*

Maklumat peribadi individu / *Personal background information*

Maklumat / *Information*	**Pilihan / *Options***
Nama / *Name*	
Umur / *Age*	☐☐ tahun / *years old*
Tarikh lahir / *Date of birth*	☐☐☐☐☐☐☐☐ (hh-bb-tttt) / *(dd-mm-yyyy)*
Jantina / Gender	☐ Lelaki / *Male* ☐ Perempuan / *Female*
Kaum / *Ethnicity*	☐ Melayu / *Malay* ☐ Cina / *Chinese* ☐ India / *Indian* ☐ Lain-lain / *Others*, nyatakan / *please state*: _____
Alamat rumah / *Home address*	
No. Telefon / *Phone number*	
Alamat email / *Email address*	
Tempat lahir saya / *My place of birth*	
Tempat lahir bapa / *Father's place of birth*	: _____
Tempat lahir datuk (ayah kepada bapa) *Grandfather's place of birth (father's dad)*	: _____
Tempat lahir nenek (ibu kepada bapa) *Grandmother's place of birth (father's mom)*	: _____
Tempat lahir ibu / *Mother's place of birth*	: _____
Tempat lahir datuk (ayah kepada ibu) *Grandfather's place of birth (father's dad)*	: _____
Tempat lahir nenek (emak kepada ibu) *Grandmother's place of birth (father's mom)*	: _____
Status bekerja / *Employment status*	☐ Kerajaan / *Government* ☐ Bukan kerajaan / *Non-government* ☐ Tidak bekerja / *Unemployed* ☐ Lain-lain / *Others* nyatakan / *please state*: _____
Pendapatan isi rumah bulanan / *Monthly household income*	RM _____

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ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

APPENDIX D

Seksyen B / ***Section B:*** **Sejarah kesihatan peribadi /** ***Personal health history***

No.	Maklumat / *Information*	Pilihan / *Options*
1.	Adakah di kalangan **ahli keluarga** anda **menghidapi** penyakit **Kencing Manis**? / *Is any of* ***your family*** *members* ***has or had diabetes****?*	☐ Ya / *Yes* (**Jika Ya**, sila nyatakan siapa / *If Yes, please state who*): _____ _____ ___ (ibu/bapa/kakak/abang/adik) *(mother/father/sister/brother)* ☐ Tidak / *No*
2.	Adakah anda **merokok**? / *Are you* ***smoking****?*	☐ Ya / *Yes* ☐ Tidak / *No* ☐ Ex-smoker / *Bekas perokok*
3.	Bilakah **kali terakhir** anda **mengambil antibiotik**? / *When was* ***last you took antibiotics****?*	: _____
4.	Adakah anda seorang ***vegetarian***? *Are you a* ***vegetarian****?*	☐ Ya / *Yes* ☐ Tidak / *No*

Seksyen C / ***Section G:*** **Ukuran antropometri /** ***Anthropometry measurement***
(akan diisi oleh penyelidik / *fill in by researcher*)

Pengukuran / ***Measurement***	**Bacaan /** ***Reading***
Berat / *Weight* (kg)	
Tinggi / *Height* (cm)	
Indeks Jisim Tubuh / *Body Mass Index* (kg/m²)	
Ukur lilit pinggang / *Waist circumference* (cm)	

Soalan tamat di sini. Terima kasih atas penyertaan anda. ***The questions end here. Thank you for participating.***

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APPENDIX E

1st BASE
YOUR RESEARCH, OUR BUSINESS
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Next Generation Sequencing
Genomic DNA Quantification Report

Customer Details
Customer Name : Siva Gowri Pathmanathan
NGS ID : 12470
Date of Submission : 28-Jun-21
Date Completed : 30-Jun-21

Sample Information

		Nanodrop (water as blank)					Fluorometric				
No.	SampleName	OD260/280	OD260/230	Conc. (ng/uL)	Vol (uL)	Total amount (ug)	Initial Conc. (ng/ul)	Dilution factor	Final Conc. (ng/uL)	Total amount (ug)	Comment
1	NM5813M49	1.939	1.395	86.20	10	0.86	73.54		73.54	0.74	gDNA showed smearing without intact band
2	NM5173M30	1.922	1.695	181.45	10	1.81	88.59	2	177.18	1.77	gDNA showed smearing without intact band
3	NM5317M28	1.942	1.197	78.25	11	0.86	66.21		66.21	0.73	gDNA showed smearing without intact band
4	NM5255M41	2.124	1.681	101.30	67	6.79	30.05		30.05	2.01	gDNA showed smearing without intact band
5	NM5467M60	1.984	1.191	54.95	11	0.60	44.70		44.70	0.49	gDNA showed smearing without intact band
6	NM5594F44	2.172	0.464	17.05	40	0.68	7.88		7.88	0.32	gDNA showed smearing without intact band
7	NM5306F36	1.939	1.419	86.50	12	1.04	75.05		75.05	0.90	gDNA showed smearing without intact band
8	NM5250F52	1.937	1.538	94.60	18	1.70	84.04		84.04	1.51	gDNA showed smearing without intact band
9	NM5618F25	2.033	0.822	21.45	45	0.97	15.05		15.05	0.68	gDNA showed smearing without intact band
10	NM5282F52	2.587	0.301	5.95	95	0.57	1.62		1.62	0.15	gDNA band is not visible on agarose gel
11	NM5978F30	1.998	1.146	64.75	10	0.65	55.10		55.10	0.55	gDNA showed smearing without intact band
12	NM5068F27	2.128	1.391	78.30	14	1.10	17.37		17.37	0.24	gDNA showed smearing without intact band
13	NM6948F57	1.972	1.901	78.60	10	0.79	65.91		65.91	0.66	gDNA showed smearing without intact band
14	NM5176F63	2.255	1.020	15.45	42	0.65	6.11		6.11	0.26	gDNA showed smearing without intact band
15	NM5378F56	2.173	1.219	52.05	15	0.78	10.66		10.66	0.16	gDNA showed smearing without intact band
16	NI5961M34	2.450	0.326	9.80	73	0.72	1.82		1.82	0.13	gDNA band is not visible on agarose gel
17	NI5953M48	1.912	1.955	155.80	10	1.56	76.36	2	152.72	1.53	gDNA showed smearing without intact band
18	NI5745M29	2.350	0.339	4.70	30	0.14	1.16		1.16	0.03	gDNA band is not visible on agarose gel
19	NI6077M50	1.940	1.900	92.05	10	0.92	84.34		84.34	0.84	gDNA showed smearing without intact band
20	NI5216F46	1.921	1.928	256.40	10	2.56	88.38	3	265.14	2.65	gDNA showed smearing without intact band
21	NI5752F38	2.042	1.666	159.50	10	1.60	40.05	2	80.10	0.80	gDNA showed smearing without intact band
22	NI7024F27	2.100	0.923	28.25	34	0.96	23.18		23.18	0.79	gDNA showed smearing without intact band
23	NI6152F30	2.112	0.954	18.80	41	0.77	14.90		14.90	0.61	gDNA showed smearing without intact band
24	NI5424F30	3.122	0.287	6.40	100	0.64	0.05		0.05	0.01	gDNA band is not visible on agarose gel
25	NI5706F31	2.356	0.276	6.95	114	0.79	1.36		1.36	0.16	gDNA band is not visible on agarose gel
26	NI6078F53	2.875	0.238	8.05	107	0.86	0.00		0.00	0.00	gDNA band is not visible on agarose gel
27	NI5974F39	2.678	0.648	7.90	84	0.66	4.55		4.55	0.38	gDNA showed smearing without intact band
28	NI5018F41	2.073	0.957	25.50	34	0.87	23.69		23.69	0.81	gDNA showed smearing without intact band
29	NI5958F48	1.942	1.477	87.30	17	1.48	80.86		80.86	1.37	gDNA showed smearing without intact band
30	NI5098F41	1.941	1.970	111.30	10	1.11	110.40		110.40	1.10	gDNA showed smearing without intact band
31	NC5251M48	2.029	1.175	38.65	29	1.12	35.56		35.56	1.03	gDNA showed smearing without intact band
32	NC5170F42	2.073	1.606	37.10	23	0.85	35.66		35.66	0.82	gDNA showed smearing without intact band
33	NC5672F60	2.253	0.456	8.90	80	0.71	3.08		3.08	0.25	gDNA is insufficient for library preparation
34	NC5270F48	2.234	0.649	12.40	55	0.68	10.45		10.45	0.57	gDNA showed smearing without intact band
35	NC5469F59	1.982	1.740	67.00	11	0.74	63.54		63.54	0.70	gDNA showed smearing without intact band
36	NC5242F69	2.333	0.422	8.40	76	0.64	3.59		3.59	0.27	gDNA is insufficient for library preparation
37	NC5862F64	2.170	0.911	20.40	39	0.80	17.88		17.88	0.70	gDNA showed smearing without intact band
38	NC6056F58	1.926	1.784	98.50	10	0.99	95.25		95.25	0.95	
39	NC5570F58	2.211	1.136	18.35	41	0.75	16.01		16.01	0.66	gDNA showed smearing without intact band
40	NC5902F67	1.979	1.479	55.70	17	0.95	51.97		51.97	0.88	gDNA showed smearing without intact band
41	NC5076F72	2.787	0.260	6.55	107	0.70	0.30		0.30	0.03	gDNA band is not visible on agarose gel
42	NC5494F70	1.954	1.874	105.90	10	1.06	98.99		98.99	0.99	gDNA showed smearing without intact band
43	NC6006F38	1.976	1.933	214.60	10	2.15	85.91	2	171.82	1.72	gDNA showed smearing without intact band
44	NC5092F30	1.949	0.888	96.40	13	1.25	94.34		94.34	1.23	gDNA showed smearing without intact band
45	NC5115F48	2.277	0.729	41.10	20	0.82	11.62		11.62	0.23	gDNA showed smearing without intact band
46	NC5242F35	2.854	0.245	6.85	36	0.25	0.40		0.40	0.01	gDNA band is not visible on agarose gel
47	DM5218F55	2.106	1.051	24.75	29	0.72	23.33		23.33	0.68	gDNA showed smearing without intact band
48	DM5128F57	1.963	1.985	78.80	12	0.95	81.11		81.11	0.97	gDNA showed smearing without intact band
49	DM5318F61	2.306	0.472	12.80	18	0.23	8.28		8.28	0.15	gDNA showed smearing without intact band
50	DM5396F69	1.922	1.949	207.45	10	2.07	103.59	2	207.18	2.07	gDNA showed smearing without intact band
51	DM5624F71	3.286	0.176	5.75	145	0.83	0.00		0.00	0.00	gDNA band is not visible on agarose gel
52	DM5538F42	2.119	0.913	28.40	25	0.71	24.14		24.14	0.60	gDNA showed smearing without intact band
53	DM5254F54	2.019	1.097	43.50	22	0.96	34.75		34.75	0.76	gDNA showed smearing without intact band
54	DM5616F48	1.928	1.924	198.30	10	1.98	106.06	2	212.12	2.12	gDNA showed smearing without intact band
55	DM5040F78	2.018	1.146	50.25	18	0.90	44.24		44.24	0.80	gDNA showed smearing without intact band
56	DM5688F67	2.191	0.807	24.65	19	0.47	20.15		20.15	0.38	gDNA showed smearing without intact band
57	DM5484F38	4.652	0.220	5.35	114	0.61	0.91		0.91	0.10	gDNA band is not visible on agarose gel
58	DM5704F35	1.947	1.682	124.90	11	1.37	57.98	2	115.96	1.28	gDNA showed smearing without intact band
59	DM5949M42	1.921	1.808	220.80	10	2.21	72.63	3	217.89	2.18	gDNA showed smearing without intact band
60	DM5031M67	2.605	0.243	5.60	123	0.69	1.62		1.62	0.20	gDNA band is not visible on agarose gel
61	DM5127M54	1.924	1.336	249.15	10	2.49	77.68	3	233.04	2.33	gDNA showed smearing without intact band
62	DM5003M61	1.937	1.545	134.50	10	1.35	67.17	2	134.34	1.34	gDNA showed smearing without intact band
63	DM5189M46	3.000	0.252	5.85	123	0.72	0.00		0.00	0.00	gDNA band is not visible on agarose gel

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APPENDIX E

64	DI5014F69	1.989	1.600	71.70	11	0.79	69.65		69.65	0.77	gDNA showed smearing without intact band
65	DI5008F72	2.321	1.006	15.55	52	0.81	11.92		11.92	0.62	gDNA showed smearing without intact band
66	DI6086F61	4.826	0.257	5.55	123	0.68	0.00		0.00	0.00	gDNA band is not visible on agarose gel
67	DI5442F67	1.923	1.861	229.25	10	2.29	68.18	3	204.54	2.05	gDNA showed smearing without intact band
68	DI5642F49	1.993	1.174	75.75	12	0.91	63.94		63.94	0.77	gDNA showed smearing without intact band
69	DI7104F55	2.287	0.591	14.75	57	0.84	7.37		7.37	0.42	gDNA showed smearing without intact band
70	DI6712F61	1.989	1.758	72.50	12	0.87	60.71		60.71	0.73	gDNA showed smearing without intact band
71	DI5076F57	1.965	1.788	92.25	11	1.01	89.90		89.90	0.99	gDNA showed smearing without intact band
72	DI6228F45	2.047	1.363	26.30	32	0.84	24.90		24.90	0.80	gDNA showed smearing without intact band
73	DI5678F53	2.725	0.145	9.40	76	0.71	2.37		2.37	0.18	gDNA is insufficient for library preparation
74	DI5537M56	1.919	2.149	187.70	10	1.88	76.11	2	152.22	1.52	gDNA showed smearing without intact band
75	DI5083M61	1.962	2.029	153.80	10	1.54	56.16	2	112.32	1.12	gDNA showed smearing without intact band
76	DI5191M69	2.932	0.225	6.45	100	0.65	0.00		0.00	0.00	gDNA band is not visible on agarose gel
77	DI5279M63	1.948	1.700	108.60	10	1.09	85.81		85.81	0.86	gDNA showed smearing without intact band
78	DI5013M57	2.034	1.310	35.50	22	0.78	26.16		26.16	0.58	gDNA showed smearing without intact band
79	DI7061M61	1.972	1.565	52.75	14	0.74	40.96		40.96	0.57	gDNA showed smearing without intact band
80	DC5234F68	1.994	1.210	35.20	21	0.74	30.81		30.81	0.65	gDNA showed smearing without intact band
81	DC5196F70	2.064	0.525	11.35	27	0.31	5.00		5.00	0.14	gDNA showed smearing without intact band
82	DC5688F73	2.077	1.119	21.60	37	0.80	12.83		12.83	0.47	gDNA showed smearing without intact band
83	DC5716F69	2.005	1.212	38.60	22	0.85	23.84		23.84	0.52	gDNA showed smearing without intact band
84	DC5146F64	2.250	0.535	13.05	50	0.65	5.61		5.61	0.28	gDNA showed smearing without intact band
85	DC5646F37	2.289	0.383	13.05	50	0.65	3.59		3.59	0.18	gDNA is insufficient for library preparation
86	DC5890F64	1.948	1.985	183.10	10	1.83	69.29	2	138.58	1.39	gDNA showed smearing without intact band
87	DC5904F51	2.110	0.875	23.10	35	0.81	15.45		15.45	0.54	gDNA showed smearing without intact band
88	DC5104F43	1.922	1.590	151.75	10	1.52	74.39	2	148.78	1.49	gDNA showed smearing without intact band
89	DC7272F54	2.471	0.279	8.65	76	0.66	1.72		1.72	0.13	gDNA band is not visible on agarose gel
90	DC5917M66	3.059	0.416	5.20	64	0.33	0.45		0.45	0.03	gDNA band is not visible on agarose gel
91	DC5961M60	2.386	0.321	9.90	64	0.63	2.37		2.37	0.15	gDNA is insufficient for library preparation
92	DC8059M57	1.958	1.478	74.10	10	0.74	48.64		48.64	0.49	gDNA showed smearing without intact band
93	DC5081M44	2.424	0.118	10.30	64	0.66	1.57		1.57	0.10	gDNA band is not visible on agarose gel
94	DC5787M66	2.119	1.265	54.15	13	0.70	17.53		17.53	0.23	gDNA showed smearing without intact band
95	PM5428F61	2.854	0.359	5.85	123	0.72	0.81		0.81	0.10	gDNA band is not visible on agarose gel
96	PM5050F65	2.049	1.712	111.05	10	1.11	39.39		39.39	0.39	gDNA showed smearing without intact band
97	PM5316F59	2.243	0.508	8.30	84	0.70	2.22		2.22	0.19	gDNA is insufficient for library preparation
98	PI5092F61	1.958	1.885	115.05	10	1.15	76.16		76.16	0.76	gDNA showed smearing without intact band
99	PI5045M73	2.094	0.949	28.90	27	0.78	13.89		13.89	0.38	gDNA showed smearing without intact band
100	PI5624F53	2.082	0.318	18.95	40	0.76	6.06		6.06	0.24	gDNA showed smearing without intact band
101	PC5652F51	2.153	0.608	64.15	12	0.77	10.25		10.25	0.12	gDNA showed smearing without intact band
102	NDM5966F65	2.303	1.629	43.75	10	0.44	8.13		8.13	0.08	gDNA showed smearing without intact band
103	NDM5262F61	2.371	0.728	14.70	100	1.47	1.57		1.57	0.16	gDNA band is not visible on agarose gel
104	NDM5335M48	4.032	0.352	6.25	107	0.67	0.00		0.00	0.00	gDNA band is not visible on agarose gel
105	NDI5556F43	2.079	1.208	35.65	23	0.82	21.16		21.16	0.49	gDNA showed smearing without intact band
106	NDI5452F59	2.160	0.526	8.75	80	0.70	2.47		2.47	0.20	gDNA is insufficient for library preparation
107	NDI6091M41	2.110	2.004	181.75	10	1.82	18.08	2	36.16	0.36	gDNA showed smearing without intact band
108	NDC5896F60	2.242	0.810	13.45	53	0.71	5.40		5.40	0.29	gDNA showed smearing without intact band
109	NDC5658F67	1.967	1.782	89.70	10	0.90	56.26		56.26	0.56	gDNA showed smearing without intact band
110	NDC5193M58	1.912	1.755	177.30	10	1.77	63.84	2	127.68	1.28	gDNA showed smearing without intact band
111	Water_1	0.080	0.034	0.00	100	0.00	0.00		0.00	0.00	gDNA band is not visible on agarose gel
112	Water_2	4.818	0.123	2.65	100	0.27	0.00		0.00	0.00	gDNA band is not visible on agarose gel

DNA Samples QC Criteria

Library Type	Sample Type	Amount (Qubit®)		Volume	Concentration	Purity (NanoDrop™/agarose gel)
		Strongly Recommended	Required			
PCR-Free Library	Genomic DNA	≥ 800 ng	≥ 400 ng	≥ 30 µL	≥ 20 ng/µL	OD260/280 = 1.8 – 2.0, no degradation, no contamination

1. Strongly Recommended*: In case unforeseen happening (lib prep failure, low quality, low amount, etc), the double amount requirements are highly recommended to avoid potential sample re-sending.
2. Required*: Samples amount required for one time library preparation

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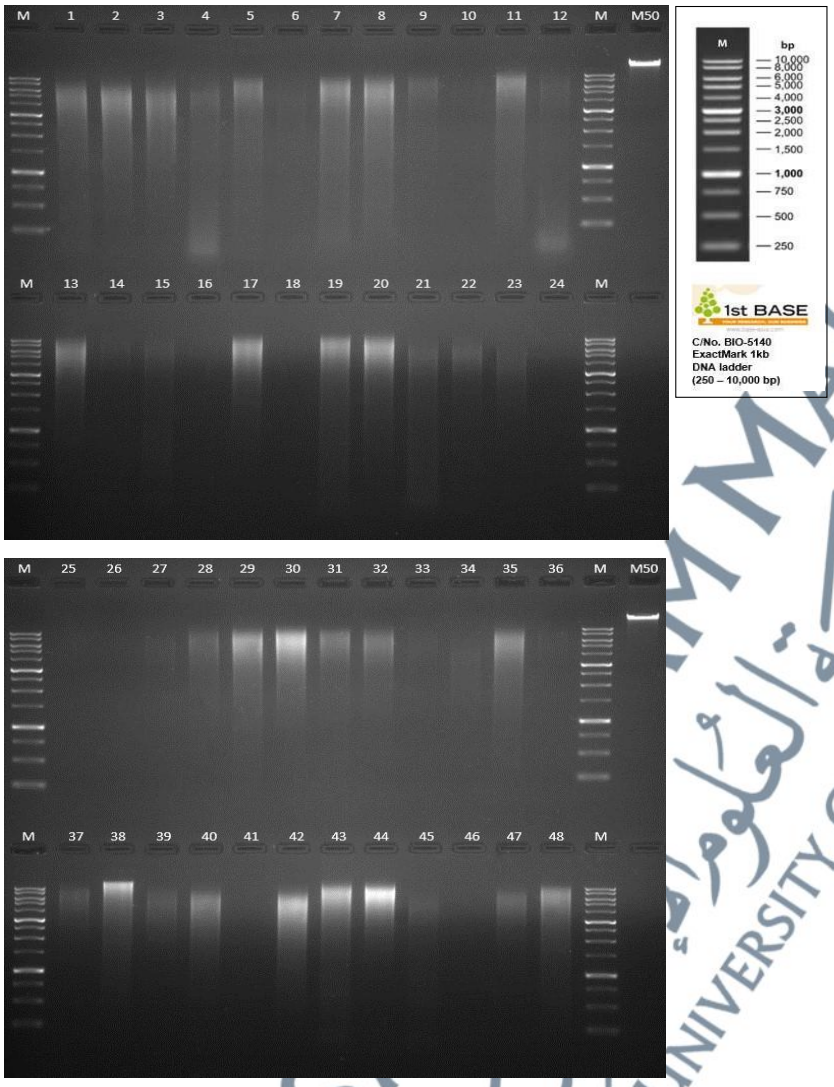
APPENDIX E

Agarose Quantification



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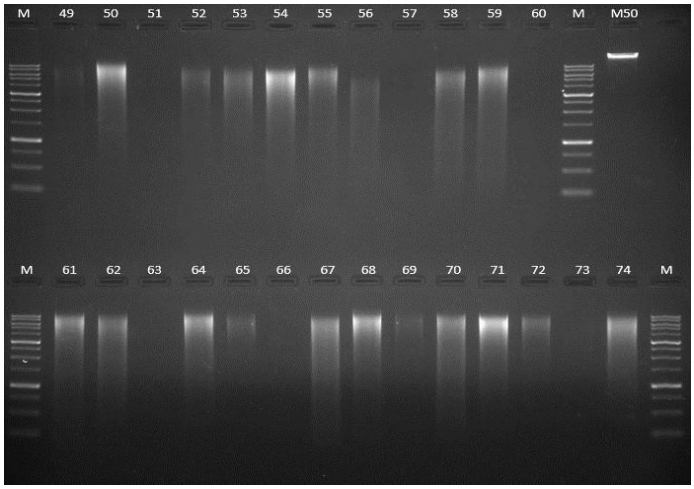
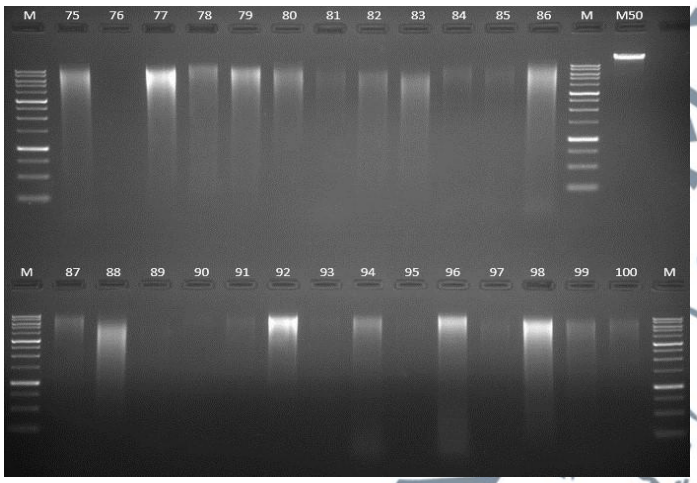
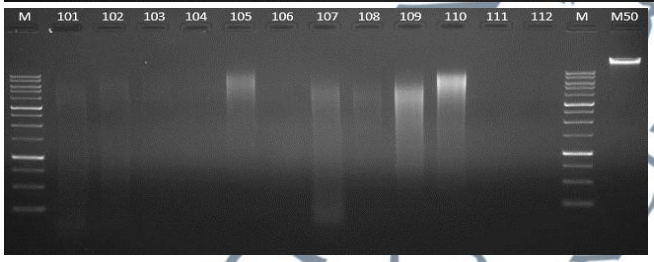
APPENDIX E







Aliquots of 1 ul gDNA were run on 1% TAE agarose gel at 100V for 60 min.
M50 is positive control (Bacterial gDNA template, 50 ng, according to fluorometric quantification).

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End of Report

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APPENDIX F

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Next Generation Sequencing
PCR to QC Genomic DNA Report

Customer Details

Customer Name : Siva Gowri Pathmanathan
NGS ID : 12470-Part 2
Date of Submission : 16-Jul-21
Date Completed : 23-Jul-21

Sample Information

No.	Lane	SampleName	16S gene (V3-V4) QC PCR Results
1	1	NM5813M49	PASS
2	2	NM5173M30	PASS
3	3	NM5317M28	PASS
5	4	NM5467M60	PASS
6	5	NM5594F44	PASS
7	6	NM5306F36	PASS
8	7	NM5250F52	PASS
9	8	NM5618F25	PASS
10	9	NM5282F52	PASS
11	10	NM5978F30	PASS
12	11	NM5068F27	PASS
13	12	NM6948F57	PASS
14	13	NM5176F63	PASS
15	14	NM5378F56	PASS
16	15	NI5961M34	PASS
17	16	NI5953M48	PASS
19	17	NI6077M50	PASS
20	18	NI5216F46	PASS
21	19	NI5752F38	PASS
22	20	NI7024F27	PASS
23	21	NI6152F30	PASS
24	22	NI5424F30	PASS
26	23	NI6078F53	PASS
27	24	NI5974F39	PASS
28	25	NI5018F41	PASS
29	26	NI5958F48	PASS
30	27	NI5098F41	PASS
31	28	NC5251M48	PASS
32	29	NC5170F42	PASS
33	30	NC5672F60	PASS
34	31	NC5270F48	PASS
35	32	NC5469F59	PASS
36	33	NC5242F69	PASS
37	34	NC5862F64	PASS
39	35	NC5570F58	PASS
40	36	NC5902F67	PASS
42	37	NC5494F70	PASS
43	38	NC6006F38	PASS
44	39	NC5092F30	PASS
45	40	NC5115F48	PASS
46	41	NC5242F35	PASS
47	42	DM5218F55	PASS
48	43	DM5128F57	PASS
49	44	DM5318F61	PASS
50	45	DM5396F69	PASS
51	46	DM5624F71	PASS
52	47	DM5538F42	PASS
53	48	DM5254F54	PASS
54	49	DM5616F48	PASS
55	50	DM5040F78	PASS
56	51	DM5688F67	PASS
57	52	DM5484F38	PASS

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APPENDIX F

58	53	DM5704F35	PASS
59	54	DM5949M42	PASS
60	55	DM5031M67	PASS
61	56	DM5127M54	PASS
62	57	DM5003M61	PASS
63	58	DM5189M46	PASS
64	59	DI5014F69	PASS
65	60	DI5008F72	PASS
66	61	DI6086F61	PASS
67	62	DI5442F67	PASS
68	63	DI5642F49	PASS
69	64	DI7104F55	PASS
70	65	DI6712F61	PASS
71	66	DI5076F57	PASS
72	67	DI6228F45	PASS
73	68	DI5678F53	PASS
74	69	DI5537M56	PASS
75	70	DI5083M61	PASS
76	71	DI5191M69	PASS
77	72	DI5279M63	PASS
78	73	DI5013M57	PASS
79	74	DI7061M61	PASS
80	75	DC5234F68	PASS
82	76	DC5688F73	PASS
83	77	DC5716F69	PASS
84	78	DC5146F64	PASS
86	79	DC5890F64	PASS
88	80	DC5104F43	PASS
89	81	DC7272F54	PASS
90	82	DC5917M66	PASS
91	83	DC5961M60	PASS
92	84	DC8059M57	PASS
93	85	DC5081M44	PASS
95	86	PM5428F61	PASS
96	87	PM5050F65	PASS
97	88	PM5316F59	PASS
98	89	PI5092F61	PASS
99	90	PI5045M73	PASS
100	91	PI5624F53	PASS
101	92	PC5652F51	PASS
103	93	NDM5262F61	PASS
104	94	NDM5335M48	PASS
105	95	NDI5556F43	PASS
106	96	NDI5452F59	PASS
107	97	NDI6091M41	PASS
108	98	NDC5896F60	PASS
109	99	NDC5658F67	PASS
110	100	NDC5193M58	PASS
111	101	Water_1	FAIL

Note

Pass : The sample is qualified for amplicon library construction.

Hold : The sample does not totally meet the requirements but can be proceed to amplicon library construction at risk. Failure of amplicon library construction will incurred additional charge.

Fail : It is highly recommended to

1) Additional purify the genomic DNA to remove impurities at separate charge

OR

2) Resend purified genomic DNA

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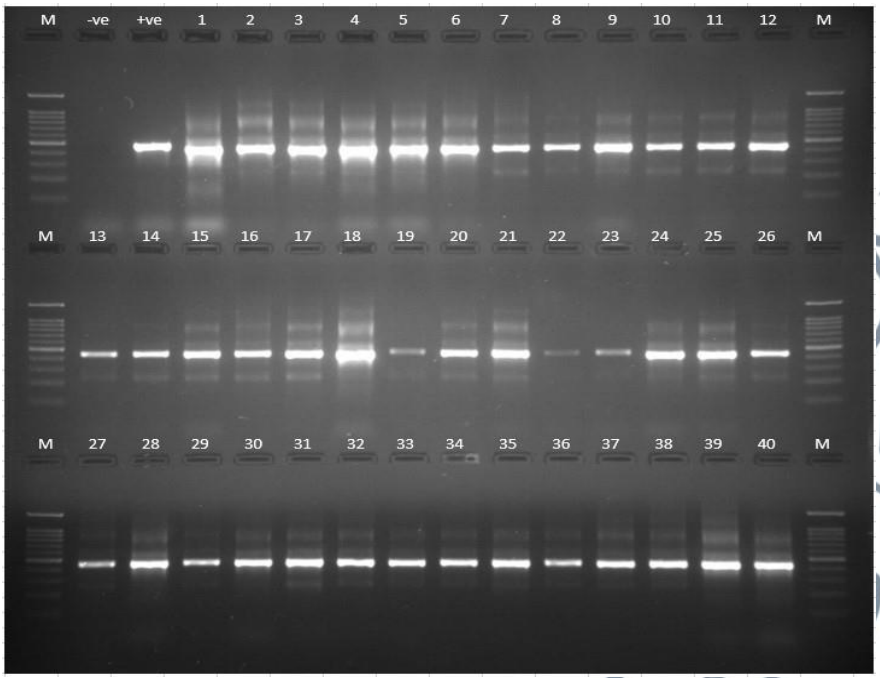
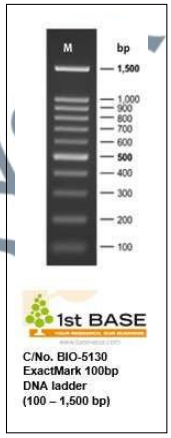
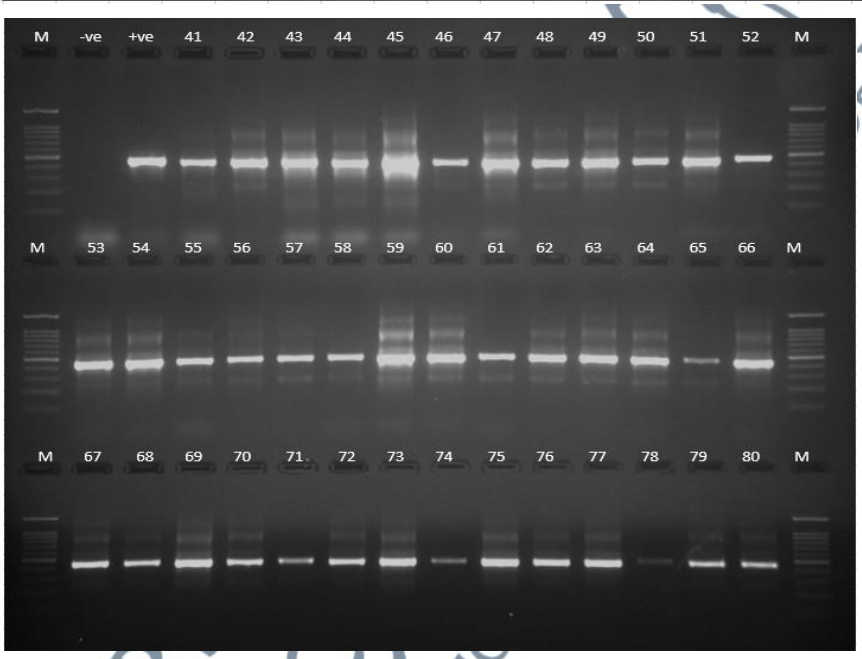
APPENDIX F

Agarose Quantification







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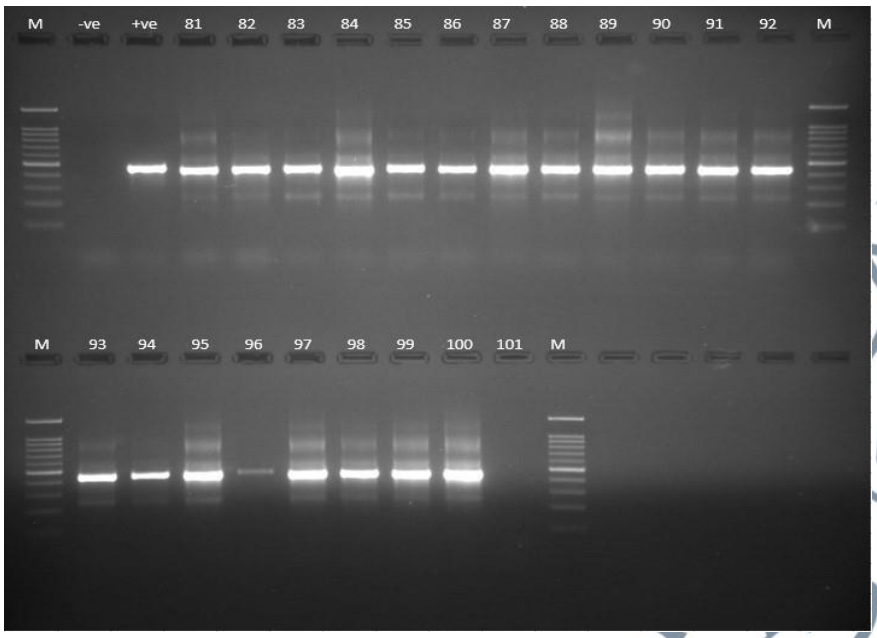
APPENDIX F



Aliquots of 3 ul of PCR product was run on 1.7% TAE agarose gel at 100V, 65 min.
"-ve" is no-template control (water to replace DNA template).
"+ve" is positive control (gDNA template, 10 ng, according to fluorometric quantification). .
A total 1 ul of gDNA template was used in one 25 ul PCR reaction.

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End of Report

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APPENDIX G

Clinical Parameters In Diabetic And Non-Diabetic Patients: A Case-Control Study At A Health Clinic In Klang Valley

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Introduction
Type-2 diabetes mellitus (T2DM) is prevalent in Malaysia and often associated with deranged biochemical profile.

Objective(s)
This study aimed to compare the sociodemographic and biochemical profile between T2DM and non-diabetic patients at a health clinic in Klang Valley. This is part of an ongoing research studying the gut microbiota involvement in T2DM.

Methodology
This is an unmatched case control study conducted between November 2019 and August 2020. Cases were randomly selected diabetic patients while controls were from the outpatient department with normal blood glucose levels. Patients were subjected to anthropometry and biochemical investigations including fasting blood glucose (FBG), serum lipids, liver function tests (LFT) and renal profile.

Results
A total of 49 T2DM cases and 49 non-diabetic controls participated in the study. The gender and ethnicity distributions were not significant, but BMI was slightly higher in T2DM [OR (95%CI) = 1.09 (1.01-1.19)]. As expected, the FBG was significantly higher among T2DM patients (9.35 ± 3.34 mmol/L) vs non-diabetic patients (5.18 ± 0.43 mmol/L) [OR (95%CI) = 9.15 (3.18-26.39)]. Triglyceride (TG) was markedly higher in T2DM patients [OR (95%CI) = 2.86 (1.41-5.80)], with no significant difference on other lipid profile. T2DM patients had significantly altered LFT with higher alkaline phosphatase (ALP) [OR (95%CI) = 1.02 (1.02-1.05)] and alanine aminotransferase (ALT) [OR (95%CI) = 1.04 (1.01-1.07)]. Creatinine was similar in both groups however urea was higher in T2DM [OR (95%CI) = 1.34 (1.04-1.72)] with lower chloride [OR (95%CI) = 0.83 (0.71-0.97)].

Conclusion(s)
T2DM patients demonstrated altered biochemical profile in particular higher TG, ALP, ALT, urea and lower chloride levels, depicting pathophysiological changes in the disease. Whether or not these alterations are mediated by gut microbiota is subjected to further studies. This study was funded by the Fundamental Research Grant Scheme from the Ministry of Education, Malaysia (FRGS/1/2018/SKK08/USIM/02/1).

Keywords
diabetes, sociodemographic, biochemical profile, serum biomarkers, microbiota

APPENDIX H

GUT MICROBIOTA PROFILE OF MAJOR ETHNIC GROUPS IN KLANG VALLEY, MALAYSIA

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INTRODUCTION

The profiling of gut microbiota composition in healthy individuals is important to identify compositional changes or dysbiosis that occurs in a disease state. Ethnicity is found to be one of the factors influencing the microbiota composition of people in a given geographical region[1]. Understanding specific microbiota profiles by ethnicity could assist with microbiota-based disease prevention and management. Thus, this study aimed to compare gut microbiota composition in in healthy adults of the three major Malaysian ethnic groups (Malay, Chinese and Indians) residing in Klang Valley, Malaysia.

METHODOLOGY

An observational study conducted from November to August 2020 in a health clinic in Klang Valley, Malaysia

↓

Recruitment of randomly selected participants attending non-communicable disease (NCD) screening with informed consent

↓

All participants, n=45
Malay 15
Chinese 15
Indian 15

↓

Stool for microbiota analysis

↓

DNA Extraction & 16S rDNA sequencing (V3 – V4 region) in Illumina Miseq platform

↓

Data Analysis (QIIME2 & MicrobiomeAnalyst[2])

↓

Taxonomy Assignment (phylum & genus) | Diversity Analyses (alpha & beta diversity)

↓

Discussion & Conclusion

RESULTS

1. A total of 45 Malaysian adults with 15 participants of each Malay, Chinese and Indian ethnic groups were enrolled in the study. The mean age was 45.1 ± 12.8 years.
2. Between the ethnic groups, higher age and weight was found in Chinese and Indian ethnic groups, respectively (Table 1).

Table 1 The Characteristics of Study Participants, n=45.

Socio-demography and anthropometry	Malay Mean ± SD n=15	Chinese Mean ± SD n=15	Indian Mean ± SD n=15	*p*-value
Age (years)	43.33 ± 13.22	52.87 ± 12.64	39.00 ± 8.59	**0.007**
Gender (male/female)*	5/10	1/14	4/11	0.188
Height (m)	1.59 ± 0.11	1.57 ± 0.07	1.65 ± 0.10	0.102
Weight (kg)	66.10 ± 11.97	60.13 ± 11.10	75.80 ± 16.25	**0.009**
BMI (kg/m²)	25.99 ± 2.60	24.31 ± 4.50	28.12 ± 6.65	0.113

continuous variables analysed using ANOVA[post-hoc test (Bonferroni)] and categorical variables analysed using *Chi-square test in SPSS, *p*-value < 0.05 is significant. Pairwise comparison: Age; Chinese vs Indian *p*=**0.007**; Weight; Chinese vs Indian *p*=**0.007**

3. As shown in Figure 1 & 2, the study found no differences in alpha (Shannon, p=0.273) and beta diversity (PERMANOVA, pseudo-F=1.177, p=0.077) indices between the three ethnic groups.

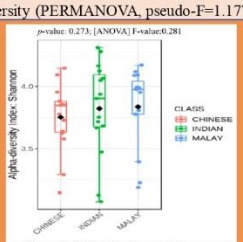

Figure 1 Alpha diversity with Shannon index n=44.

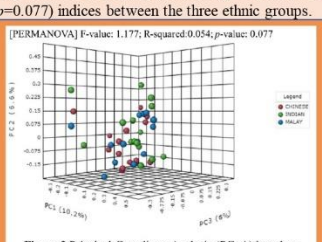

Figure 2 Principal Coordinate Analysis (PCoA) based on Bray-Curtis Dissimilarity Matrix, n=44.

4. Overall, 12 phyla and 237 genera were identified across all samples.
5. The dominant phyla across all samples were *Firmicutes* (73.75%), *Bacteroidetes* (21.17%), *Actinobacteria* (2.35%) and *Proteobacteria* (1.61%) (Figure 3A). No significant differences in bacterial phyla abundances were found between the ethnic groups.
6. The top six genera were *Faecalibacterium* (12.77%), *Blautia* (12.35%), *Bacteroides* (11.23%), *Prevotella* (7.88%), *Agathobacter* (4.04%) and *Eubacterium* (3.92%) (Figure 3B).
7. Genus *Bacteroides* which was found to be higher among Chinese participants lost its significance after adjustment for multiple comparisons (FDR>0.05).

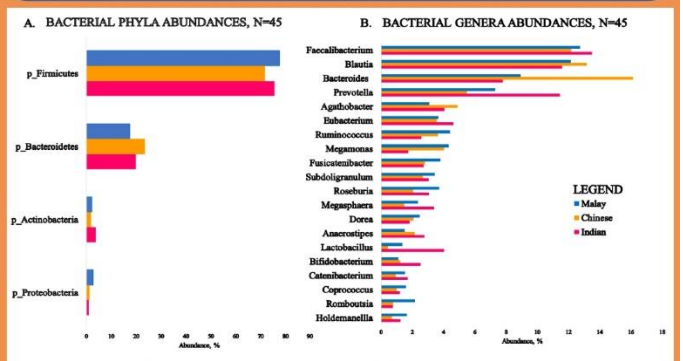

Figure 3 The Gut Microbiota Composition at Phylum Level and Top 20 Genera with more than 1% abundance in all ethnic groups.

DISCUSSION & CONCLUSION

1. In the present study, the dominant phyla found among the three ethnic groups were *Firrmicutes, Bacteroidetes, Actinobacteria* and *Proteobacteria*, with >90% of the total gut bacteria represented by *Firmicutes* and *Bacteroidetes*. This is in accordance with past reported studies[2].

2. No significant differences were noted in diversity and gut microbial abundance between the ethnic groups.

3. This indicates that the study population residing in the same geographic region in this study possibly shared similar gut microbiota composition despite ethnicity differences.

4. This could be contributed by similarities in dietary intake and lifestyle factors between the ethnic groups as Malaysians are exposed to and share different ethnic-specific cultures and food.

5. The lack of differences in bacterial abundance between the ethnic groups may have also been contributed by the small number of samples. Hence, larger studies are required to identify and compare microbiota composition between different communities and regions in Malaysia.

ACKNOWLEDGEMENTS

This study was funded by the Fundamental Research Grant Scheme from Ministry of Education, Malaysia (FRGS/1/2018/SKK08/USIM/02/1)

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