بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ
بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ إِلَيۡكَ وَحۡيُهُۥۖ
وَقُل رَّبِّ زِدۡنِي عِلۡمٗا ١١٤﴾

صدق الله العلي العظيم

سورة طه الآية (١١٤)

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Heba Salah

Dedication

To my Prophet.....**Muhammad**

To the owner of the waiting time……. **Imam Mahdi**

To whom she dedicated her life to me ……..**Mother**

To those who left their studies for my future.....**Brother**

To those who loved me, encouraged me and supported me……**Husband**

To those who are the reason for my joy in life..... **Children**

To my love and my friend..... **Zainab**

Heba salah

Abstract:

Type 2 Diabetes mellitus (T2D) is a chronic, multi-organ, multifactorial metabolic disease. It is characterized by the body's inability to uptake glucose (hyperglycemia) as a result of defects in insulin secretion, insulin action (insulin resistance) or combination of both.. Environmental and genetic predispositions are the major cause of T2D. A primary nursing role to reduce the prevalence of a disease, such as type 2 diabetes, by identifying and addressing the underlying cause. Some recent research findings point to the gut microbiome as a key player in developing many chronic diseases. The relationship between, the gut microbiome progression and severity of diabetic Mellitus have not been thoroughly studied. In the current study compared the gut microbiota of diabetes mellitus type 2 and control individuals. Moreover, detected the most abundance of bacteria in diabetes mellitus type 2 and compared with normal individuals. Thirty-six stool specimens were collected from participant's patient (20) and control (16) who attended Alemara laboratory in Misan province. The investigation period has been extended from September 2021 to February 2022.

The results showed that there are many types of bacteria in the human intestine, such as *firmicutes, Bacteroide, Verrucomicrobia*, *Proteobacter, Lentisphaerae, Elusimicrobia, Tenericutes, actinobacteria* and *Fusobacteria*. Furthermore, despite the fact that disease can effect on the abundance of the bacterial species in the human intestine. The study findings show that there are no statistically significant differences in the microbiota between diabetes mellitus type 2 and controls (P=0.099) by using different bioinformatics approaches. The *Verrucomicrobia* (2.9%), *Proteobacteria* (12.70%) and *Fusobacteria* (0.47%) display the highest percentages in diabetes mellitus type 2 compared with control ((0.5%;9.06%;0%) respectively), the *Firmicutes* (36.78%), *Bacteroide* (44.89%) *Tenericutes* (0.195%)

and *actinobacteria* (0.34%) reveled the lowest percentages in diabetes mellitus type 2 compared with control ((39.9%;47,6%;1.7%;0.48%) respectively.

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List of Abbreviations

Abbreviations	Key
C	Celsius degree
CA	Community assembly
CNS	Central nervous system
CVD	Cardio vascular diseases
DM	Diabetes mellitus
DM2	Diabetes mellitus type two
DNA	Deoxyribonucleic acid
DsDNA	Double strand deoxy Ribonucleic acid
EDTA	Ethylendiemintetraacetate
ETOH	Ethanol
FLASH	Fast length adjustment short
GIT	Gastro intestinal tract
IBD	Inflammatory bowel diseases
LADA	Latent autoimmunity diabetes adults
LMIC	Low middle income countries
MAX	Maximum
Min	Minute
Min	Minimum
Ml	Milliliter
Mm	Millimeter
MODY	Maturity onset diabetes young
Mpcr	multiplexPolymerase Chain Reaction
N	Number
NGS	Next generation sequencing
Nm	Nanometer

OUT	Operational taxonomic units
Pb	Base pair
PCOA	Principal coordinate analysis
PCR	Polymerases Chain Reaction
QC	Quality checkpoints
QIIME	Quantitative Insights into microbial ecology
QIM	Quantitative Insights microbial
RA	Rheumatoid arthritis
RNA	Ribonucleic acid
Rpm	rotation per minute
rRNA	ribosomal Ribonucleic acid
Sec	Second
SPSS	Statistical Package for the Social Sciences
T1D	Type one diabetes
T2D	Type two Diabetes
TBE	Tris Borate EDTA
US	United States
USA	United States of America
UV	Ultra Violet
V3	Variation three
V4	Variation four
W1	Wash one
W2	Wash two
WHO	The World Health Organization
XT	NextEra
µg/gm	Microgram/gram
ML	Microliter

MGC	Mammalian Gene collection

List of Appendices

NO.	**Title**
1-	The questionnaire of population of diabetes mellitus type 2
2-	Results of pre-processing and clustering of OUT picking method (denovo) of diabetes mellitus type 2 and control
3-	OUT abundance
4-	Results of Community richness and diversity of microbiome in diabetes mellitus type 2 and control,
5-	Percentages of bacteria in diabetes mellitus type 2 patients and control;

Chapter one

Introduction

Introduction:

The intestinal tract of human is colonized by complex microbial communities called the gut microbiota. The microbiota in the gut has been shown to have important physiological functions in the body and can change and causes many diseases in human. (De Palma *et al*., 2017; Wiley *et al*., 2017). Aside from aiding resistance to pathogens and influencing the immune system, the gut microbiota also regulates digestion and metabolism, controls epithelial cell differentiation and proliferation, modulates insulin resistance and secretion (Sekirov *et al*., 2010; Chung *et al*., 2012; Arpaia *et al*., 2013; Kelly *et al*., 2015; Rothschild *et al*., 2018),

Type 2 diabetes mellitus (T2D) is a chronic, multifactorial metabolic disease. It is characterized by the body's inability to absorb glucose (hyperglycemia) due to a decrease in insulin secretion, insulin action (insulin resistance), or a combination of both. T2D patients have insulin resistance or relative insulin deficiency. Environmental and genetic predispositions are the important causes of T2D. A sedentary lifestyle, and excessive feeding of unhealthy diet, may be causes of T2D, and with the incidence of T2D is rising exponentially. Because the pathogenesis of T2D is multifactorial, the alternation of different gene products may be considered (Stumvoll *et al*., 2005).

In addition to gastrointestinal diseases, the microbiota directly effects on the development of diseases such as type 2 diabetes mellitus DM2. (Relman, 2015). However, it has been suggested that an adequate balance between Firmicutes and Bacteroidetes that is necessary to avoid the appearance of this type of disease (Magne *et al*., 2020). The gut microbiome plays a central role in regulating glucose and energy balance. It also plays a prominent role in obesity and controlling blood sugar, Namely, type 2 diabetes mellitus (Harris *et al*., 2012).

Nucleic acids (RNA and DNA) that were directly isolated from stool in recent years have allowed researchers to identify the constituents of gut microbes through analysis. Most of these methods rely on DNA extraction and *16srRNA* gene (rRNA) amplification (Mizrahi-Man *et al*., 2013; Poretsky *et al*., 2014). A frequently helpful technique for demonstrating the diversity and abundance of the microbiome is 16srRNA sequencing. For the characterization of microbial strains, gene sequencing can be used in conjunction with polymerase chain reaction (PCR) and metagenomic sequencing (Nelson *et al*., 2010).

This study aimed to:

The Study the impact of differences in gut bacterial microbiome on DM type 2 patients as compared with healthy which was achieved by the following objectives:

1- Isolation and genetic identification of bacteria isolated from patients with type 2 diabetes and healthy subjects.

2-DNA extraction from stool specimens to DM2patients and compared with healthy control individual for detection some Bacterial microbiome.

3-Amplification of identification gene 16SrRNA for all Bacterial microbiome in DM2 patients and healthy control individual.

4-Metagenomic and meta-analysis using next-generation sequencing and software packages concerning assembly, processing, clustering, alpha, and beta diversity.

Chapter two

Literature Review

2. Literature Review

2.1 **Microbiome**.

Former Nobel Prize winner Joshua Lederberg introduced the term "human microbiome" in 2001 (Lederberg and McCray, 2001). Microorganisms frequently live in communities and can establish symbiotic, commensal, mutualistic, parasitic, or pathogenic relationships with complex living things such as plants and humans. This collection of microorganisms is called the microbiome.

The microbiome refers to a group of microbes and their genomic content with microorganisms (Uresell *et al*., 2012). The human body's microbiome can be found everywhere, from the skin to the intestines (proal *et al*., 2014). According to various statistics, various human body parts are home to more than 10,000 different microbial species. (Blaser,2006., Ley *et al*., 2006). While the variety of microbes in the skin and vaginal sites is relatively low, it can be found in great diversity in other sites, such as the gut (Jiang *et al*., 2009).

The "gut microbiome," a group of bacteria, fungi, viruses, archaea, and eukaryotes that colonize the GI tract, has co-evolved with the host over thousands of years to develop a complex and beneficial interaction. (Backhed, 2005; Neish, 2009).

2.2. The gut microbiome.

The human gut microbiome co-evolves with its host for thousands of years, and thus widely participated in the group diverse of basic activities in the host, such as digestion and nutrition (Hehemann, *et al*., 2010), detoxification and protect of the body (Relman, 2012). The maturation of the host immune system (Turnbaugh and

Gordon, 2009) and disease mediation (Kau *et al*., 2011; Baldrige *et al*., 2015). In general, the diet of the family and its evolution contributes to the adjustment of the composition of the gut microbial community in mammals and other species (Rawls *et al*., 2006; Ley *et al*.,2008; Miyake *et al*., 2015).

2.3. Microbiota.

The term "microbiota" refers to the diverse range of bacteria, archaea, viruses, fungi, and protozoans that live in a particular location on or within the body. (Marchesi and Ravel, 2015; Gilbert and Lynch, 2019), human health is strongly influenced by microbiota that co-habiting with our body (Redman and Falkow, 2001). An adult human is colonized by approximately 100 trillion microbes found predominantly in the gastrointestinal tract (GIT), of which the largest population resides in the colon (Clave *et al*., 2014). The vast majority of gut microbiota belong to four main families (phyla): —*Firmicutes, Bacteroidetes, Proteobacteria* and *Actinobacteria* (Delzenne and Cani,2011; Kootte *et al*., 2012). Other smaller but relevant phyla include the *Verrucomicrobia* and *Fusobacteria* (Turn Baugh *et al*., 2007). Under normal physiological conditions *Firmicutes* make up the greatest proportion of the gut microbiota (64%), followed by the *Bacteroidetes* (23%), *Proteobacteria* (8%) and lastly *Actinobacteria* (3%), evidence suggests that gut microbiota can influence human health either directly or indirectly (Kaur *et al*., 2011; Clemente *et al*., 2012). Disruption to stable communities may increase the prevalence of pro-inflammatory conditions such as obesity, inflammatory bowel disease, T2DM, arthritis and cancer (Guarner and Malagelada, 2003). Figure (2-1): Definitions of the microbiome, microbiota.

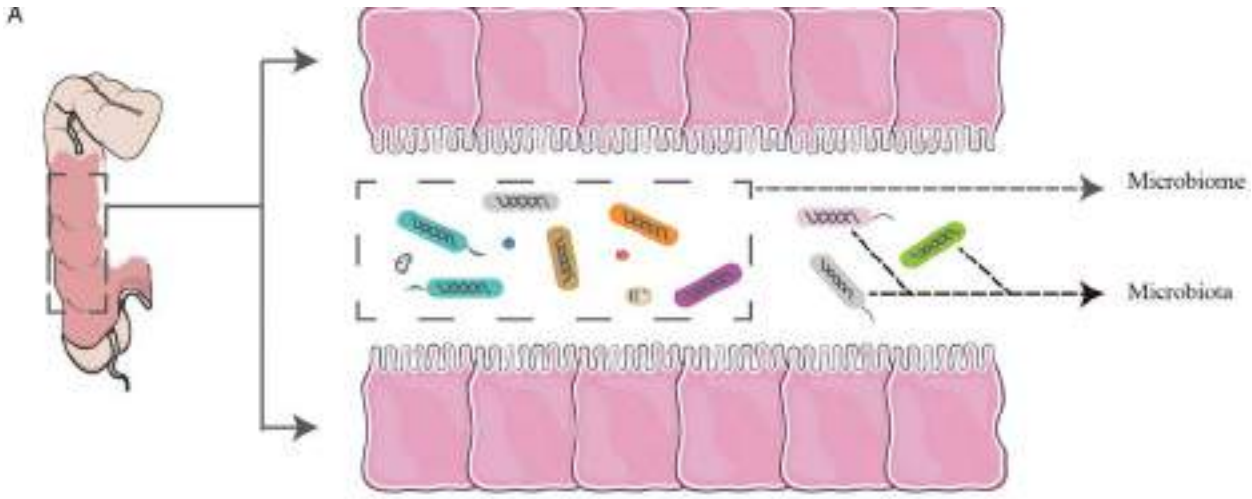


Figure (2-1): Definitions of the microbiome, microbiota. (Qian *et al*., 2020).

The concept of microbiome covers not only the microorganisms but also the surrounding environmental conditions. Microbiota only means microorganisms.

2.4. Importance and composition of gut microbiota.

The gut microbiota is a group of microorganisms that settle down the gastrointestinal tract at a higher ration than the cells of the human body (Breban, 2016). About half of the faecal mass comprises the microorganisms that are principally grouped into five phyla, Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia, and Actinobacteria*:* essentially with a predominance of 90% of the first two (Wu *et al*., 2020). because the diversity of microorganisms, makes it the most main environmental agent, and the gut microbiota is directly linked to the health of the host and some diseases (Thakur *et al*., 2016).

Once the microbiota is created in an individual, they often change in a short time, and even changes are generated in the stages during a person's life, although their great variability changes the composition of the gut microbiota has significant implications for the Pathogenesis of a wide range of diseases from chronic

gastrointestinal diseases to neurological disorders. The influence of gut microbiota in the development of diseases is so clear that some studies have appearance changes in the serotonergic neurotransmitter of the central nervous system (CNS) which were secondary to disruption of the gut microbiota transport which were secondary to disruption of the gut microbiota imbalance (symbiosis), (Margolis *et al*., 2021). The gut microbiota is involved in a variety of metabolic functions in food such as fermentation and absorption of undigested carbohydrates, absorption of salts and minerals, modulation of intestinal motility, and the synthesis of some micronutrients. (Gill *et al*., 2006),changing the gut microbiota, including host genes, diet, and age (Jandhyala *et al*., 2015; Odamaki *et al*., 2016), due to its role in the development of these functions, it has been suggested, that microbial changes in the human gut as a possible cause of obesity. Addition to their metabolic functions, germs participate in interaction with the system immunoglobulin, produced signals to promote immune cell maturation and normal development for its functions, as well as destroying toxins and carcinogens, avoiding colonization of pathogenic bacteria (Ley *et al*., 2005).

The gut microbiota is a group of organisms that provide various usefulness and make up resistance to colonization of new species, saving a symbiotic relationship with the host. However, an imbalance can produce in this complex community leading to recolonizing by pathogenic microorganisms, causing inflammatory processes and the development of various diseases (Gibson *et al*., 2014). This indicates that the gut microbiota maintains the homeostasis of the human gut (Clemente *et al*., 2012), and provides numerous effects such as protection from pathogens, digestion of carbohydrates, regulation of fat storage, and production of

essential vitamins, modulating the immune response, representing an environmental factor of great use in human homeostasis (Martinez *et al*., 2013).

2.5. Microbiota phyla in life stages in human.

The human body harbours more than 1,000 phylotypes on the level of species, but most of the intestinal bacteria belong to a few phyla. In adults, Bacteroidetes and Firmicutes often dominate the intestinal microflora, while Proteobacteria, Actinobacteria and Verocombemicrobes are in a rather small percentage. Methanogenic archaea (represented by methanobrevibacter), eukaryotes (mainly yeast) viruses (mainly phages) are also components of this microbiota (Kim *et al*., 2013; Eckburg *et al*., 2015) Despite the consistency in composition globally, the intestinal microbiota appears to be highly variable between individuals at the species level; phylotypes; usually *Faecalibacterium prausnitzii* (*F. prausnitzii*), *Roseburia intestinalis*, *Bacteroides uniformis*, and species of bifidobacteria and lactobacilli are present in most people (Lozupone *et al*., 2012). Intestinal microbial colonization starts in infants immediately after birth. facultative anaerobes, such as bacteria *Enterococcus*, and lactobacilli are the first colonize.The anaerobic Bacteroides and Bifidobacterium, including *Clostridium* create a gradual, contribution to the gradual decline of facultative anaerobes to a strict anaerobic ratio with time (Arboleya *et al*., 2012), about 3 years of age, spores arrive intestines are of same composition and diversity to adults and remain stable even somewhat over time into adulthood, new changes appear old. The microbes of the elderly differ from those of the basic microbiome and levels of diversity in younger adults (Claesson *et al*., 2012; Salazar *et al*., 2013), the microbiome has been identified and proposed to be a major factor in changing human health, so much so that it has been suggested to be an 'essential organ' in the human body (Kashyap *et al*., 2017, Wang *et al*., 2017). while It has

been "dysbiosis" distinct changes in the composition of the microbiome a condition known as describing it in various diseases, determining the distinct composition of the "healthy" microbiome was a matter. is difficult, due to the variance between individuals (Lloyd-Price *et al*., 2016)

The human body contains a variety of bacterial species, with a lower representation of viral and eukaryotic microbes, which is indicated It is referred to as the "Microbe Bank". Because the human intestine houses most microbes and can be considered a "microbe" It consists of a complex set of microbial communities that interact with each other as well as with the host, in a way that affects the health of the host (Clemente *et al*., 2012). The major bacterial divisions, Bacteroidetes and Firmicutes with subgroups, Proteobacteria, Cyanobacteria, Fusobacterial, Actinobacteria , Verrucomicrobia and a few others. A healthy intestinal tract is relatively stable throughout puberty, but with the ageing process, disturbances occur with external factors Such as the use of antibiotics, diet and internal factors such as cellular stress. ageing and related complications relevance is a major public health concern worldwide (Kohl *et al*., 2012). Ageing is accompanied by significant physiological changes such as the change in the gut microbial composition, (dysbiosis) and immune responses and metabolism that may lead to different inflammatory conditions (Li and Jasper, 2016), and autoimmune disorders (Cherubini *et al*., 2012). Any compositional differences with age have a direct impact on intestinal motility and digestion (Kleessen *et al*., 1997). Fermentation processes in the colonic gut are altered adversely with variations in the microbiota. This affects the homeostasis in the gut, leading to immunosenescence (decline in immune responses) and inflame-ageing, that is, low-grade inflammatory response (Franceschi, 2007, Magrone and Jirillo,2013) in the early childhood stage (between 1 and 5 years), the expansion of bacterial diversity slows down, and diversity

remains gut microbiome is lower compared to adults. Gut microbiota Childhood is more stable and dominated by many members of Bacteroidetes in healthy pre-adolescence (from 7 to 12 years), the gut microbiome is rich in species, containing many bacterial taxa and similar functional genes, of adult microbes enriched with, Lachnospiraceae, Anaerovorax, Bifidobacterium, and Faecalibacterium The bacterial. composition in adults mostly from Firmicutes and Bacteroidetes, (Saraswati and Sitaraman,2015), other groups of researchers have reported having bacteria of the phylum, Actinobacteria and, Proteobacteria, Verrucomicrobia Fusobacteria and Cyanobacteria in addition to methanogenic archaea, multiple phages and Eucarya in healthy individuals (Hayashi *et al*., 2002, Wang *et al*., 2007, Virgin *et al*., 2009). Figure (2-2) gut microbiota phyla in different life stages.

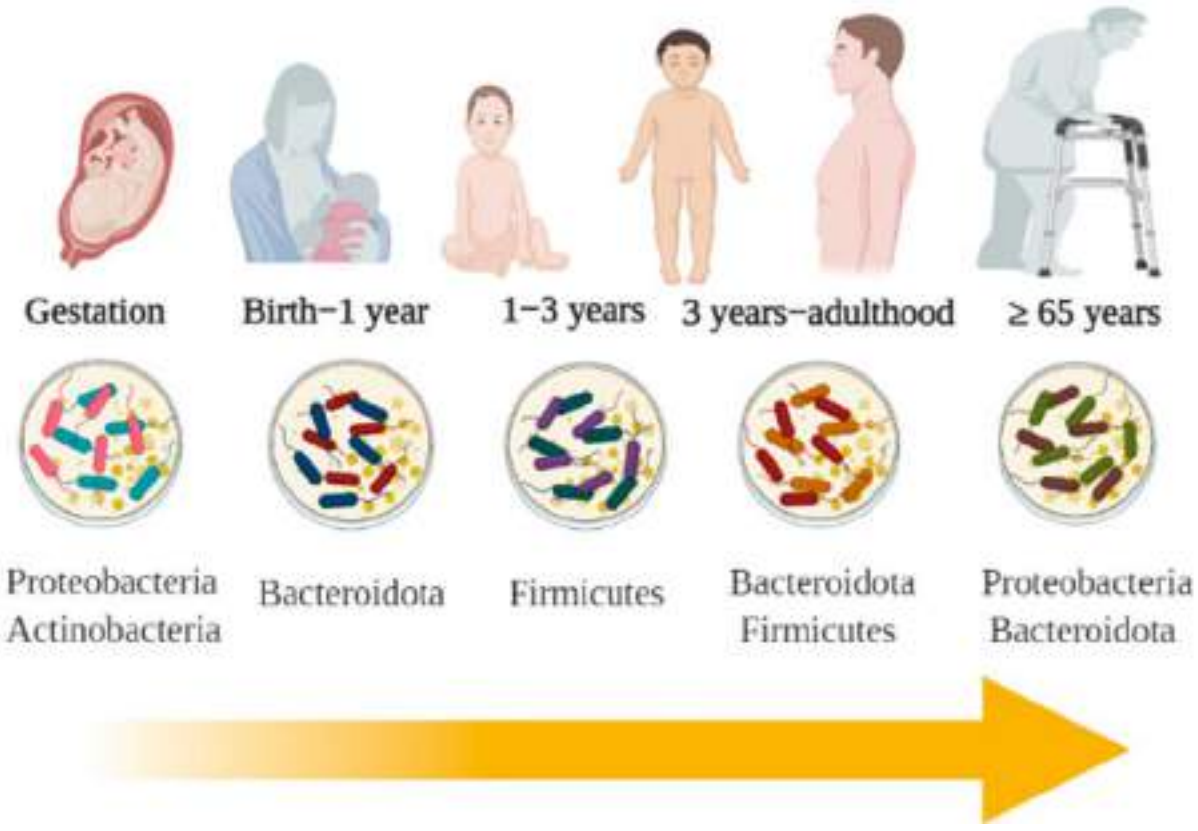


Figure (2-2). Dominant gut microbiota phyla in different life stages. (Olvera-Rosales *et al*., 2021)

2.6. Microbiota in health and diseased humans

The balance of intestinal microbes has a direct bearing on human health and disease. compared to other parts of the body, the human GI tract is home to a large microbial ecosystem that contains about 100 trillion microbes. (Ley *et al*., 2006), Numerous research has been conducted to determine the crucial connection between the gut microbiota and fundamental human biological functions. For instance, recent research has demonstrated how intimately linked the human microbiota is to nutritional absorption, immunity, and metabolism. (Derrien*et al*., 2019). There are numerous ways that microbiota might influence biological processes. Microbiota is essential for nutrient and energy extraction from food due to the adaptable metabolic genes that produce separate, distinct enzymes and biochemical pathways. (Relman, 2016). The gut microbiota plays a critical role in the creation of bioactive compounds like lipids, vitamins, and amino acids. (Roberfroid *et al*., 1995) The human microbiota plays an important role in the development of the immune system and intestinal mucosa in addition to protecting the host from foreign pathogens by creating antimicrobial compounds.

The gut microbiota displays stability, toughness, and symbiotic relationships with the host when things are healthy. The idea of "healthy" gut microbiota and its connection to host physiological processes are the subject of extensive investigation. Bacteria, viruses, yeasts, and viruses make up the gut microbiota. High taxonomic diversity, high microbial gene richness, and stable core microbiota are frequently seen in a healthy microbiota community. (Fan and Pedersen, 2021).

2.6.1. Hypertension

Emerging evidence has further reinforced the claim that gut microbiota is critical to maintaining physiological balance. Dysbiosis has been reported in richness, diversity, proportion Persistence/biomass and Firmicutes in the gut microbiome of hypertension in both studies of animal and human (Yang *et al*., 2015)

2.6.2. Cardiovascular Disease

Despite encouraging advances in the prevention and treatment of arterial thrombosis cardiovascular diseases (CVD), it's still a leading cause of death and disability in the world and will continue to grow mainly because of the increase in low- and middle-income countries (LMIC) (Bansilal *et al*., 2015). the potential role of the microbiome in metabolic diseases, especially cardiovascular disease, is a focus of recent investigations (Liu *et al*., 2016) It has been clear that the composition of the gut microbiome is associated with both atherosclerosis and arterial stiffness markers (Kashtanova *et al*., 2017)

2.6.3. Cancer

The human microbiome is receiving a lot of attention for its complex relationship to the development of cancer It is believed to account for about 20% of all cancer cases worldwide (Wong *et al*., 2018), with a better understanding of the role of the microbiome in causing cancer, the potential for microbiome-based therapies in the treatment of cancer is a topic of growing research. In patients with, prostate cancer was as *F. prausnitzii* and *Eubacterium* were observed in lower abundance suggesting a possible role of these bacteria in the development of prostate cancer (Golombos *et al*., 2018).

2.6.4. Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is often associated with symbiosis by a shift toward an elevated abundance of microbes capable of dealing with oxidative stress with increased facultative anaerobic bacteria of the Enterobacteriaceae family.

Ruminococcus gnavus abundance the intestinal family abundance also found elevated in IBD has also may some strains have evolved to thrive in the gut environment of IBD through mechanisms of oxidative stress responses (Hall *et al*., 2017).

2.6.5. Gout

Gout is an inherited or acquired metabolic disease with severe arthritic symptoms caused by Increased synthesis of uric acid caused by abnormalities of purine metabolism (Zhou *et al*., 2014). The gut microbiome of gout patients is dysregulated compared to healthy individuals with an increased abundance of opportunistic pathogens, a similar enrichment was also observed in autoimmune diseases (Shao *et al*., 2017).

2.6.6. Depression

The gut microbiome is increasingly being described as being implicated in many neuropsychiatric disorders, with particularly strong evidence for its role in depression (Bastiaanssen *et al*., 2019). Researchers have exploring the potential and efficacy of using probiotics to provide mental health benefits to patients diagnosed with mental illness (Ostlund *et al*., 2016).

2.6.7. Arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joints It causes bone and cartilage damage and even disability. (Gibofsky, 2014; Smolen *et al*., 2016). however, a lot of the aetiology is still unknown, and it has been suggested that the gut microbiome plays a role in causing disease. (Scher and Abramson, 2011) Intestinal and oral microbiome dysbiosis is observed in patients with rheumatoid arthritis and changes in the microbiome were able to distinguish between inflammatory patients and healthy individuals, *Haemophilus* spp was found in low abundance and was negatively correlated with the level of autoantibodies in the blood of rheumatoid arthritis patients. on the other hand, the abundance of saliva Lactobacillus was found to be high (Zhang *et al*., 2015).

2.6.8. Obesity

Obesity is a chronic systemic disease characterized by excessive fat accumulation. has increased tremendously over the past 40 years affecting nearly 40% of adults and 19 of young people in the united states. It is defined as a body mass index of 30 or more for adults and more than 95 per cent for the respective age group and gender among youth. (Yanovski *et al*, 2015; Bischoff *et al*., 2017). Obesity component of metabolic syndrome, it is a major risk factor for type 2 diabetes which accounts for 90-95% of all cases of diabetes (Gill *et al*., 2006).

2.6.9. Diabetes mellitus

Diabetes is a chronic disease caused by a genetic or acquired deficiency in the production of insulin by the pancreas or the inability of the body to proper use produced insulin (WHO, 2016). Diabetes affects more than 420 millpons people worldwide, and this number may continue to increase in the future. World Health Organization (2016), expected to be affected About 630 million people will fall ill

worldwide in 2045. (IDF 2017). Diabetes is a Global severely affects public healthcare expenditures at an estimated cost of $827 billion worldwide (Seuring *et al*., 2015). three main types of diabetes are type 1 and type 2 and gestational diabetes. However, there are some other types of disease, for example, diabetes-like latent autoimmunity in adults, (LADA).

Type 1 diabetes (also called insulin-dependent, youth or early childhood) is characterized by reduced production of insulin and requires daily intake of this hormone. The cause of the injury type 1 diabetes is unknown and cannot be prevented with current knowledge (WHO, 2016).

Diabetic LADA, (latent autoimmunity, diabetes in adults) is a special subtype of type 1 diabetes that is characterized by slow β cell damage in the islets damage. (Xiang *et al*., 2015) usually appear early signs of diabetes mellitus from LADA patients with type 2, resulting in a non-negligible prognosis rate. It is about 6% among Ladas, and it is estimated that the incidence of patients newly diagnosed with type 2 diabetes. (Martinell *et al*., 2016).

Diabetes, Maturity-onset diabetes of the young (MODY) It is type that begins at maturity in young adults A subtype of diabetes mellitus is characterized by early-onset (usually under 25 years of age) and autosomal dominant transmission (determined in three generations at least). It corresponds to a fundamental defect in insulin secretion associated with pancreatic β-cell dysfunction (Nyunt *et al*., 2009).

Gestational diabetes is characterized by hyperglycemia (high blood sugar) that appears during pregnancy and reaches values of, on Although it is higher than normal, it is lower than that Specific for the diagnosis of diabetes. women with diabetes Pregnancy is more likely to have complications during pregnancy and

childbirth. In addition, they and their children are more likely to get sick Type 2 diabetes in the future (WHO 2016.).

Clinical (DM2) type 2 diabetes mellitus syndrome with variable phenotypic expression, with no specific aetiology. It is a disease of a polygenic nature mediated by the environment and characterized by bi-hormonal dysfunction in the pancreas (Santos *et al*., 2014) and the consequent release of homeostasis mechanisms for blood glucose levels (American Diabetes Association, 2009). DM2 is a Chronic disease considered habits, lifestyle, social and economic factors that in essence, it has a deep relationship with inflammatory mechanisms (Peter and, Izakovicova, 2014). It is a major risk factor for heart disease and stroke has become leading, disease burden worldwide (Xu *et al*., 2013).

T2D is a metabolic disease, related to other factors such as the gut microbiota, genetic predisposition, and physical inactivity. and psychological pressure. (Wellen and Hotamisligil, 2005).

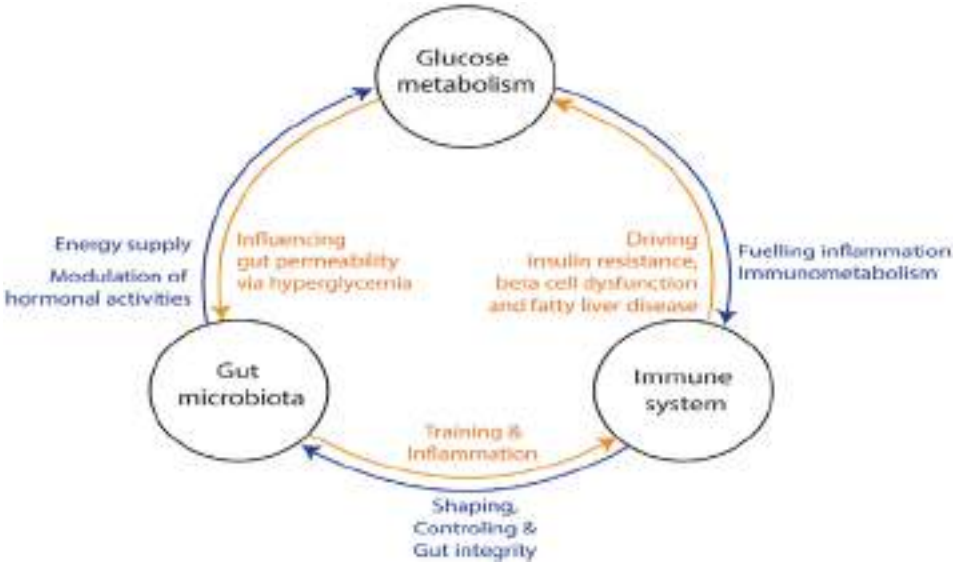


Figure (2-3) Three-way interaction between the gut microbiota, glucose metabolism, and the immune system. (Scheithauer *et al*., 2020)

The gut microbiota influences the host ‘s glucose metabolism and hormone production via the production of several metabolites. Hyperglycemia increases gut permeability and thereby translocation of bacterial components into the circulation. In turn, bacterial translocation is fueling a (pro) inflammatory response of the immune system. Under normal conditions, the gut microbiota is training the immune system via several bacterial components and metabolites.The immune system is shaping and controls gut microbiota to keep a symbiotic relationship between host and microbiota. Further, it prevents bacterial translocation viabyomoting gut integrity. Bacterial translocation may lead to inflammation in several tissues and consequential loss of function (e.g., beta-cell dysfunction, insulin resistance and fatty liver disease). The glucose metabolism can induce a pro-inflammatory response of the immune system through the interplay of metabolic and inflammatory pathways (immunometabolism). Thereby, all three factors affect each other and may drive metabolic diseases.

2.7. Diabetes and Gut Microbiome

It is becoming increasingly clear that the gut microbiota is contributing to many human diseases including diabetes (T1D) type I and type II. type 1 diabetes, is an autoimmune disease caused by the destruction of pancreatic cell it is primarily caused by the immune system. Although the T1D result from genetic defects, it has been shown that epigenetic and environmental factors play important role in this disease, have been reported at higher rates in recent years. That is not explained by genetic factors and is attributed to changes in our lifestyles such as diet and hygiene and the use of antibiotics that can directly affect germs (Gulden *et al*., 2018). It has been proven that the incidence of diabetes in non-obese diabetic patients or germ-free patients. T1D has increased significantly. That is in line with the observation

that rates are higher in countries with strict sanitary practices (Gülden, *et al*., 2015), In 2010 Larsen et al, performed the first study to demonstrate the strong relationship between the human gut microbiota and individuals with type 2 diabetes. (Larsen *et al*., 2010).

2.8. Factors affecting the formation of the gut microbiota

The composition of the gut microbiota is affected by many factors such as the diet system, disease status, medications as well as host genes as a result the composition changes. the gut microbiome is constantly affecting the health and well-being of the host such as the disease state as well as the use of various drugs like antibiotics (Tai *et al*., 2015), as showing in figure (2-4).

2.8.1. Age and delivery style

The intestinal microbial colonization process starts in utero by microbiota in the amniotic fluid and placenta (Collado *et al*., 2016). Studies have reported that there are bacteria and bacterial products such as DNA in meconium; (Nagpal *et al*., 2017; Wampach *et al*., 2017) amniotic fluid (DiGulio *et al*., 2008; Collado *et al*., 2016). and the placenta (Friedrich, 2013; Collado *et al*., 2016) Studies have shown that pregnant mice that ingested orally labelled *Enterococcus fecium* firm stare led to its isolation from a newborn stool sample, (Jiménez *et al*., 2008) this result is consistent with the evidence maternal microbes being transferred into the amniotic fluid (Jiménez *et al*.,2008; Collado *et al*., 2016) and the placenta (Goldenberg and Andrews, 2000). After childbirth, the manner of delivery affects the early development of the gut microbiota. newborns who are born vaginally have the primary gut microbiota dominated by *Lactobacillus, prevotella* which is derived from the mother's vaginal microbiota, while those born by caesarean section derive

the gut microbiota from the skin, which) leads to the dominance of *Streptococcus, Corynebacterium, and Propionibacterium* (Mackie, 1999; Dominguez *et al*., 2010), these primary microbes evolve to become more diverse and stable relatively, at 3 years of age, it becomes similar to the gut microbiome of adults (Yatsonenko *et al*., 2012).

2.8.2. Antibiotics

Antibiotics are a double-edged sword: they destroy both pathogenic and beneficial microbes randomly, allowing the loss of gut microbiota or the so-called dysbiosis and the growth, of unwanted microbes (Klingensmith and Coopersmith, 2016). studies have shown conducted on experimental mice showed that administration of antibiotics affected secondary bile acid and also serotonin in the colon resulting in delayed bowel movement by causing exhaustion antibiotics disrupt the competitive exclusion mechanism, a property of germs (Ge *et al*., 2017). Antibiotics disrupt the competitive exclusion machinery, a basic property by which microbiota eliminate pathological microbes (Hehemann *et al*., 2010), and this disturbance the growth of other pathogens, such as *Clostridium difficile* (Ramnani *et al*., 2012).

2.8.3. Diet

After birth, the first effect on the gut bacteria is the infant's diet (breast milk or artificial milk). milk composition influences the formation of the early gut microbiota (Guaraldi and Salvatore, 2012: Groer *et al*., 2014). In the breastfed infant, the species that dominate the gut microbiota; are *Lactobacillus* and *Bifidobacterium*. breast milk contains oligosaccharides that can they are easily degraded by these species, resulting in an excess of short-chain fatty acids, which directs the immune system to increase the expression of immunoglobulin G

(Ouwehand, and Salminen, 2002).Whereas in infant's predominant species Bacteroides, *Enterobacteria*, Clostridia, Enterococcus and Streptococcus (Stark, Lee and Parsonage, 1982; Yoshioka, Iseki and Fujita, 1983). Primary germs needed during childhood may play a basic role in primary immunity throughout the development of children,for this reason, the formation of primary germs during this period is very important to keep children from diseases., related to immunosuppression (Groer *et al* 2014., Sherman, Zaghouani, and Niklas, 2014).

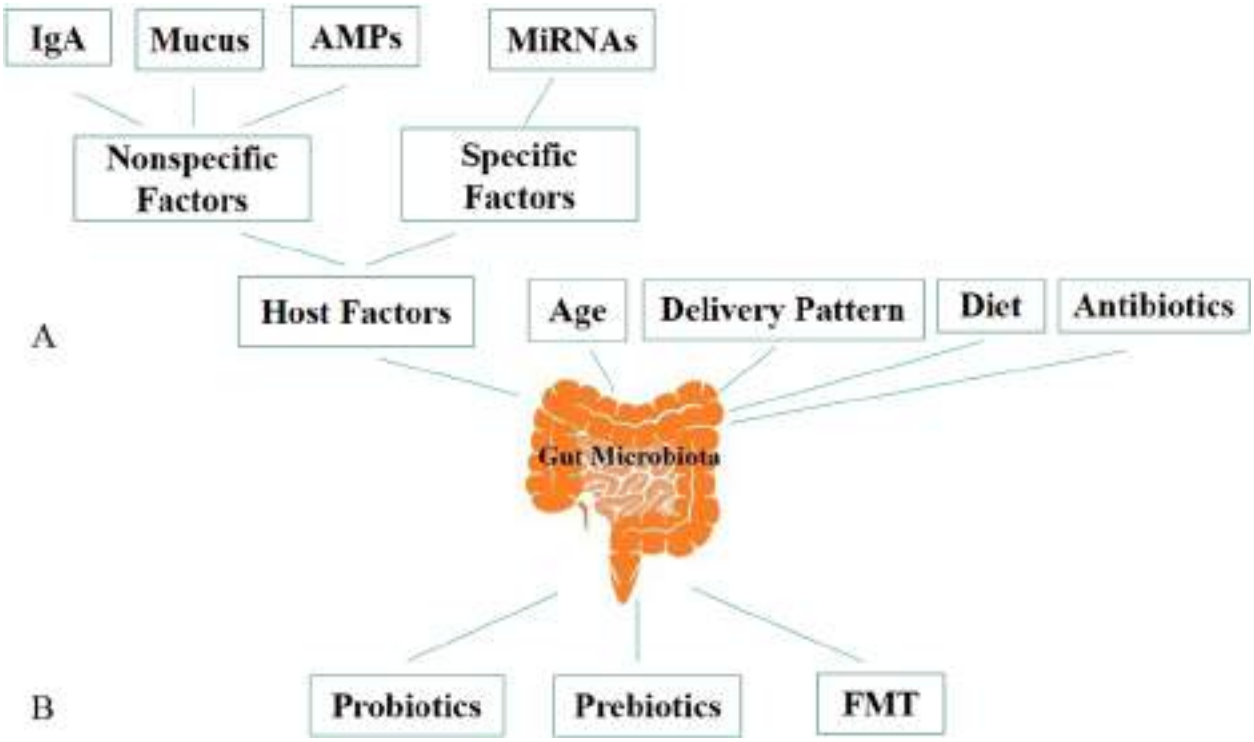


Figure (2-4) Factors affecting gut microbiota and ways to modulate it. (A) Factors affecting gut microbiota. (B)Ways to modulate gut microbiota. AMPs, antimicrobial peptides; IgA, immunoglobulin A; miRNA, microRNA; FMT, faecal microbiota transplantation. (Cunningham *et al*., 2021)

2.9. Next Generation Sequencing.

2. 9 .1. Library preparation and quality control conduction.

The majority of DNA sequencing projects are currently being using different platforms the Illumina platforms series for DNA sequencing compares high-throughput short reading procedures with favourable performance. The short parts that HiSeq and Illumina MiSeq prefer, ranging from 150 to 550 basis points, are where these approaches are constrained. Due to this, numerous unique primer combinations have been developed to specifically target various regions of 16S rRNA on various marker genes, such as bacterial genes (Kumar *et al*., 2011; Huse *et al*., 2020).

Currently, the bulk of gun sequence projects uses the short reading frame and high-throughput DNA Illumina metagenomics sequencing (Olson *et al*., 2020). There are several creation techniques that, generally speaking, use the various series libraries from three to four phases from Illumina: DNA fragmentation targeted DNA fragment repair, and adapter ligation optional collection (Sato *et al*., 2020). Platform-specific PCR was carried out and was amplified using a variety of techniques, including Enzymatic digestion, mechanical shearing, and the use of transposons are a few of them (Sinha *et al*., 2017). The normal analysis starts with a quality filter to weed out data with critical calls that are unclear or prone to inaccuracy. This move is no longer used because of a significant volume of bad data, although it has been shown to have very little bias against object recognition in areas of low abundance (Nearing *et al*., 2021). Readings must be placed into an analytical unit using either noise reduction methods for error correction or operational classification unit selection once the quality has been filtered (OUT).

2.9.2. Bioinformatics Analysis.

The 454-pyrosequencing data sets are analyzing with the Quantitative Insights into microbial Ecology (QIIME) program as previously described by Moreno-Indias *et al* (2015). After going through the 454-amplicon processing pipeline, raw reads are filtered first. The split library.py script of QIIME are used to further de-multiplex and filter the pyrosequencing reads. To increase accuracy, readings having an average quality score of less than 25, ambiguous base calls, primer mismatches, or lengths of less than 100 bp were not included in the study. Following the quality filter, the following pipeline analysis are applying to examine the 16S gene reads: Singletons are remove and sequences are denoised. By grouping sequences with a similarity of >97%, operational taxonomic units (OTUs) were chosen. The representative sequences, chosen as the most abundant in each cluster, are then send to the UCLUST, which use the green genes 16S rRNA gene database to determine the taxonomy assignment and relative abundance of each OTU. As mentioned, QIIME is use to assess alpha and beta diversity (De Filippis et al., 2013). When samples are determined using 16S ribosomal RNA (rRNA) gene (also known as rDNA) sequencing.

2.9.2.1.Alpha-diversity.

The term "a-diversity" describes the variety found in a sample, such as a faecal sample. (Gilbert and Lynch, 2019). There are many of indices as following:

2.9.2.1.1. The Chao 1 index.

A richness indicator that counts the number of species in a sample (Xia and Chen, 2018). The three factors taken into account are the number of species, the number of singleton taxa, and the number of doubleton taxa, as a result, it is unable to record the microbiota's abundance. (Xia and Chen, 2018).

2.9.2.1.2.The Shannon-Wiener index.

Possesses both richness and evenness. (Borcard *et al*., 2018) Rare species are given additional consideration (Xia and Chen, 2018). which indicates that it rises when more uncommon species are discovered. Its value often doesn't go above 5, and the greater it is, the more a-diversity there is.

2.9.2.1.3 Simpson index.

Richness and evenness are combined in the Simpson index as well. However, it prioritizes common species more so than the Shannon-Wiener index does. Its value spans from 0 to virtually 1, and the higher it is, the more a-diversity there is in the world. (Xia and Chen,2018) Richness in the aforementioned indices refers to the total number of species in a sample, whereas abundance refers to the raw read counts of a species (Knight et al., 2018, Xia and Chen, 2018). When the raw read counts are normalized or converted to percentages, relative abundance is employed (Xia and Chen,2018).

2.9.2.2.Beta-diversity.

B-diversity describes variations in the microbiome found in various samples or groups. (Gilbert and Lynch, 2019). The compositional dissimilarity between two samples or groups is measured statistically using the following

1- Bray-Curtis dissimilarity.

Its value is 0 to 1, with 0 being the minimum. signifies that all species are shared by the two samples or groups, and translates to "they don't share any." (Bray and Curtis, 1957) Additionally, it provides more proportional to common species. (Borcard *et al*., 2018).

2- Jaccard Distance.

This is the proportion of members to the total are similar in both samples, and the number of distinct members; it is a gauge of similarity for the spans between 0 and two communities (the communities are distinct) and 1 (the two communities are identical). A statistic for evaluating the diversity and similarity of sample sets is the Jaccard index, commonly referred to as the Jaccard similarity coefficient. Grove Karl Gilbert created it in 1884 (Murphy, 1996).

3-UniFrace Distances

Distances in UniFrac the weighted or unweighted UniFrac distance estimates variations between samples or groups when weighted based on phylogenetic distance. (Lozupone and Knight 2005) The unweighted UniFrac distance considers the existence and lack of taxa. It has richness detection sensitivity. Adaptations in endangered species while ignoring abundance a computation's use of data. (Chen *et al*., 2012).The weighted UniFrac Distance takes into account the wealth of information (Lozupone *et al*., 2007) and minimizes the impact of uncommon species. (Xia *et al*., 2018). The phylogenetic tree is taken into account by the UF and weighted unifrac distances between two samples, which results in phylogenetic distances between community members (Lozupone *et al*., 2007).

In weighted unifrac, branch lengths are weighted by the relative abundance of sequences, whereas in UF, the distance is determined as the fraction of the branch length. (Lozupone *et al*., 2007).

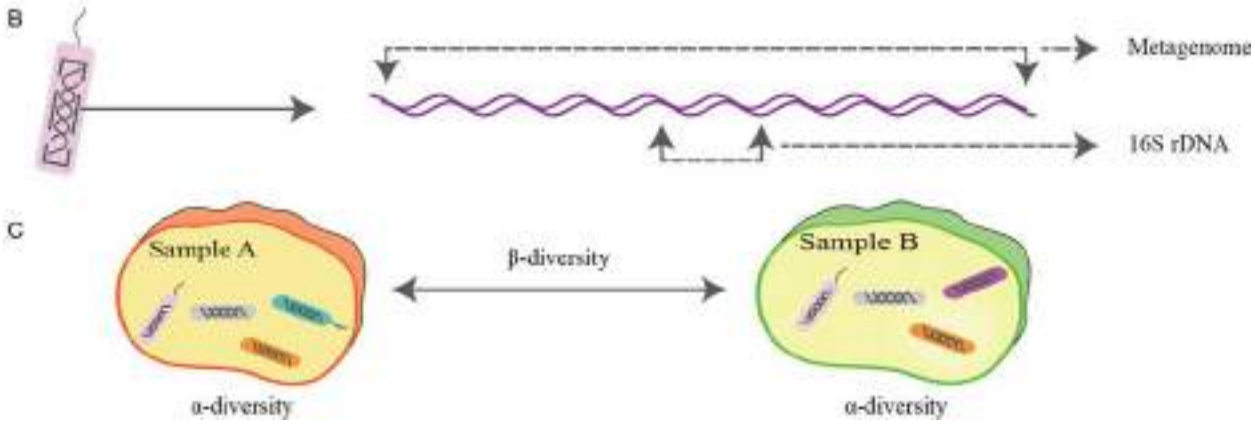


Figure (2-5): Definitions of, metagenome, and 16S rDNA. (B) Metagenome means all genomes of the microorganisms, while 16S rDNA only covers a segment of the genomes. (C) a-diversity measures the diversity within a sample, while b-diversity compares the difference between samples. (Qian *et al*., 2020).

Chapter Three

Materials and Methods

3. Materials and Methods:

3.1. Materials:

3.1.1: Apparatuses and Tools.

Apparatuses and tools used in this study are summarized in table (3-1).

Table (3-1): Apparatuses and tools used in this study.

No	Tools and Apparatus	Company/Origin
1	Autoclave	Hirayama/ Japan
2	Beakers	Iso Lab/ Germany
3	Biosafety Cabinet	Lab Tech/ France
4	Cylinder	Iso Lab/ Germany
5	Electrophoresis apparatus	Consort/ Belgium
6	Eppendrof tubes	Bione/ South Korea
7	Flask (250, 500, 1000)	Iso Lab/ Germany
8	Gel Documentaion	Vilber lourmat/ France
9	Gloves	Broche/ Malaysia
10	Incubator	Human Lab/South Korea
11	Micropipettes	Dragon MED/ China
12	Oven	Memmert/ Germany
13	Water Path	Memmert/ Germany
14	Sensitive Balance	Sartorius/ Germany
15	Tips	Sterling/Ltd./UK
16	Vortex mixture	Medilab/ South Korea
17	Spectro photometer	Shimadzu/ India
18	Thermal cycler apparatus	Prime/UK

3.1.2: Chemical and Biological materials.

The chemical and biological materials that used in the current study are in tables (3-2).

Table (3-2): Chemical and Biological materials used in the present study.

No	Material	Company/Origin
1	Absolute Ethanol	Scharlau /Spain
2	Agarose	Biobasic / Canada
3	50X TBE (Tris- Boric acid EDTA	
4	DNA Ladder (100bp)	Bioneer/South Korea
5	Ethidium bromide	Promega/ USA
6	Distal water	

3.1.3: The Kits.

The kits used in the current study are listed in the table (3-3).

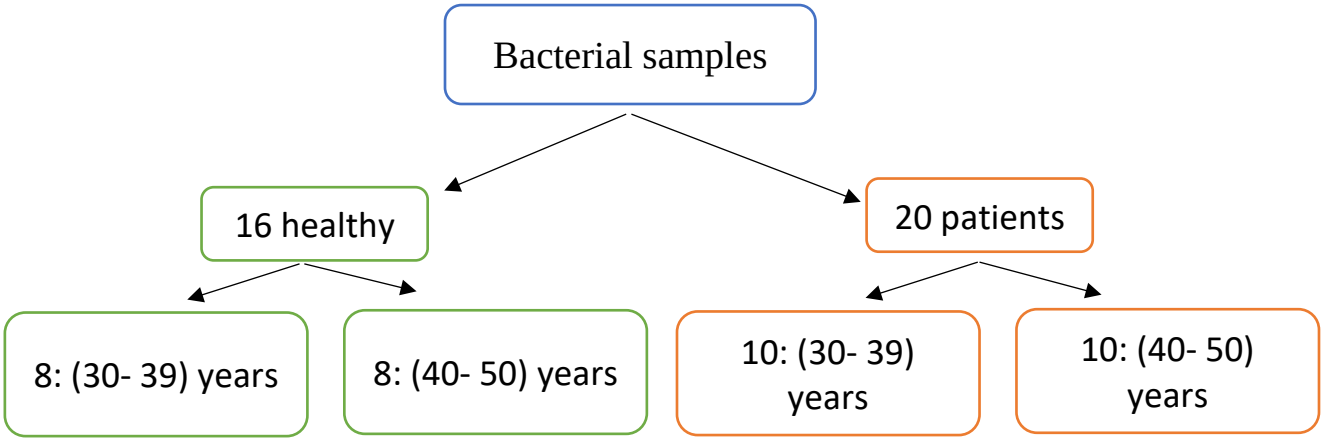
Table (3-3): kits used in the present study.

No	Kits type	Purpose	Company/Origins
1	QIA amp (fast DNA stool)	Extraction of DNA from stool	Qiagen/ Germany
2	Accupower^{R} PCR premix	Primer for amplified PCR	Bioneer/ South Korea

3.2: Methods:

Steps of study.

1) Thirty-six collected stool samples were divided as followed layout:



2) DNA were extracted by using Minikit QIAamp fast DNA stool.
3) 16S rRNA gene was amplified by PCR.
4) PCR products were electrophorized by gel electrophoresis.
5) Analysis of results by bioinformatics software by macrogen NGS manual – OUT (Bioinformatics)
   a) Assembly- Misq PE,by(flash).
   b) Pre-processing (denoi sing) by CD-HIT-OTU\rDna tools.
   c) Clustering by CD-HIT-OTU\ rDna tools.
   d) Diversity analysis by QIIME
      i) Alpha-diversity ⟶ Alpha-diversity
      ii) Taxonomy ⟶ UNITE(ITS).
      iii) Alpha- Rarefaction ⟶ Alpha- Rarefaction
      iv) PCOA ⟶ make-2d-plots.py.
      v) UPGMA Tree ⟶ upgma- cluster.py.

Figure (3-1) The study layout.

Table: (3-4) Processes programs and results of macrogen

Process		Program		Result
Assembly -MiSeq PE		FLASH		Assembled reads (SE, fastq)
Pre-processing (denoising)		CD-HIT-OTU / rDnaTools		Removed noise data
Clustering		CD-HIT-OTU / rDnaTools		OTU cluster
Diversity Analysis	Alpha-diversity	QIIME	alpha_diversity.py	OTUs, Chao1, Shannon, Simpson…
	Taxonomy		UCLSUT / RDP(16S) or UNITE(ITS)	Taxonomy Composition
	Alpha-Rarefaction		alpha_rarefaction.py	Rarefaction curve graph
	PCoA		make_2d_plots.py	PCoA graph - 2D, 3D
	UPGMA Tree		upgma_cluster.py	UPGMA Tree graph

3.2.1: Sterilization Methods:

3.2.1.1: Sterilization by dry Heat.

The glassware was sterilized by oven at 150 °C for two and a half hours.

3.2.1.2: Sterilization by autoclaving.

The culture media were sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 psi.

3.2.2: Genomic DNA extraction.

Genomic DNA was extracted from a stool by using a Genomic DNA mini kit fast DNA stool

Step 1: Sample Preparation:-

- Fresh stool (0.2 mg) was added to 2 ml microcentrifuge tube.
- one ml was added to inhibit Ex buffer by micropipette to each stool sample and vortexed continuously for 1 minute or until the stool sample was thoroughly homogenized.

- Microcenterifuge tube was microcentrifuged for 1 minute at 14000 rpm.
- Twenty-five µl of proteinase K (which should be diluted by added of distilled water) were added and tubes were incubated for 10 minutes at 60º C, the tubes were inverted during incubation every 3 minutes.
- Six hundred microliters of supernatant were added to proteinase K.

Step 2: Lysis Step: -

- Six hundred microliters of AL buffer were added to the sample and mixed well for 15 seconds by the vortex.
- The tubes were incubated at 70ºc for less than 10 minutes to ensure the sample lysate is clear, the tubes were inverted every 2 minutes during incubation.

Step 3: DNA Binding: -

- Six hundred microliters of absolute ethanol were added to the sample lysate and mixed immediately by shaking vigorously.
- Six hundred microliters of lysate from the step above were added to the QIAamp spin column.
- QIAamp spin column was centrifuged for 1 min.
- The QIAamp spin column was put in a new 2 ml collection tube and the tube containing the filtrate was discarded.

Step 4: Washing Step: -

- Five hundred microliters of W1 Buffer were added to the QIAamp spin column and then centrifuged at 1 min.
- The QIAamp spin column was put in a new 2 ml collection tube, and the tube containing the filtrate was discarded.
- Five hundred microliters of W2 Buffer were added to the QIAamp spin column and then centrifuged at 3 min.

- The QIAamp spin column was put in a new 2 ml collection tube, and the tube containing the filtrate was discarded.

Step 5: Elution: -

- Two hundred microliters of ATE Buffer were added to QIAamp spin column and incubated at 37 ºc for 1 min,then centrifuged for 1 min.

3.2.3: Detection of DNA Content by Agarose Gel Electrophoresis**: -**

Agarose gel was prepared according to Sambrook and Russell (2006) with some modifications used to confirm the integrity and presence of extracted DNA of bacterial isolates and performed as follows: -

1. One hundred of (1X TBE buffer) was placed in a flask.
2. One gram of agarose powder was added to 100 ml of (1X TBE buffer).
3. The solution was heated up to a boiling point by using a microwave until all the gel particles were dissolved.
4. Four microliters of ethidium bromide (0.5 mg./ ml) were added to the agarose solution, then stirred in the agarose to get mixing.
5. The solution was left to cool at room temperature.
6. The agarose solution was poured into the gel tray, and fixing the comb 1 cm away from one edge.
7. The agarose was left until solidified for 30 minutes at room temperature. After that, the fixed comb was removed carefully and the gel tray was placed in the gel tank. The tank was filled with 1X TBE buffer, until the buffer covered 3-5 mm the surface of the gel.
8. Five microliters of DNA sample were transferred to an Eppendorf tube and 2µl of loading dye was added to the tube and mixed well, the mixture was loaded into the wells of the agarose gel. Electric current was applied, 80 volts

for 1 hour. Finally, the bands were visualized at a wavelength of 350 nm on a UV transilluminator apparatus.

3.2.4: Master Mix.

The master mix components were mentioned in tables (3-5):

Table (3.5): Master Mix (AccuPower®PCR PreMix) used in Monoplex PCR**.**

No.	Component	Reaction volume 25 µl reaction
1-	Taq DNA polymerase	1U
2-	dNTPs (dATP, dCTP, dGTP, dTTP)	250 µM each
3-	Tris-HCL (PH 9.0)	10 mM
4-	KCl	30 mM
5-	MgCl2	1.5 mM
6-	Sterilizer and tracking dye1	Trace

3.2.5: Monoplex PCR Protocol.

The protocol was used according to the instructions of the manufacturer Bioneer. All components of PCR were assembled in a PCR tube and mixed by cooling microcentrifuge for 10 seconds at 850 rpm.

The Steps of Monoplex PCR were conducted as follows: -

1- Template DNA and Primers were dissolved before usage.

2- Primers and template DNA was added to the AccuPower®. Taq premix tubes as shown in table (3-6).

3-The lyophilized blue pellets were completely dissolved and spin down by using vortex apparatus.

4- The PCR Eppendorf tubes were placed in the thermocycler apparatus.

The appropriate PCR program conditions with some modifications according to Miyoshi *et al*., (2005) for universal primer are shown in table (3-6).

Table (3.6): The mixture of PCR.

NO.	PCR Master mix	Volume (µl)
1	DNA template	4 µ
2	Forward primer	1 µ
3	Reverse primer	1 µ
4	Master Mix	5 µ
5	Free ionized water	14 µ
6	Final volume	25 µ

Table (3-7): Monoplex PCR program**.**

PCR step	Temperature(°C)	Time	Repeat	Reference
Initial denaturation	94	1min	1	Miyoshi *et al.* (2005)
Denaturation	94	1min	30 cycles	
Annealing	52	35sec		
Extension	72	1min		
Final extension	72	7min	1	

3.2.6.16SrDNA Library Preparation Workflow.

The workflow for the 16SrDNA Library Preparation Protocol is shown in Figure (3- 1), Between each step, safe halting points were marked.

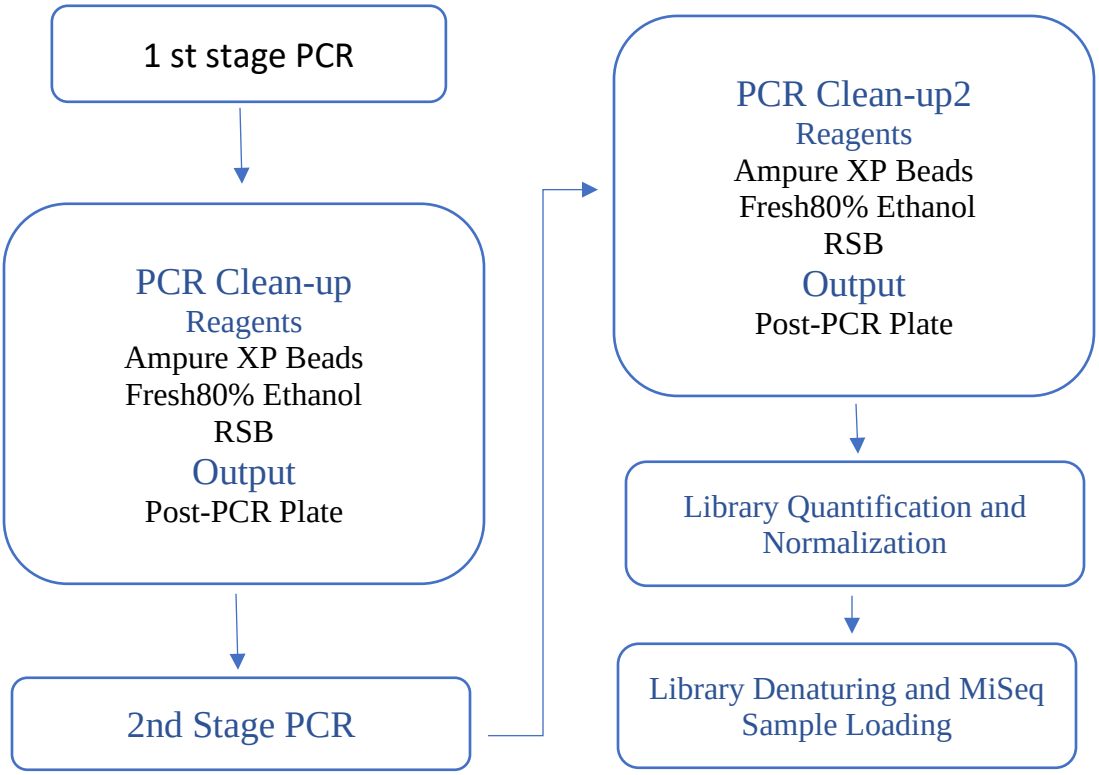


Figure (3- 1) 16SrDNA Library Preparation Workflow

In this phase, PCR is used to amplify the DNA sample's template using region-specific primers and overhang adapters. Table (3-8)

Table (3-8) PCR Mixture used to amplify the DNA sample's template using region-specific primers and overhang adapters.

Item	Quantity	Storage
Microbial Genomic DNA (5 ng/µl in 10 mM Tris pH 8.5)	2.5 µl per sample	-15° to -25°C
Amplicon PCR Reverse Primer (1 µM)	5 µl per sample	-15° to -25°C
Amplicon PCR Forward Primer (1 µM)	5 µl per sample	-15° to -25°C
2x KAPA HiFi HotStart ReadyMix	12.5 µl per sample	-15° to -25°C
Microseal 'A' film		
96-well 0.2 ml PCR plate	1 plate	

- **Procedure:**

1- The following reaction was set up using primers, two times the KAPA HiFi HotStart Readymix, and DNA: table (3-9).

Table (3-9) Reaction was set up using primers, two times the KAPA HiFi HotStart Readymix, and DNA.

Item	Volume
Microbial DNA (5 ng/µl)	2.5 µl
Amplicon PCR Forward Primer 1 µM	5 µl
Amplicon PCR Reverse Primer 1 µM	5 µl
2x KAPA HiFi HotStart ReadyMix	12.5 µl
Total	25 µl

2. The following program was used for PCR in a thermal cycler while sealing the plate:table (3-10).

Table (3-10) Program of PCR in a thermal cycler while sealing the plate.

NO.	Temperature(ºC)	Time	Repeat
1	95	3 min	**1**
2	95	30 seconds	25 cycles
3	55	30 seconds	
4	72	30 seconds	
5	72	5 min	**1**
6	Hold at 4°C		

3.2.7.PCR Clean-Up.

In this step, primers and primer dimer species were removed free from the 16S V3 and V4 amplicon using AMPure XP beads as shown in table (3-11)

Table (3-11) PCR Clean-Up 1.

Item	Quantity	Storage
10 mM Tris pH 8.5	52.5 µl per sample	-15° to -25°C
AMPure XP beads	20 µl per sample	2° to 8°C
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	
96-well 0.2 ml PCR plate	1 plate	

Preparation

• AMPure XP beads were warmed to room temperature

- **Procedure:**

1-To collect condensation,The Amplicon PCR plate was centrifuged at 1000 g for 1 minute at 20 °C. Carefully the seal was pried off.

2-To ensure that the AMPure XP beads are distributed equally, the beads for 30 seconds were vortexed. Depending on how many samples were processed, a trough with the appropriate number of beads was filled.

3- Twenty µl of AMPure XP beads were added to each well of the amplicon PCR plate using a multichannel pipette. the columns' tips were alternated.

4. The pipette volume was shaking up and down ten time.

5- Plate was incubated at room temperature for five minutes without shaking.

6-After the supernatant has cleared for two minutes, the plate was placed on a magnetic stand.

7- The supernatant with a multichannel pipette was removed while the Amplicon PCR plate was mounted on the magnetic platform. Tip was swapped between samples.

8- The beads with freshly 80% ethanol were cleaned while the Amplicon PCR plate was mounted on the magnetic platform as shown below**:**

A- Two hundred µl of freshly 80% ethanol was added to each sample well using a multichannel pipette.

b. The dish was incubated for thirty seconds on the magnetic stand.

and disposed of the supernatant with caution removing.

9- A second ethanol wash was conducted while the Amplicon PCR plate was on the magnetic platform.

a- Two hundred µl of newly 80% ethanol was added to each sample well using a multichannel pipette.

b-The plate on the magnetic stand was incubated for 30 minutes.

c- The supernatant with caution was removed and discarded.

10- The Amplicon PCR plate was removed on the magnetic stand for the beads could air-dry for 10 minutes

A- Two hundred µl of freshly 80% ethanol was added to each sample well using a multichannel pipette.

b. The dish was incubated. for thirty seconds on the magnetic stand.

and disposed of the supernatant with caution was removed.

11 –The Amplicon PCR plate was off and the magnetic stand removed, each well of the Amplicon PCR plate with 52.5 ml of 10 mM Tris pH 8.5 using a multichannel pipette.

12-Gently pipetted 10 times while alternating the tip after each column (or seal plate and shake at 1800 rpm for 2 minutes). Beads were fully resuspended, which was a guarantee.

13 – Plate was incubated at room temperature for 2 minutes.

14- After the supernatant had cleared, the plate was left on the magnetic stand for two minutes.

15- Fifty µl of the supernatant was transferred carefully from the amplicon PCR plate to a brand-new 96-well PCR plate using a multichannel pipette, to prevent contamination between samples, adifferent tips were used.

3.2.8. Index PCR.

Using the Nextera XT Index Kit, dual indices and Illumina sequencing adapters in this phase were attached as in table (3-12).

Table (3-12) Index PCR

Item	Quantity	Storage
2x KAPA HiFi HotStart ReadyMix	25 µl per sample	-15° to -25°C
Nextera XT Index 1 Primers (N7XX) from the Nextera XT Index kit (FC-131-1001 or FC-131-1002)	5 µl per sample	-15° to -25°C
Nextera XT Index 2 Primers (S5XX) from the Nextera XT Index kit (FC-131-1001 or FC-131-1002)	5 µl per sample	-15° to -25°C
PCR Grade Water	10 µl per sample	
TruSeq Index Plate Fixture (FC-130-1005)	1	
96-well 0.2 ml PCR plate	1 plate	
Microseal 'A' film	1	

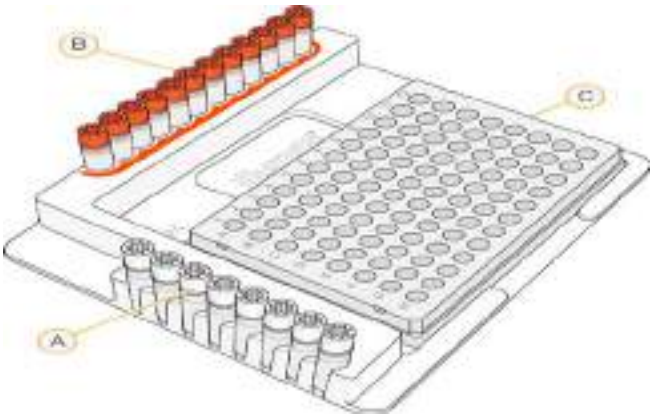
- **Procedure.**

1-Five µl of the mixture was transferred from each well to a new 96-well plate using a multichannel pipette.

2- Using the TruSeq Index Plate Fixture, the Index 1 and 2 primers on a rack were arranged using the following configurations as necessary:

a- A through H with Index 2 primer tubes were set up in rows (white caps, clear solution) vertically arranged.

b. The Index 1 primer tubes were placed (yellow solution, orange caps) horizontally, in alignment with columns 1 through 12**.**



A Index 2 primers (white caps)

B Index 1 primers (orange caps)

C 96-well plate

Figure (3-2) TruSeq Index Plate Fixture

3- 96-well PCR plate was inserted into the TruSeq Index Plate Fixture along with 5ml of resuspended PCR product DNA.

4- The following reaction with PCR-grade water was set up, DNA, Index 1 and 2 primers, and 2x KAPA HiFi HotStart ReadyMix (Table 3-13).

5- Pipette was ten times up and down gentle to combine.

6- Microseal "A" was used to seal the plate.

7-The plate was Centrifuged for one minute at 1,000 g in 20 °C.

Table (3-13) Reaction with PCR-grade water was set up, DNA, Index 1 and 2 primers, and 2x KAPA HiFi HotStart ReadyMix

Item	Volume
DNA	5 µl
Nextera XT Index Primer 1 (N7xx)	5 µl
Nextera XT Index Primer 2 (S5xx)	5 µl
2x KAPA HiFi HotStart ReadyMix	25 µl
PCR Grade water	10 µl
Total	**50 µl**

8- (3-14). The PCR program table

NO.	Temperature(ºC)	Time	Repeat
1	95	3 min	**1**
2	95	30 seconds	8 cycles
3	55	30 seconds	
4	72	30 seconds	
5	72	5 min	**1**
6	Hold at 4°C		

3.2.9.PCR Clean-Up 2.

In this phase, the resulting library was cleaned with AMPure XP beads before measurement.

Table (3-15) PCR Clean-Up 2

Item	Quantity	Storage
10 mM Tris pH 8.5	27.5 µl per sample	-15° to- 25°C
AMPure XP beads	56 µl per sample	2° to 8°C
Freshly Prepared 80% Ethanol (EtOH)	400 µl per sample	
96-well 0.2 ml PCR plate	1 plate	

- **Procedure:**

1. The Index PCR plate was centrifuged at 280 g for 1 minute at 20 °C to collect condensation.

2. Index PCR product was transferred fully from the PCR plate to the MIDI plate using a multichannel pipette set to 50 ml. between samples, the tips were changed.
3. To ensure that the AMPure XP beads are distributed equally, the beads was vortex for 30 seconds, through with the required number of beads was filled.
4- Each well of the Index PCR plate was filled with 56 ml of AMPure XP beads using a multichannel pipette.
5- Gently, the mixture was pipette 10 times up and down.
6- Plate was incubated at room temperature for Five minutes without shaking.
7- After the supernatant was cleared for two minutes, the plate was placed on a magnetic stand.
8- Using a multichannel pipette, the supernatant was removed while the Index PCR plate is still mounted on the magnetic platform. The tips are switched between samples.
9- While the Index PCR plate was mounted on the magnetic platform, the beads were cleaned as follows using newly 80% ethanol:
A-Two hundred ml of newly 80% ethanol was added to each sample well using a multichannel pipette.
b. The plate was incubated on the magnetic stand for 30 seconds.
c- The supernatant was carefully collected and discarded.

3.2.10. Statistical analysis.

The abundance of the bacterial populations of the healthy and patient groups was assessed by the independent samples Permanova and PCO plots. The R software package was used for cluster analysis. $P > 0.05$ was considered as no statistically significant differences.

CHAPTER FOUR

RESULTS

&

DISCUSSION

4- Result and Discussion:

4.1- Specimens collection and study population.

The volunteer's in this study were residents of the Misan governorate, and their ages ranged was between (30-50years). In a period of six months from September 2021 till February 2022, the samples were collected and immediately transferred under aseptic conditions to the laboratory. Thirty-six samples of stool (16) healthy and (20) patients were collected. Stool specimen were collected according to strict guidelines designed to minimize the influence of outside variables. The study did not include in any patients who meet the following criteria: Pregnant and lactating women, not allowed to patients with the following conditions: blood pressure, duodenal ulcer, cancer, autoimmune, diseases such as atherosclerosis, pneumonia and colitis, as in questionnaire shown in the appendix (1).

4.2. 16S r RNA gene amplification.

A small subunit ribosomal gene known as 16S rRNA was first sequenced in the late 1970s to evaluate bacterial phylogeny (Fox *et al*.,1997; Woese and Fox 1977). Given that it is a conserved element of the transcriptional machinery found in all DNA-based lifeforms, this gene is very valuable (Moffatt and Cookson 2017). Nine regions are stable, whereas nine (V1-V9) are hypervariable (Chakraborty *et al*., 2007). The ability to distinguish between distinct bacteria is enabled by the evaluation of these hypervariable regions; typically, one or two hypervariable regions are chosen. The bacterial makeup can quickly and affordably analyze from several samples using NGS of 16S rRNA using gene amplicon sequencing. Quantitative estimations of the number of bacteria present in a sample can then be obtained by mapping the amplified sequences to a database.

In the scope of molecule biology, polymerase chain reaction (PCR) is widely regarded as a straightforward and cost-effective method. Moreover, the polymerase chain reaction (PCR) is a useful technique that has revolutionized molecular biology research and has important applications in the diagnosis of microbial infections and genetic diseases, as well as in the detection of pathogens in stool specimens (Armany et al., 2016). The highly conserved region in the prokaryotic genome has been used successfully for many purposes. Thus, in the present study V3-V4 region of the *16s rRNA gene* was amplified by PCR from the isolated sample to diagnose the microbiome and its relationship to diabetic mellitus type II disease.

All bacterial isolates were successfully identified using the universal bacterial primer pair specific to the *16SrRNA gene* fragment (Figure 1), positive result recorded for all bacterial by amplification band corresponding to 1500 bp.

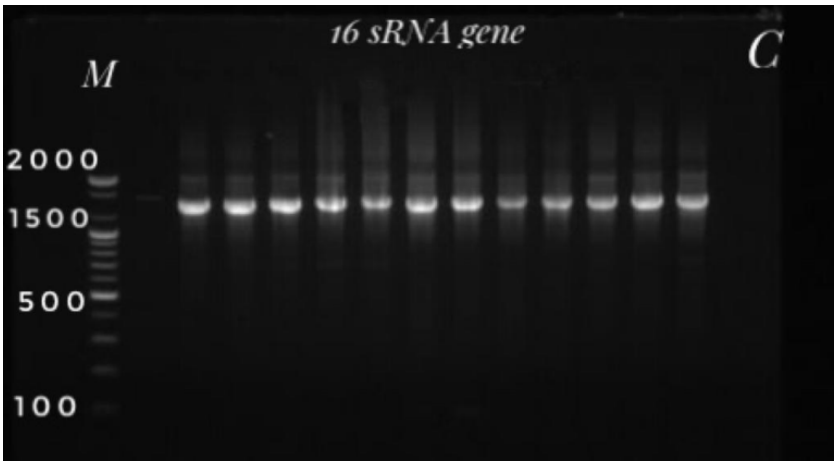


Figure (4-1) Agarose gel electrophoresis of PCR product 16sRNA gene, (1500bp), where M: ladder (100bp), All sample positive results, C: Blank (only primer+ Master Mix), the gel stained by ethidium bromide (0.5 μg/ml) the electrophoresis was running in 70 volts for one hour.

According in accordant with many research's it hypothesized that people with type 2 diabetes would have a different composition of gut microbiota compared with healthy. Furthermore, by taking advantage from universal primers,

Pyrosequencing of the V3-V4 region of the 16S rRNA gene was used to test the hypothesis in a large group of adults across a wide age range. Using the Illumina sequencing platform, Masella *et al* (2012) distinguished between healthy and diseased individuals through the study fecal microbes by sequencing the region v3-v4 of the gene. The 16S rRNA gene has been a mainstay of sequence-based bacterial analysis for decades. However, high-throughput sequencing of the full gene has only recently become a realistic prospect. Here, it sequence-based experiments to critically re-evaluate the potential of the 16S gene to provide taxonomic resolution at species and strain level (Johnson *et al*., 2019).

4.3. Library Quality Control Result of DNA.

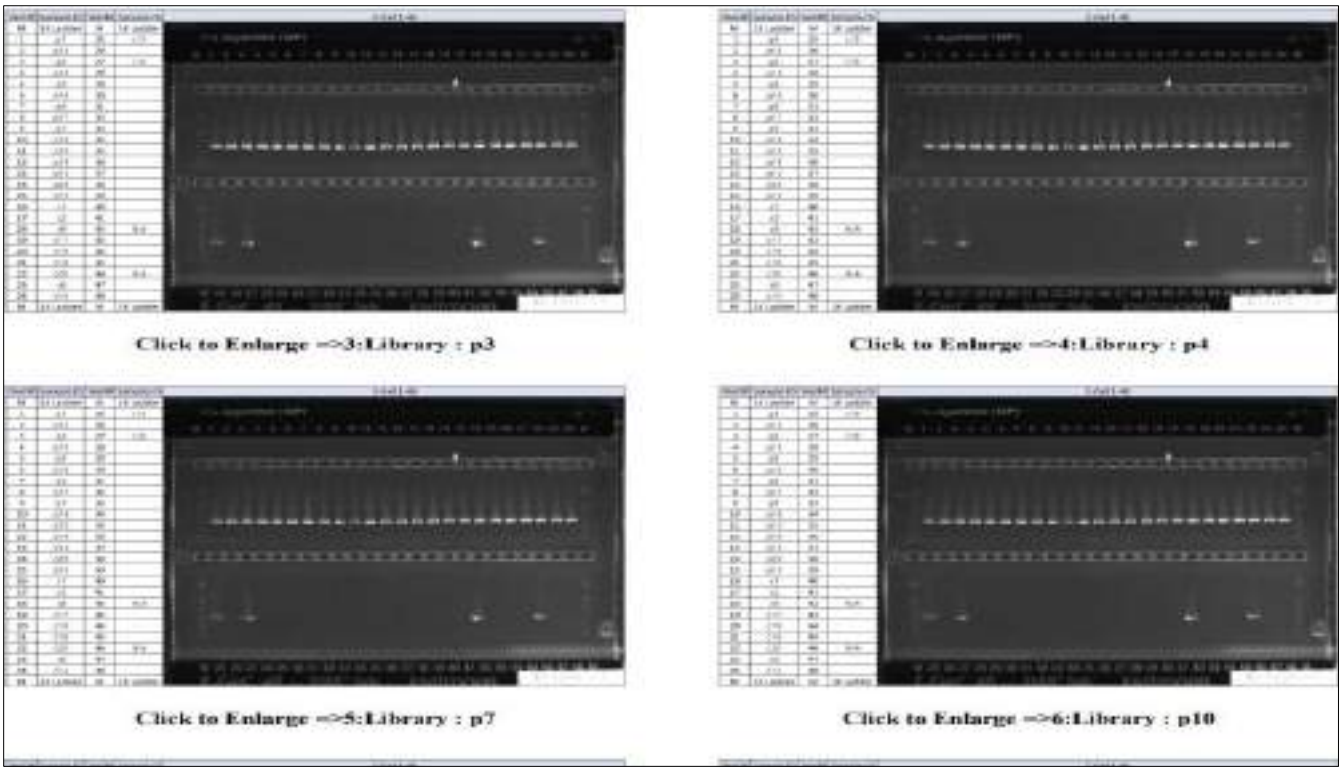
In order to proceed with bioinformatics, the need to ensure that the quality of samples falls within a certain range. This was achieved by checking the DNA fragment against the QC Library Result., as shown in table (4-1) and figure (4-2) revealed that a metagenomics amplicons were passed successfully below.

Table (4-1) Library QC Result of DNA

No	**Sample Name**	**WSID**	**Conc. (ng/ul)**	**Final Volume (ul)**	**Total Amount (ug)**	**Result***	
1	p3	ANW220217S007AS0000015832	4.6	70	0.322	Pass	
2	p4	ANW220217S007AS0000015833	2.66	30	0.08	Pass	
3	p5	ANW220217S007AS0000015834	2.75	80	0.22	Pass	
4	p6	ANW220217S007AS0000015835	6.97	90	0.627	Pass	

5	p8	ANW220217S007 AS0000015837	5.01	90	0.451	Pass	
6	p9	ANW220217S007 AS0000015838	0.93	90	0.084	Pass	
7	p10	ANW220217S007 AS0000015839	2.24	10	0.022	Pass	
8	p11	ANW220217S007 AS0000015840	5.35	80	0.428	Pass	
9	p13	ANW220217S007 AS0000015842	4.56	10	0.046	Pass	
10	p15	ANW220217S007 AS0000015844	3.49	90	0.314	Pass	
11	p16	ANW220217S007 AS0000015845	18.13	10	0.181	Pass	
12	p17	ANW220217S007 AS0000015846	0.54	80	0.043	Pass	
13	p18	ANW220217S007 AS0000015847	1.15	70	0.081	Pass	
14	c1	ANW220217S007 AS0000015850	1.68	10	0.017	Pass	
15	c2	ANW220217S007 AS0000015851	3.92	40	0.157	Pass	
16	c3	ANW220217S007 AS0000015852	3.09	80	0.247	Pass	
17	c17	ANW220217S007 AS0000015853	5.52	20	0.11	Pass	
18	c6	ANW220217S007 AS0000015855	1.68	30	0.05	Pass	
19	c7	ANW220217S007 AS0000015856	0.82	70	0.057	Pass	
20	c8	ANW220217S007 AS0000015857	4.95	40	0.198	Pass	

21	c9	ANW220217S007 AS0000015858	3.83	80	0.306	Pass	
22	c10	ANW220217S007 AS0000015859	1.56	80	0.125	Pass	
23	c20	ANW220217S007 AS0000015861	1.37	30	0.041	Pass	
24	c13	ANW220217S007 AS0000015862	0.8	50	0.04	Pass	
25	c14	ANW220217S007 AS0000015863	0.45	50	0.022	Pass	
26	c16	ANW220217S007 AS0000015865	2.28	50	0.114	Pass	



(A) (B)

Figure (4-2) Library QC Result of DNA in T2D patient (A) and control(B)

Nucleic acid QC is an essential component of next-generation sequencing workflows. Confirming nucleic acid quality at checkpoints throughout NGS library preparation – from incoming nucleic acid QC to final library validation – helps ensure successful sequencing outcomes, reliable QC of DNA and RNA samples requires an accurate understanding of the size and concentration, as well as visual confirmation of sample quality (Bal *et al*., 2018).

4.4. Next Generation Sequencing.

The next-generation sequencing (NGS) technologies such as the 16S rRNA gene sequencing yield useful data for describing microbial compositions in an ecosystem (Lin and Peddada, 2020).

4.4.1 Assembly of microbiome.

The results of Assembly microbiome as shown in table (4-2) and figure (4-3) elucidated the total bases,read count ,Nucleotide (N)% ,GC content % and phred quality score (Q20% and Q30%) which used to indicate the measure of base quality in DNA sequencing where high consistency of a sequenced base indictated by greater values of phred. A phred score of 20 indicates the likelihood of finding incorrect base call among 100 bases (Goswami and Sanan-Mishra,2022).

The score of 30 means that the error probability is 1 (1000 or 99.9% accuracy for a bases in the assembled sequence) (Lapidus,2009).

Table (4-2) Assembly of microbiome in diabetes mellitus type 2 and control

Sample Name	Total Bases	Read Count	N (%)	GC (%)	Q20 (%)	Q30 (%)
c1	53,010,949	116,029	0	50.25	97.91	92.67
c10	90,873,118	202,877	0	52.83	98.38	94.31
c13	43,645,152	96,974	0	52.17	98.25	94.08
c14	51,532,664	115,210	0	51.97	98.05	93.19

c15	60,460,655	131,997	0	50.84	97.75	92.39
c16	53,911,054	120,129	0	52.79	97.58	91.66
c17	68,373,284	151,809	0	52.58	97.97	92.98
c18	1,284,231,582	2,859,409	0	52.78	97.68	92.34
c19	79,084,649	175,824	0	52.71	97.77	92.47
c2	70,141,187	154,264	0	51.3	97.88	92.81
c20	53,252,160	118,248	0	52.64	97.92	92.81
c3	151,840,257	336,005	0	51.96	98.3	94.19
c6	74,813,384	165,009	0	52.46	97.95	92.95
c7	91,653,125	200,726	0	52.23	98.16	93.83
c8	65,338,242	143,989	0	52.64	97.77	92.38
c9	101,720,959	225,151	0	52.49	98.1	93.76
p1	39,355,039	85,614	0	52.03	97.21	90.43
p10	73,465,479	162,087	0	52.59	97.66	91.97
p11	81,374,582	180,001	0	51.97	98.24	94.07
p12	54,122,177	119,773	0	51.98	97.8	92.38
p13	49,016,532	108,385	0	51.17	97.94	92.9
p14	93,451,345	207,252	0	52.46	97.73	92.23
p15	47,440,539	104,819	0	52.55	97.31	90.47
p16	88,503,229	195,548	0	53.04	97.85	92.54
p17	65,248,526	144,522	0	53.03	97.76	92.23
p18	69,372,180	153,043	0	53	97.74	92.28
p19	17,917,498	39,790	0	52.18	96.65	88.29
p2	88,791,904	196,417	0	53	97.85	92.68
p20	58,797,674	130,020	0	53.07	97.88	92.51
p3	74,339,320	164,173	0	51.08	97.96	93.06
p4	65,490,853	144,692	0	52.23	97.78	92.28
p5	70,767,758	156,917	0	52.47	98.42	94.52
p6	38,072,241	83,078	0	51.41	98.29	94.28

p7	75,375,204	166,808	0	52.3	97.9	92.79
p8	36,387,373	80,613	0	52.4	98.05	93.46
p9	25,562,273	56,022	0	51.73	98.28	94.17

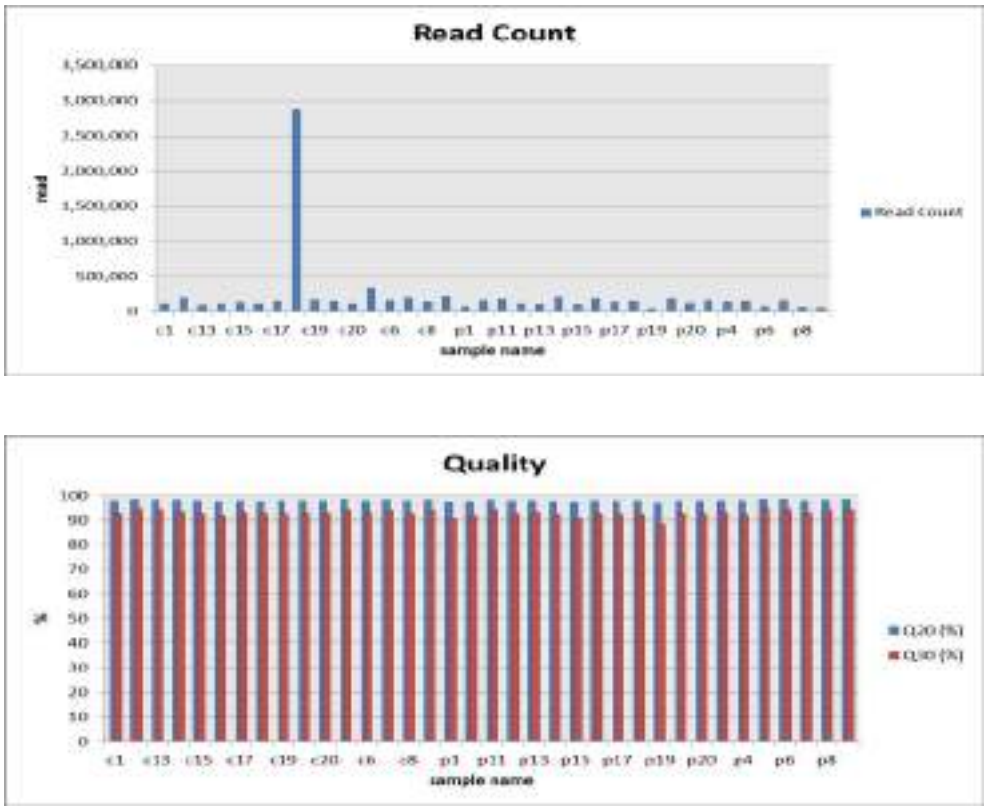


Figure (4-3) Assembly of microbiome in diabetes mellitus type 2 and control.

Rojo, (2021) mentioned the community assembly (CA) is a topic of growing interest in ecology due to global change, among other reasons. Jones *et al*., (2022) stated the Microbiome assembly give rise to an individual's microbiome.

4.4.2: Pre-processing and clustering by CD-HIT-OUT.

The results of pre-processing and clustering of OUT picking method (denovo) of diabetes mellitus type 2 and control, as shown in table (4-3), Appendix (2).

Table (4-3) Results of pre-processing and clustering of OUT picking method (denovo) of diabetes mellitus type 2 and control:

Results of pre-processing	
Sample count	36
Read count	1,433,100
Gamma diversity	1,042
Counts/Sample summary	
Min	3,371.0
Max	419,760.0
Median	21,595,5
Mean	39,808,333
Filtered Read Count	
Ambiguous	0
Wrong prefix or primer	383,206
Sequence of prefix or primer	CCTACGGG(ACGT)GGC(AT)GCAG
Low-Quality	40.105
Chimera	229,673
Other	5,907,140

Sample count: The total number of sample(36), Read count: The total number of sequence reads(1,433,100) alpha diversity corresponds to species diversity in sites habitats at a local scale, Beta diversity comprises species diversity among sites / habitats, Min: Minimum number of sequence per sample(3,371.0), Max: Maximum number of sequences per samples(419,760.0), Median: The number separating the higher half of a data samples(21,595,5), Mean: The average number of the sequence of samples(39,808,333), Ambiguous: Filtered seqs with ambiguous bases calls, Low –Quality: Filtered seqs with low-quality bases (Quality score offset 33)(40,105), Chimera: Filtered seqs with chimeric reads(229,673), Denoising : Filtered seqs with all other noise.

Fu *et al*., (2012) clarified the CD-HIT is a widely used program for clustering biological sequences to reduce sequence redundancy and improve the performance of other sequence analyses. In response to the rapid increase in the amount of sequencing data produced by the next-generation sequencing technologies.

Metzker, (2010) and Casey *et al*., (2013) are of the latest developments in science and technology that affect medical Next-generation sequencing research, which has the potential to enhance clinicians' diagnostic and treatment methods targeted treatments. Andermann *et al* (2022) Explained that the Alpha diversity refers to diversity on a local scale, describing the species diversity (richness) within a functional community. For example, alpha diversity describes the observed species diversity within a defined plot or within a defined ecological unit, such as a pond, a field, or a patch of forest, while Beta diversity, on the other hand, describes the amount of differentiation between species communities. Unlike the other levels of species diversity, the exact interpretation and quantification of beta diversity varies substantially across studies, originally beta diversity was defined as the ratio between gamma and alpha diversity.

4.5.3. Diversity Analysis:

4.5.3.1. Taxonomy Assignment.

Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers (Liu *et al*., 2008).

4.4.3.1.1.OTU Abundance.

Several characteristics of OTU tables that reflect bacterial kinds, as well as data from 16S ribosomal RNA (rRNA) amplicon sequencing, create obstacles for ecological and statistical interpretation (Weiss *et al*., 2017).

The studys results of OTU abundance, as shown in appendix (3).Which showed the abundance of different bacteria and their taxonomy and accession number.

4.4.3.1.2. Taxonomy Abundance.

The assessment of microbiome biodiversity is the often common application, 16S sequencing stayes standard procedure for taxonomic profiling of genomic data (Khachatryan *et al*., 2020).

The results of taxonomy abundance, shows the taxonomic composition for each samples from phylum to genus. Shown in table (4-4). Which revealed the Taxonomy abundance to the level of species?

Table (4-4). Taxonomy abundance.

	A	B	L	M	N	O	P	Q	R	S	T
1	Kingdom	Phylum	c2	c20	c3	c6	c7	c8	c9	p1	p10
2	Archaea	__"Euryarchaeota"	0	0	0	0	0	0	0	0	0
3	Bacteria	Other	59	0	0	338	0	4	0	0	0
4	Bacteria	__	39	50	324	40	8	0	162	0	73
5	Bacteria	__"Actinobacteria"	331	0	499	508	71	42	211	12	38
6	Bacteria	__"Bacteroidetes"	14,599	7,177	58,454	9,204	71,158	6,858	34,911	3,479	10,837
7	Bacteria	__"Deinococcus-Thermus"	2	0	0	163	0	2	0	0	0
8	Bacteria	__"Elusimicrobia"	0	658	0	0	14	0	0	1	0
9	Bacteria	__"Fusobacteria"	0	0	0	39	74	0	0	0	25
10	Bacteria	__"Lentisphaerae"	8	41	26	20	2	0	2,833	0	119
11	Bacteria	__"Proteobacteria"	659	1,225	4,136	3,228	2,888	2,617	4,218	1,601	1,487
12	Bacteria	__"Tenericutes"	4	177	39	155	14	0	594	0	43
13	Bacteria	__"Verrucomicrobia"	126	0	78	341	255	4	26	3	4,066
14	Bacteria	__Candidatus Saccharibacteria	1	0	19	1	0	0	7	1	1
15	Bacteria	__Cyanobacteria/Chloroplast	0	0	1	2	0	0	0	0	0
16	Bacteria	__Firmicutes	6,860	6,713	43,393	9,034	12,403	7,286	23,300	5,124	9,471
17	Unassigned	Other	9	6	0	5	0	6	12	0	3

	A	B	C	D	E	F	G	H	I	J	K
1	Kingdom	Phylum	c1	c10	c13	c14	c15	c16	c17	c18	c19
2	Archaea	__"Euryarchaeota"	0	14	0	0	0	0	0	0	0
3	Bacteria	Other	0	0	28	0	0	4	0	12	0
4	Bacteria	__	0	230	264	3	0	1	140	21,120	5
5	Bacteria	__"Actinobacteria"	37	1,340	44	20	76	3	15	129	10
6	Bacteria	__"Bacteroidetes"	11,852	14,648	12,254	5,355	13,288	4,958	9,495	117,500	10,355
7	Bacteria	__"Deinococcus-Thermus"	19	1	5	0	4	0	0	1	2
8	Bacteria	__"Elusimicrobia"	0	1,046	0	0	1	0	216	6,434	0
9	Bacteria	__"Fusobacteria"	0	25	0	0	0	0	0	0	0
10	Bacteria	__"Lentisphaerae"	0	1,200	5	2	0	2	23	8,128	0
11	Bacteria	__"Proteobacteria"	3,596	1,904	2,799	262	688	670	3,696	77,850	2,955
12	Bacteria	__"Tenericutes"	0	58	690	3	1	6	376	64,466	1,063
13	Bacteria	__"Verrucomicrobia"	24	3,259	53	7	4	7	3	668	0
14	Bacteria	__Candidatus Saccharibacteria	0	1	13	0	0	4	15	21	0
15	Bacteria	__Cyanobacteria/Chloroplast	0	0	0	0	1	0	0	0	0
16	Bacteria	__Firmicutes	3,141	40,011	14,062	8,131	7,530	8,956	6,175	123,191	7,874
17	Unassigned	Other	3	0	43	0	6	0	10	240	3

	A	B	AE	AF	AG	AH	AI	AJ	AK	AL
1	Kingdom	Phylum	p20	p3	p4	p5	p6	p7	p8	p9
2	Archaea	__"Euryarchaeota"	0	0	0	0	0	0	0	0
3	Bacteria	Other	1	0	0	0	0	0	0	0
4	Bacteria	__	125	35	1	3	1	96	243	11
5	Bacteria	__"Actinobacteria"	98	15	0	693	145	62	416	69
6	Bacteria	__"Bacteroidetes"	6,017	12,976	7,551	20,849	35,151	9,069	10,204	11,245
7	Bacteria	__"Deinococcus-Thermus"	0	0	0	3	0	1	0	0
8	Bacteria	__"Elusimicrobia"	8	0	0	0	0	9	0	287
9	Bacteria	__"Fusobacteria"	1	1,178	11	0	31	27	0	0
10	Bacteria	__"Lentisphaerae"	1	11	1	98	0	80	7	25
11	Bacteria	__"Proteobacteria"	1,714	4,849	1,038	9,080	707	3,009	2,077	171
12	Bacteria	__"Tenericutes"	1	1	0	31	2	143	1	0
13	Bacteria	__"Verrucomicrobia"	602	22	2,691	1,321	1	30	129	0
14	Bacteria	__Candidatus Saccharibacteria	1	1	1	1	1	0	1	0
15	Bacteria	__Cyanobacteria/Chloroplast	0	0	0	0	0	0	1	0
16	Bacteria	__Firmicutes	7,222	5,576	9,716	22,864	2,207	8,655	8,513	3,556
17	Unassigned	Other	9	9	3	3	1	3	0	0

	A	B	U	V	W	X	Y	Z	AA	AB	AC	AD
1	**Kingdom**	**Phylum**	**p11**	**p12**	**p13**	**p14**	**p15**	**p16**	**p17**	**p18**	**p19**	**p2**
2	Archaea	__"Euryarchaeota"	0	0	0	0	0	0	0	0	0	0
3	Bacteria	Other	0	0	0	0	0	0	1	0	0	0
4	Bacteria	__	62	77	79	21	0	2,489	137	7	0	1,085
5	Bacteria	__"Actinobacteria"	98	59	9	4	12	10	55	35	11	25
6	Bacteria	__"Bacteroidetes"	30,536	7,335	8,473	10,578	3,592	10,680	8,892	7,596	1,246	10,314
7	Bacteria	__"Deinococcus-Thermus"	0	0	0	0	0	0	0	0	0	1
8	Bacteria	__"Elusimicrobia"	7	8	0	0	0	0	0	0	0	2,894
9	Bacteria	__"Fusobacteria"	48	19	517	2	0	0	11	0	22	3
10	Bacteria	__"Lentisphaerae"	86	93	3	3	0	4	1	2	0	155
11	Bacteria	__"Proteobacteria"	6,669	1,604	2,510	7,264	1,451	1,984	871	6,534	298	6,503
12	Bacteria	__"Tenericutes"	2	4	1	0	0	0	0	0	0	841
13	Bacteria	__"Verrucomicrobia"	0	3	7	45	3,622	0	5	0	0	27
14	Bacteria	__Candidatus Saccharibacteria	2	1	0	0	0	0	0	0	0	0
15	Bacteria	__Cyanobacteria/Chloroplast	0	0	0	0	0	1	0	0	0	0
16	Bacteria	__Firmicutes	18,344	5,315	4,281	6,570	6,203	9,531	7,850	5,846	1,794	6,845
17	Unassigned	Other	0	0	6	9	0	18	6	6	0	3

4.4.3. 2.Alpha Diversity.

Alpha diversity measurements provide an overview of the richness (number of taxonomic categories), evenness (distribution of group abundances), or both, of an ecological community's structure. A typical first step in microbial ecology is to analyse the alpha diversity of the data from amplicon sequencing to identify differences between environments (Willis, 2019).

4.4.3.2.1.: Community richness & diversity.

The results of Community richness and diversity of microbiome in diabetes mellitus type 2 and control, as shown in table (4-4), appendix (4), figure (4-4).

Figure (4-4) Community richness and diversity of microbiome in patient and control samples

OTUs : Operational Taxonomic Unit is an operational definition of a species or group of species often used when only DNA sequence data is available, Chao1 : returns the Chao1 richness estimate for an OTU definition, Shannon : The Shannon index takes into account the number and evenness of species, Gini-Simpson : The Gini-Simpson index represents the probability that two randomly selected individuals in the habitat will belong to the same species.

Scrosati *et al*. (2011) mentioned that the Species diversity is an important property of communities because it is often related to their functioning and potential for change. Diversity is a measure of how likely two randomly selected individuals in a community belong to different species, Thus, diversity is affected by two other properties of communities: richness, which is the total number of species, and evenness, which is the degree of similarity in abundance among the species.

4.4. 3.2. 2. Alpha Rarefaction.

Chao1, Shannon and Simpson samples, as shown in the table (4-5); Figure (4-6).

Table (4-5) Alpha Rarefaction of microbiome.

OTUs					
Community Diversity					
Sample Name	**OTUs**	**Chao1**	**Shannon**	**Gini-Simpson**	**Good's Coverage**
c1	123.0	136.571428571	3.62167794166	0.866788771882	0.998928877464
c10	307.0	345.153846154	6.28286548856	0.975420146724	0.999497936834
c13	317.0	377.272727273	5.73474766423	0.951692153353	0.998281559815
c14	175.0	217.5	4.61555607955	0.918701434617	0.997460639919
c15	141.0	182.052631579	2.64702916631	0.753196980788	0.99814806241
c16	171.0	214.05	4.40132615758	0.9274750553	0.997125453426
c17	261.0	299.896551724	5.15926939423	0.93515969585	0.997619519937
c18	603.0	644.0	5.63250025801	0.960695433409	0.999899942824
c19	182.0	210.5	4.20151929359	0.890998139445	0.998248529214
c2	216.0	229.5	5.17393875199	0.95214099834	0.998766356787
c20	173.0	225.0	4.88677583412	0.925521877416	0.997507322241
c3	313.0	352.0	5.63211298855	0.956464213156	0.999635408389
c6	358.0	419.0	6.24437245062	0.974847516331	0.997313458705
c7	188.0	210.884615385	2.98132664204	0.752837262157	0.999597177944
c8	152.0	173.083333333	4.40446020402	0.905262272412	0.99863249896

c9	221.0	242.9375	4.86911411341	0.919994152892	0.999592600416
p1	73.0	112.428571429	3.49468219258	0.864043222227	0.997651893161
p10	249.0	282.0	4.85090048748	0.917618908162	0.99828001376
p11	249.0	284.0625	5.06118630156	0.935298229538	0.999391270097
p12	258.0	320.666666667	5.76478901069	0.960787328036	0.996693759471
p13	242.0	281.2	4.3022447642	0.855433572095	0.996915523102
p14	191.0	222.071428571	4.98679331549	0.94081890121	0.998775310255
p15	97.0	108.0	4.27974051454	0.904812695109	0.999193548387
p16	256.0	289.476190476	5.49908489225	0.959238391678	0.998462596593
p17	252.0	308.4	5.45634398393	0.952752817592	0.997307757025
p18	173.0	195.142857143	4.26386983929	0.884492634358	0.998452012384
p19	126.0	149.25	5.03556750452	0.947449501441	0.990803915752
p2	256.0	279.375	4.84338661131	0.919038976611	0.998815165877
p20	277.0	351.391304348	5.51993182863	0.953247524435	0.996265822785
p3	255.0	281.857142857	4.13812596164	0.865525501066	0.998054553561
p4	154.0	217.0	4.76128306065	0.932129699896	0.998286774854
p5	266.0	297.5	5.18298084899	0.943026773941	0.999344811269
p6	200.0	241.777777778	2.35342835894	0.512122664303	0.998744999608
p7	274.0	305.538461538	5.63005971577	0.957541529555	0.998064577039
p8	220.0	257.058823529	5.30383989793	0.936837957048	0.998332715821
p9	128.0	170.857142857	3.7321057095	0.805364549079	0.998372819578

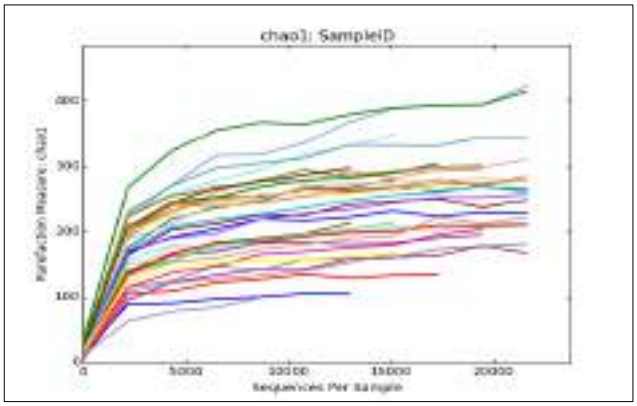


Figure (4-5) Chao1 sample ID of Alpha Rarefaction of microbiome

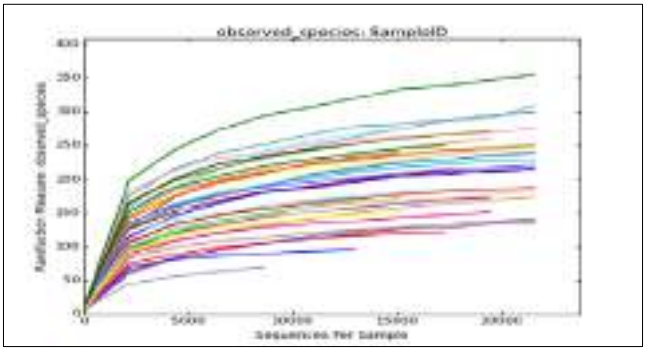


Figure (4-6) Observed sample ID of Alpha Rarefaction of microbiome.

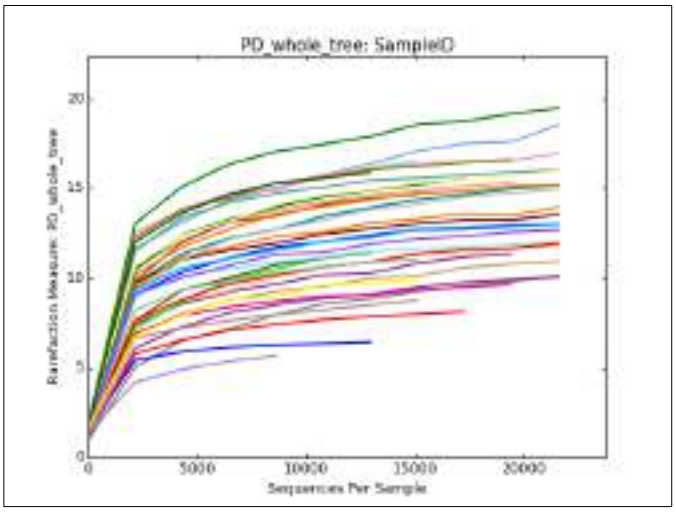


Figure (4-7) PD sample ID of Alpha Rarefaction of microbiome.

Table (4-6) Community richness and diversity of microbiome in patient and control samples.

	Total		**Control**		**Patient**	
	Mean	**Median**	**Mean**	**Median**	**Mean**	**Median**
Variables						
OTUs	224.9166667	220.5	243.8125	202	209.8	245.5
Chao1	262.0682361	249.9981618	279.9626646	227.25	247.7526934	280.2875
Shannon	4.74858159	4.877944974	4.780537027	4.877944974	4.72301724	4.918846901
Gini-simpson	0.903188263	0.929802378	0.910449757	0.926498466	0.897379069	0.933713965
Good's coverage	0.998124033	0.998309745	0.998515959	0.998457029	0.997810492	0.998309745

Willis, (2019) showed by selecting a set number of samples that are equal to or fewer than the number of samples in the smallest sample, the rarefaction method corrects for differences in library sizes across samples to facilitate comparisons of alpha diversity. Once the set number of samples is reached, reads from larger samples are randomly discarded until the set number of samples is reached.

4.4.3.2.3. PCoA Graph - weighted unifrac.

The results of PCoA Graph - weighted unifrac of microbiome, as shown in figure (4-8).

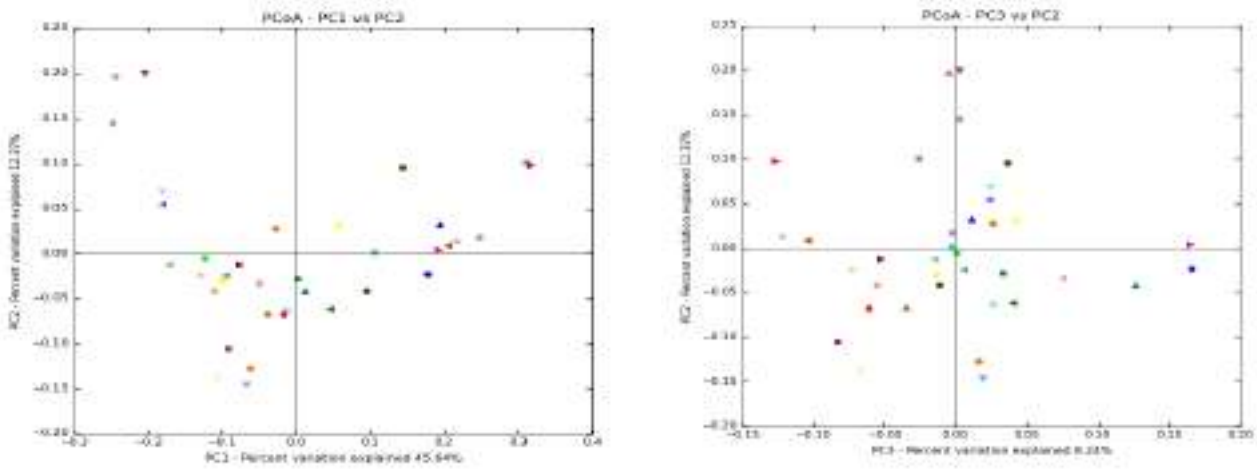


Figure (4-9) PCoA Graph - weighted unifrac.

Principal Coordinate Analysis (PCoA) is a technique that helps to extract and visualize a few highly informative components of variation from complex, multidimensional data. This is a transformation that maps the samples present in the distance matrix to a new set of orthogonal axes such that a maximum amount of variation is explained by the first principal coordinate, the second largest amount of variation is explained by the second principal coordinate. The two dimensional principal coordinates provide an intuitive visualization of the data structure and look at differences between the samples. Lozupone *et al*. (2007) stated that the use of weighted and unweighted measures of β diversity revealed markedly different factors influencing the microbial communities. The original, unweighted UniFrac measure is well suited to detecting differences in the presence or absence of lineages of bacteria in different communities. Wong *et al.* (2016) mentioned that the UniFrac distance metric is often used to separate groups in microbiome analysis, but requires a constant sequencing depth to work properly,

uniFrac is highly sensitive to rarefaction instance and to sequencing depth in uniform data sets with no clear structure or separation between groups. We show that this arises because of subcompositional effects. We introduce information UniFrac and ratio UniFrac, two new weightings that are not as sensitive to rarefaction and allow greater separation of outliers than classic unweighted and weighted UniFrac.

4.4.3.2.4. UPGMA Tree – unweighted and unweighted unifrac.

To compare the differences between microbial compositions between healthy and patient groups, beta diversity was analyzed using weighted and unweighted distances, as shown in figures (4-10).

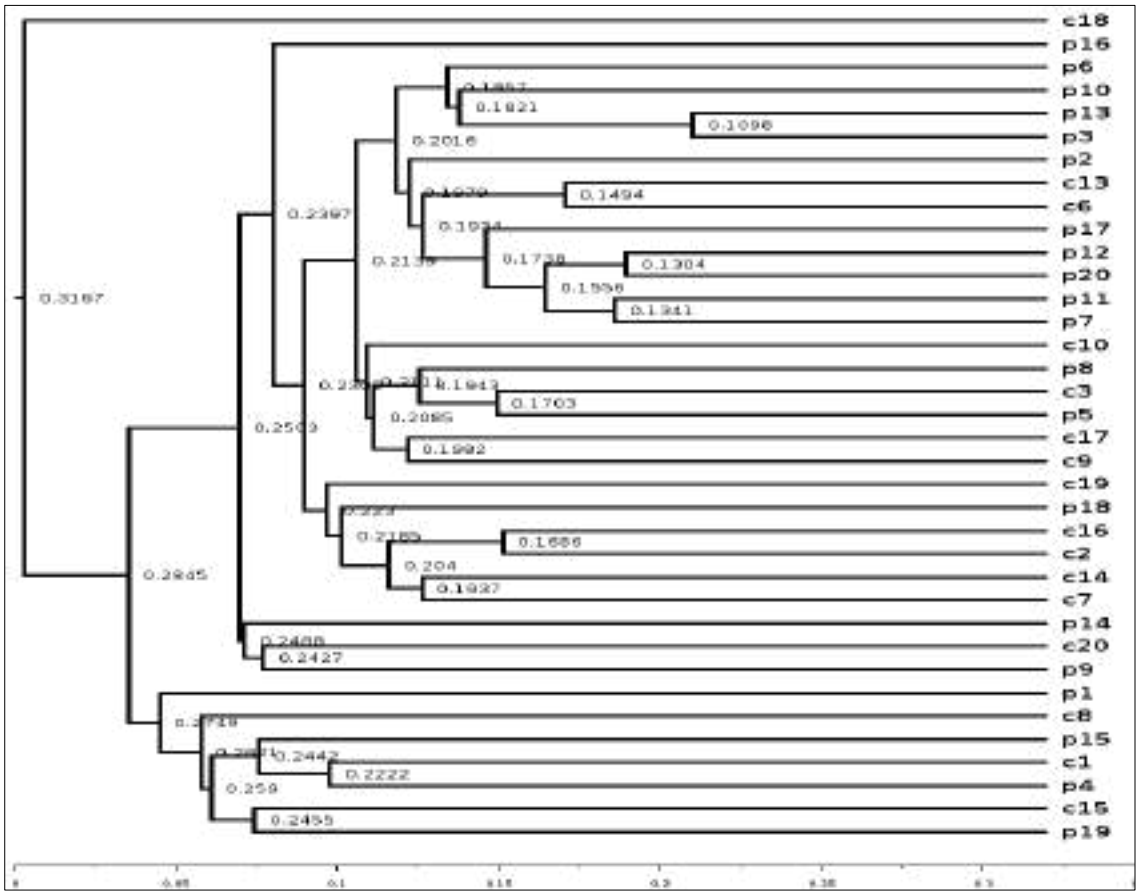


Figure (4-9) UPGMA Tree - unweighted unifrac of microbiome.

Unweighted Pair Group Method with Arithmetic mean (UPGMA) is type of hierarchical clustering method using average linkage and can be used to interpret the distance matrix (weighted_unifrac_dm.txt).

The output is a file that can be opened with tree viewing software, the distance matrix file can be used to draw your own phylogenetic tree using a suitable tool.

4.5.3.2.5. UPGMA Tree –weighted unifrac.

The results of UPGMA Tree –weighted unifrac of microbiome, as shown in Figure (4-11).

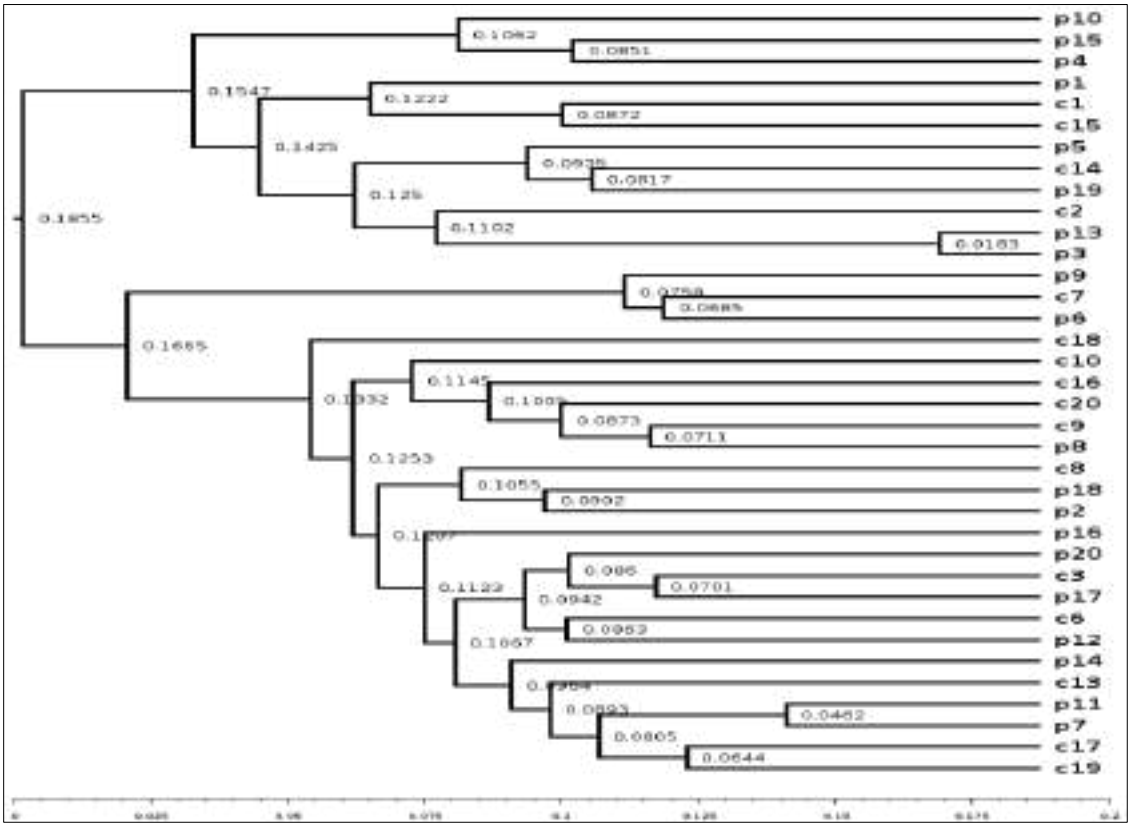


Figure (4-10) UPGMA Tree - weighted unifrac of microbiome.

Unweighted Pair Group Method with Arithmetic mean (UPGMA) is type of hierarchical clustering method using average linkage and can be used to interpret the distance matrix (unweighted_unifrac_dm.txt).

The output is a file that can be opened with tree viewing software, the distance matrix file can be used to draw your own phylogenetic tree using a suitable tool. Mohit *et al*., (2014) mentioned that the UPGMA tree indicating the unweighted-UniFrac clustering of the attached and free-living communities based on UniFrac phylogenetic distance.

4.4.3.3. Beta Diversity.

It is also important to use a beta diversity measure (similarity between multiple communities/samples) that captures changes in community composition (Wagner et al., 2018).

4.4.3. 3.1. PCoA Graph - unweighted unifrac.

The results of PCoA Graph - unweighted unifrac of microbiome, as shown in figure (4-11).

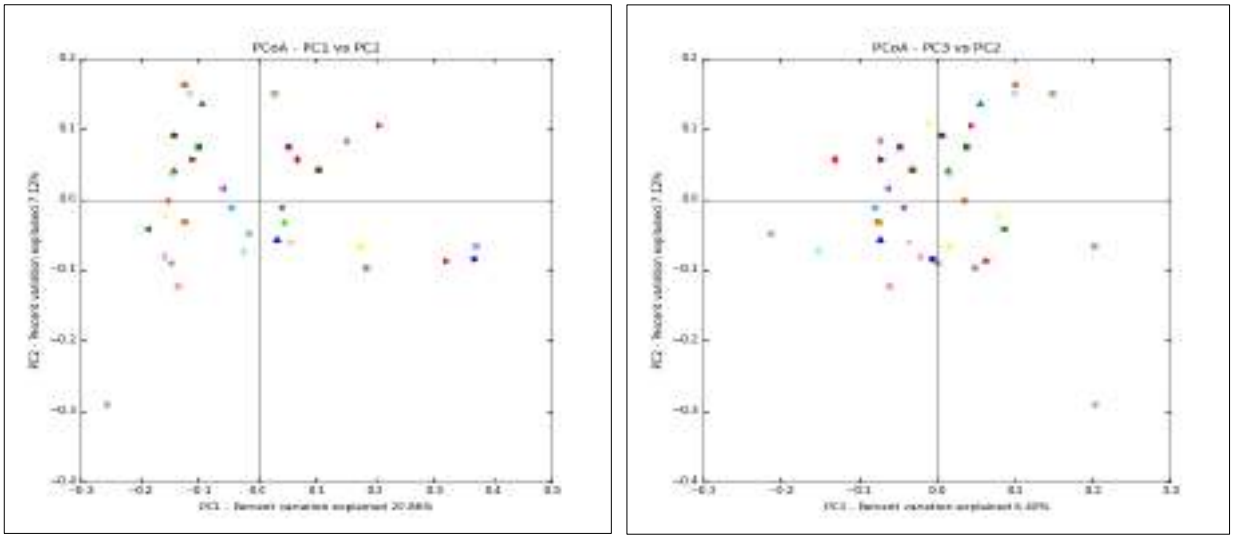


Figure (4-11) PCoA Graph - unweighted unifrac of microbiome.

4.4.3.3.2. Jaccard index.

The study results of statistical analysis of Jaccard perman of permanova test showed no significant differences between diabetes mellitus type 2 patients and control in micobiome, as shown in table (4-7) and figure (4-13).

Table (4-7) Statistical analysis of Jaccard perman of permanova test between microbiome of diabetes mellitus type 2 patients and control.

Permanova	
Sample size	36
Number of groups	2
Test statistics	1.451428957
P-value	0.061
Permanova: Permutational multivariate ANOVA	

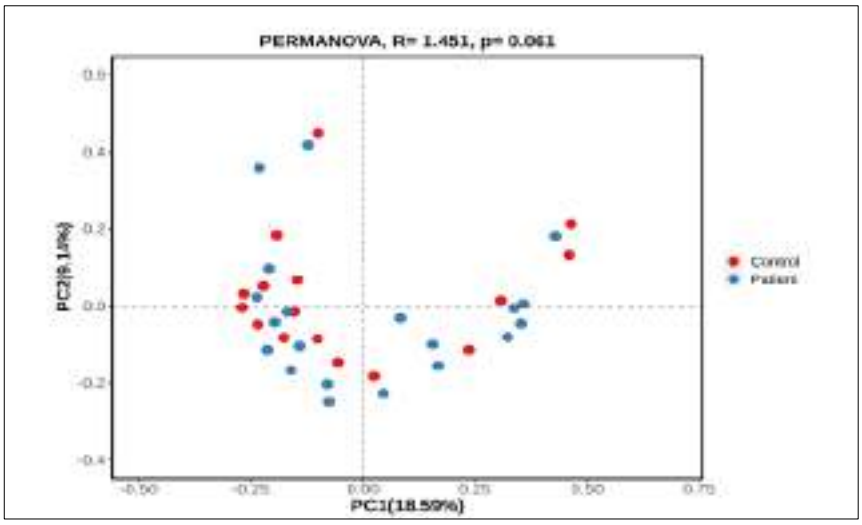


Figure (4-12) Jaccard perman of permanova test between microbiome of diabetes mellitus type 2 patients and control.

PCoA: Principal Coordinate Analysis) is a technique that helps to extract and visualize a few highly informative components of variation from complex, multidimensional data.

4.4.3.3.3. Bray Cruits index.

The study findings result of Bray cruits of permanova test showed no significant differences between diabetes mellitus type 2 patients and control in microbiome, as shown in tables (4-8), figure (4-14).

Table (4-8): Statistical analysis of Bray Cruits of permanova test between microbiome of diabetes mellitus type 2 patients and control.

Permanova	
Sample size	36
Number of groups	2
Test statistics	1.629500134
P-value	0.099
Permanova: Permutational multivariate ANOVA	

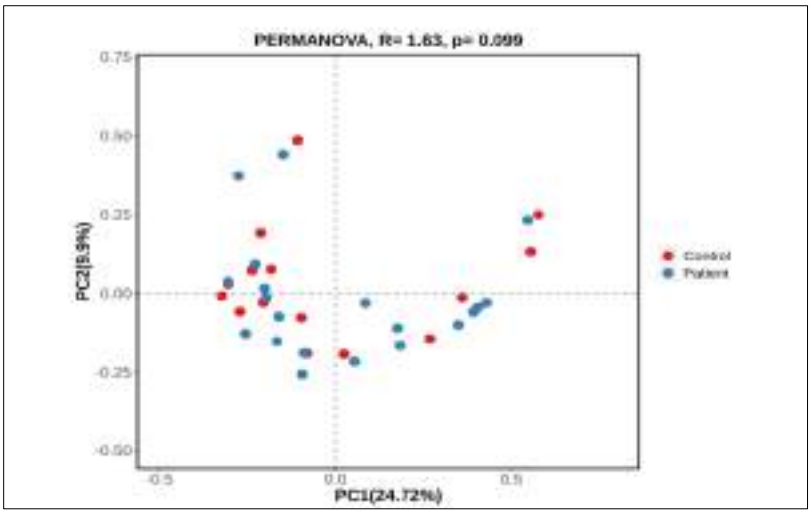


Figure (4-13) Statistical analysis of Bray cruits of permanova test between microbiome of diabetes mellitus type 2 patients and control,

PCoA: Principal Coordinate Analysis) is a technique that helps to extract and visualize a few highly informative components of variation from complex, multidimensional data.

Marzinelli *et al*., (2018) showed that the permanova analyses based on Bray-Curtis and Jaccard measures of relative abundances of OTUs moreover Bishop *et al*., (2017). Explain that the Permanova is the most popular and considered to be the most powerful statistical tool.

4.4.3.3.4. Bacterial Phyla Distribution:

Table (4-9) Percentages of bacteria in diabetes mellitus type 2 patients and control.As showed in appendix (5).

Type Of Bacteria	Patient%	Control%
Firmicutes	36.78	39.9
Bacteroides	44.89	47.6
Actinobacteria	0.34	0.48
Verrucomicrobia	2.9	0.5
Proteobacter	12.7	9.06
Lentisphaerae	0.1	0.5
Elusimicrobia	0	0.5
Tenericutes	0.195	1.7
Fusobacteria	0.47	0

Interestingly, our study as stated in table (4-9) showed that was high percentage of Firmicutes which was detected in 39.9% of control compared with 36.78 % in the patients. A similar result was shown by Larsen *et al* (2010), they mentioned that the Firmicutes reduced in the population of T2D. Surprisingly, our results disagree with Zhang *et al* (2013) they displayed that there was an increase in the abundance of Firmicutes in T2D in China population. The differences in results between Iraq and other countries may be due to lifestyle and geographic region. Han and Lin (2014) reported that the change in the abundance of Firmicutes in relation to diabetes had been attributed to differences in ancestry, geographic regions, eating habits, and research methods.

A study conducted by Karlsson *et al*. (2013) on European female T2D patients revealed decrease in the abundance of butyrate-producing bacteria, including *F. prausnitzii* were deduced to be highly discriminant for T2D by the mammalian

Gene collection (MGC) model analysis, this is in agreement with the present results.

Bacteroides was detected in 47.6 % of control samples compared with 44.89% of patients. Ley *et al* (2005) and Turnbaugh *et al* (2006) showed a decrease in the Bacteroidetes phylum believed to be associated with increased energy absorption from food and increased low-grade inflammation.

The percentages of Firmicutes and Bacteroides increased in control compared with diabetes mellitus type 2 patients. Schwiertz and colleagues (2009) wrote that the predominant bacterial types in healthy and sick patients are Firmicutes and Bacteroidetes. Qin *et al* (2012) found that the positive correlation between the ratio of Bacteroidetes to Firmicutes and lower glucose tolerance. Nookaew (2013) mentioned that the pathogens that seize opportunities as well as an enrichment of other Sulfate reduction and oxidative stress-reducing microbial activities resistance to stress in larger European population.

The present study showed other bacteria such as Verrucomicrobia, Proteobacter, and Fusobacteria unlike Firmicutes had a higher percentage in diabetes mellitus type 2 patients (2.9,12.7and 0.47% respective) compared with control (0.5,9.06and 0% respective) . Ley *et al* (2005) and Turnbaugh *et al* (2006) showed an increase in bacteria from the Firmicutes phylum may associated with increased energy absorption from food and increased low-grade inflammation.

Also the results showed large abundance of Verrucomicrobia bacteria that agree with result, studied by Fujio-Vejar *et al* (2017) in chilean where the high abundance of the phylum Verrucomicrobia was reported. This the phylum is a member of the super phylum (Verrucomicrobia), which includes relatively related bacteria with unusual properties such as having a complex and dynamic inner

membrane system It makes them, in some respects, closer to eukaryotic cells, it usually includes a few genera isolated from fresh water and soil animal feces.

Another study found that Verrucomicrobia had a significantly small abundance in both the pre-diabetic and type 2 diabetic groups (Zhang *et al*., 2013). Previous studies show that Verrucomicrobia may be a potential marker of type 2 diabetes (Barlow *et al.,* 2015). Some researchers suggest that people with type 2 diabetes have a decreased number of bacteria that product short-chain fatty acids (e.g., acetate, propionate, and butyrate) (Lv *et al*., 2018).

Studies proved that Enrichment of the gut microbiota in people with type 2 diabetes related to Gram-negative bacteria, which belong to the Proteobacteria phylum. The main components of the outer membranes in Gram negative bacteria lipopolysaccharides (LPS), (otherwise known as catalysts Strong infections, which can show endotoxinemia (Allcock *et al*.,2001).

Obesity is a major risk factor for T2D. A number of conventional faecal culture trials have shown that the gut microbiota plays an important role in energy acquisition and tissue accumulation fatty acids, and insulin resistance (Bäckhed *et al*., 2004; Ridaura *et al*., 2013). Some studies have also reported that the level of Low Firmicutes/Bacteroides Ratio Associated with Obesity and Metabolic Disorders Diet (Ley, *et al*., 2005; Turnbaugh *et al*., 2006) while other studies indicate the opposite (Duncan, *et al*., 2008; Jumpertz, *et al*., 2011).

Abundance has been reduced microbes are highly diverse in patients with depressive and anxiety disorders (Du *et al*.,2020). While Table (4-10) below show result of Beta diversity.

Table (4-10) Distribution of bacterial phyla according to Beta diversity (Bray-Curtis).

Type Of Bacteria phylum	Percentage Of Bacteria In Patient	Percentage Of Bacteria In Control
Actinobacteria	0.000823419	0.001194409
Bacteroidetes	0.030518004	0.030816386
Deinococcus Thermus	4.55509E-05	0.000971769
Elusimicrobia	0.010115794	0.010873167
Fusobacteria	0.005933969	0.000977946
Lentisphaerae	0.001002047	0.004747233
Proteobacteria	0.009039459	0.006635984
Tenericutes	0.001890485	0.007589342
Verrucomicrobia	0.032599236	0.004964936
Candidatus Saccharibacteria	4.82817E-05	0.000209292
Cyanobacteria	4.33857E-05	4.74365E-05
Firmicutes	0.007038775	0.007066767

The gut microbiota, which contains more than 1,000 different bacterial species, colonizes the gastrointestinal system about trillions further were the two main types Firmicutes and Bacteroidetes, two phyla, account for over 90% of all the number of microorganisms (Qin *et al*., 2010). Those microbes for hundreds of years, the community has coevolved with people. for countless years, they are developing a mutually beneficial partnership with their host and carrying out crucial tasks seen as crucial. It helps in the extraction of nutrients and energy from diets, vitamin production, and immune system development system anti-pathogen defense (Qin et al., 2010) Alterations of the microbial interactions with the host affect the gut barrier function as well as the local immune system, producing in the

disruption of the intestinal homeostasis and contributing to the development of many human diseases including gastrointestinal disorders (inflammatory bowel diseases (IBD), diarrheic syndrome, colorectal cancer, etc.), autoimmune diseases (multiple sclerosis, type-1 diabetes, rheumatoid arthritis), metabolic diseases (obesity, type 2 diabetes, non-alcoholic hepatic steatosis, atherosclerosis) and neurological disorders (autism, Parkinson's disease) (Bravo *et al*., 2012; Julio-Pieper *et al*., 2014; Jandhyala *et* al., 2015). In reality, many non-communicable diseases change the composition of the gut microbiota in those affected, evidenced by a decline in microbial diversity (Konturek *et al*., 2015). Because of these factors, the gut microbiota is currently being looked at as a new target to enhance patient care through therapeutic (certain antibiotics, faecal transplant) or dietary intervention (newly developed probiotics or prebiotics), allowing the return to "healthy" gut microbiota (González-Arancibia *et al*., 2016).

Therefore, to establish a baseline that can aid us in understanding the relationship between altered gut states and diseases, it is required to determine the composition of the gut microbiota of healthy patients. Since many different factors are known to influence the taxonomic makeup of the gut microbiota, it is challenging to identify a shared core microbiota that every member of a population shares. According to host genetics, dietary preferences, age, ethnic origin, location, and lifestyle, the microbiota can differ between individuals or populations (Zoetendal et al., 1998; Turnbaugh *et al*., (2008, 2009); De Filippo *et al*., 2010; Arumugam *et al*., 2011; Yatsunenko *et al*., 2012; Claesson *et al*., 2012; Cotillard *et al*., 2013; Schnorr *et al*., 2014; Suzuki and Worobey, 2014. For instance, it has been proposed that differences in the gut microbiota may be seen in people or animals living in colder climates that require them to extract more energy and store more fat than those living in warmer climates (Suzuki and Worobey, 2014).

CHAPTER FIVE

CONCLUSION & RECOMMENDATION

5-1: Conclusions.

1-Many types of bacteria have been detected in the human intestine, such as Firmicutes, Bacteroide, Verrucomicrobia, Proteobacter, Lentisphaerae, Elusimicrobia, Tenericutes, actinobacteria, and Fusobacteria.

2- The statistical analysis revealed no significant differences in the microbiota between diabetes mellitus type 2 and control

3- The Verrucomicrobia,Proteobacter and Fusobacteria gave the highest percentages in diabetes mellitus type 2 compared with control .

4- The Firmicutes, Bacteroide ,Tenericutes and actinobacteria gave the lowest percentages in diabetes mellitus type 2 compared with control .

5-2: *Recommendations.*

1. More samples must be collected in the future to provide a clear picture of the microbiota of diabetes mellitus type 2.
2. Future research should include another type of diabetes mellitus to provide a clear picture of the microbiota's relationship with diabetes mellitus.
3. Investigate the impact of other ages and body weight on the microbiota of diabetics.
4. Examine the effects of treatments on the microbiota of people with type 2 diabetes
5. Study the impact bacterial gut microbiome on diseases other than diabetes mellitus such as obesity,autoimmune diseases,depression,cancers,allergy, autism and cardiovascular dieases.

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Appendices.

Appendix. (1) the questionnaire of population of diabetes mellitus type 2

الاسم الثلاثي			
العمر			
الجنس	ذكر	أنثى	
تاريخ الاصابة بالسكري النوع الثاني			
الامراض المرافقة الأخرى	**ضغط الدم**		
	قرحة المعدة		
	قرحة الاثني عشر		
	سرطان		
	امراض مناعة ذاتية		تصلب متعدد
			التهاب مفاصل رئوي
			التهاب القولون
أمراض أخرى			
تاريخ استخدام مضادات حيوية			
علاجات أخرى			

Appendix (2) results of pre-processing and clustering of OUT picking method (denovo) of diabetes mellitus type 2 and control.

- Results of Clustering (cutoff : 97%)		
No.	**Sample Name**	**Read Count**
1	c1	18,672
2	c10	63,737
3	c13	30,260
4	c14	13,783
5	c15	21,599
6	c16	14,611
7	c17	20,164
8	c18	419,760
9	c19	22,267
10	c2	22,697
11	c20	16,047
12	c3	106,969
13	c6	23,078
14	c7	86,887
15	c8	16,819
16	c9	66,274
17	p1	10,221
18	p10	26,163
19	p11	55,854
20	p12	14,518
21	p13	15,886

22	p14	24,496
23	p15	14,880
24	p16	24,717
25	p17	17,829
26	p18	20,026
27	p19	3,371
28	p2	28,696
29	p20	15,800
30	p3	24,673
31	p4	21,013
32	p5	54,946
33	p6	38,247
34	p7	21,184
35	p8	21,592
36	p9	15,364

Appendix (3) OUT abundance.

	A	B	C	D
1	Group	Organism	Kingdom	Phylum
2	denovo0	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _"Prevotellaceae"; _Prevotella; _uncultured bacterium	Bacteria	_"Bacteroidetes"
3	denovo1	Bacteria; _"Proteobacteria"; _Alphaproteobacteria; _; _; _; _uncultured bacterium	Bacteria	_"Proteobacteria"
4	denovo2	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _"Prevotellaceae"; _Prevotella; _uncultured bacterium	Bacteria	_"Bacteroidetes"
5	denovo3	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Ruminococcaceae; _Oscillibacter; _uncultured bacterium	Bacteria	_Firmicutes
6	denovo4	Bacteria; _"Proteobacteria"; _Gammaproteobacteria; _Aeromonadales; _Succinivibrionaceae; _Succinivibrio; _uncultured bacterium	Bacteria	_"Proteobacteria"
7	denovo5	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _"Prevotellaceae"; _Prevotella; _uncultured bacterium	Bacteria	_"Bacteroidetes"
8	denovo6	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _; _; _uncultured organism	Bacteria	_Firmicutes
9	denovo7	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Ruminococcaceae; _Faecalibacterium; _uncultured organism	Bacteria	_Firmicutes
10	denovo8	Bacteria; _"Lentisphaerae"; _"Lentisphaeria"; _Victivallales; _"Victivallaceae"; _Victivallis; _uncultured organism	Bacteria	_"Lentisphaerae"
11	denovo9	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Lachnospiraceae; _; _uncultured bacterium	Bacteria	_Firmicutes
12	denovo10	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _; _; _uncultured rumen bacterium	Bacteria	_Firmicutes
13	denovo11	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Peptostreptococcaceae; _Clostridium XI; _uncultured bacterium	Bacteria	_Firmicutes
14	denovo12	Bacteria; _"Tenericutes"; _Mollicutes; _Anaeroplasmatales; _Anaeroplasmataceae; _Asteroleplasma; _uncultured organism	Bacteria	_"Tenericutes"
15	denovo13	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _"Porphyromonadaceae"; _; _uncultured bacterium	Bacteria	_"Bacteroidetes"
16	denovo14	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _Bacteroidaceae; _Bacteroides; _uncultured bacterium	Bacteria	_"Bacteroidetes"
17	denovo15	Bacteria; _"Tenericutes"; _Mollicutes; _Anaeroplasmatales; _Anaeroplasmataceae; _Asteroleplasma; _uncultured bacterium	Bacteria	_"Tenericutes"
18	denovo16	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Lachnospiraceae; _; _uncultured bacterium	Bacteria	_Firmicutes
19	denovo17	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Ruminococcaceae; _; _uncultured Firmicutes bacterium	Bacteria	_Firmicutes
20	denovo18	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _; _; _uncultured bacterium	Bacteria	_Firmicutes
21	denovo19	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Ruminococcaceae; _Oscillibacter	Bacteria	_Firmicutes
22	denovo20	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _Bacteroidaceae; _Bacteroides; _uncultured bacterium	Bacteria	_"Bacteroidetes"
23	denovo21	Bacteria; _"Verrucomicrobia"; _Verrucomicrobiae; _Verrucomicrobiales; _Verrucomicrobiaceae; _Akkermansia; _uncultured bacterium	Bacteria	_"Verrucomicrobia"
24	denovo22	Bacteria; _"Proteobacteria"; _Gammaproteobacteria; _"Enterobacteriales"; _Enterobacteriaceae; _Escherichia/Shigella; _unidentified marine bacterioplankton	Bacteria	_"Proteobacteria"
25	denovo23	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _Bacteroidaceae; _Bacteroides; _uncultured organism	Bacteria	_"Bacteroidetes"
26	denovo24	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _Bacteroidaceae; _Bacteroides; _uncultured bacterium	Bacteria	_"Bacteroidetes"
27	denovo25	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Lachnospiraceae; _; _uncultured bacterium	Bacteria	_Firmicutes
28	denovo26	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _"Prevotellaceae"; _Alloprevotella; _uncultured bacterium	Bacteria	_"Bacteroidetes"
29	denovo27	Bacteria; _"Bacteroidetes"; _"Bacteroidia"; _"Bacteroidales"; _Bacteroidaceae; _Bacteroides; _uncultured organism	Bacteria	_"Bacteroidetes"
30	denovo28	Bacteria; _"Proteobacteria"; _Deltaproteobacteria; _Bdellovibrionales; _Bdellovibrionaceae; _Vampirovibrio; _uncultured bacterium	Bacteria	_"Proteobacteria"
31	denovo29	Bacteria; _; _; _; _; _; _uncultured organism	Bacteria	_
32	denovo30	Bacteria; _Firmicutes; _Clostridia; _Clostridiales; _Lachnospiraceae; _Roseburia	Bacteria	_Firmicutes

	D	E	F	G	H	I	J	K	L
1	Phylum	Class	Order	Family	Genus	Species	Sc	H	Accession_Numb
2	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_"Prevotellaceae"	_Prevotella	_uncultured bacterium	1	3	EF400089
3	_"Proteobacteria"	_Alphaproteobacteria	_	_	_	_uncultured bacterium	1	3	JQ185437
4	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_"Prevotellaceae"	_Prevotella	_uncultured bacterium	1	3	KF232588
5	_Firmicutes	_Clostridia	_Clostridiales	_Ruminococcaceae	_Oscillibacter	_uncultured bacterium	1	3	FJ508114
6	_"Proteobacteria"	_Gammaproteobacteri	_Aeromonadales	_Succinivibrionaceae	_Succinivibrio	_uncultured bacterium	1	3	JF221486
7	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_"Prevotellaceae"	_Prevotella	_uncultured bacterium	1	3	JQ187011
8	_Firmicutes	_Clostridia	_Clostridiales	_	_	_uncultured organism	1	3	HQ821266
9	_Firmicutes	_Clostridia	_Clostridiales	_Ruminococcaceae	_Faecalibacterium	_uncultured organism	0.67	3	HQ821236
10	_"Lentisphaerae"	_"Lentisphaeria"	_Victivallales	_"Victivallaceae"	_Victivallis	_uncultured organism	0.67	3	HM013364
11	_Firmicutes	_Clostridia	_Clostridiales	_Lachnospiraceae	_	_uncultured bacterium	0.67	3	AY975204
12	_Firmicutes	_Clostridia	_Clostridiales	_	_	_uncultured rumen bacterium	0.67	3	EU463172
13	_Firmicutes	_Clostridia	_Clostridiales	_Peptostreptococcaceae	_Clostridium XI	_uncultured bacterium	0.67	3	GQ138022
14	_"Tenericutes"	_Mollicutes	_Anaeroplasmatales	_Anaeroplasmataceae	_Asteroleplasma	_uncultured organism	1	3	HQ775278
15	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_"Porphyromonadaceae"	_	_uncultured bacterium	0.67	3	DQ824142
16	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_Bacteroidaceae	_Bacteroides	_uncultured bacterium	1	3	JX850417
17	_"Tenericutes"	_Mollicutes	_Anaeroplasmatales	_Anaeroplasmataceae	_Asteroleplasma	_uncultured bacterium	1	3	AJ608228
18	_Firmicutes	_Clostridia	_Clostridiales	_Lachnospiraceae	_	_uncultured bacterium	1	3	JQ187745
19	_Firmicutes	_Clostridia	_Clostridiales	_Ruminococcaceae	_	_uncultured Firmicutes bacterium	0.67	3	GU955628
20	_Firmicutes	_Clostridia	_Clostridiales	_	_	_uncultured bacterium	1	2	EU468959
21	_Firmicutes	_Clostridia	_Clostridiales	_Ruminococcaceae	_Oscillibacter	-	1	3	FJ651577
22	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_Bacteroidaceae	_Bacteroides	_uncultured bacterium	1	3	FJ676382
23	_"Verrucomicrobia"	_Verrucomicrobiae	_Verrucomicrobiales	_Verrucomicrobiaceae	_Akkermansia	_uncultured bacterium	1	3	AY979406
24	_"Proteobacteria"	_Gammaproteobacteri	_"Enterobacteriales"	_Enterobacteriaceae	_Escherichia/Shigella	_unidentified marine bacterioplankton	1	3	KC001111
25	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_Bacteroidaceae	_Bacteroides	_uncultured organism	1	3	HQ775131
26	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_Bacteroidaceae	_Bacteroides	_uncultured bacterium	1	3	FJ509418
27	_Firmicutes	_Clostridia	_Clostridiales	_Lachnospiraceae	_	_uncultured bacterium	1	3	FJ504146
28	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_"Prevotellaceae"	_Alloprevotella	_uncultured bacterium	1	3	JQ188537
29	_"Bacteroidetes"	_"Bacteroidia"	_"Bacteroidales"	_Bacteroidaceae	_Bacteroides	_uncultured organism	0.67	3	HQ788492
30	_"Proteobacteria"	_Deltaproteobacteria	_Bdellovibrionales	_Bdellovibrionaceae	_Vampirovibrio	_uncultured bacterium	0.67	3	KC163104
31	_	_	_	_	_	_uncultured organism	1	3	HQ805959
32	_Firmicutes	_Clostridia	_Clostridiales	_Lachnospiraceae	_Roseburia	-	1	3	EU764581

	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
1	Accession_Number_2	Accession_Number_3	c1	c10	c13	c14	c15	c16	c17	c18	c19	c2	c20	c3	c6	c7	c8	c9	p1	p10	p11	p12	p13	p14
2	EF405361	EF405380	0	4415	4593	72	22	1591	3815	36589	5829	1625	3708	16368	1519	576	3573	14584	5	4090	3493	805	182	3188
3	JQ187805	JQ191148	0	0	0	0	0	0	11	41249	2	0	2	0	4	0	0	0	0	0	0	0	0	0
4	DQ797113	KF232587	0	1389	107	18	6	497	75	777	101	23	11	3	539	38367	184	2485	0	265	825	72	8	892
5	FJ508111	GU243098	14	3084	473	22	10	79	318	4148	384	365	553	6688	470	87	26	1280	0	612	1149	227	530	303
6	AB506658	JF233080	0	5	0	3	2	1	1957	13078	2291	0	342	1	24	97	0	2	0	76	5766	1065	1018	3539
7	FJ509067	FJ508794	1	7	376	16	0	1023	400	9651	308	53	0	6267	583	16136	56	1595	2	18	1122	53	3	150
8	HQ815236	HQ815115	15	201	26	173	0	1607	578	12137	290	297	147	550	38	68	132	357	2	1	165	6	3	125
9	JN187071	HQ821326	1310	4477	929	1874	13	1616	326	1765	293	495	546	2265	791	779	1035	724	128	300	1166	264	1434	165
10	HM013347	JQ191142	0	650	0	1	0	0	22	8043	0	0	39	25	0	1	0	2833	0	2	81	60	1	3
11	GU244009	GU101599	150	1050	494	1718	772	228	162	3506	324	231	91	749	303	2684	1462	761	55	71	126	130	75	103
12	GU303886	GU303621	0	32	0	0	0	0	9	17963	3	0	0	0	4	0	0	0	0	0	0	0	0	0
13	DQ809337	DQ809373	20	233	590	471	4	333	460	1011	241	50	55	1865	104	4926	124	104	13	4	255	7	14	1
14	HQ775488	HQ775296	0	0	225	3	0	5	161	30002	1060	4	176	0	113	3	0	127	0	0	1	0	0	0
15	GU101548	GU101641	1	2558	745	9	1	19	415	14278	168	87	454	3444	1143	2224	43	843	0	352	104	1921	6	1421
16	JX850418	KF866075	0	48	34	4	0	1	6	9	1	980	336	19	609	9020	1	0	3	100	101	76	617	0
17	JQ185116	JQ184599	0	0	0	0	0	1	113	30898	3	0	0	30	2	10	0	0	0	43	1	4	1	0
18	GU102848	DQ796798	0	292	1005	0	0	2	105	1217	0	7	5	1412	96	2	0	3776	0	2	0	0	1	0
19	HM013373	GU955851	0	0	190	0	1	0	1	1087	2	5	0	138	62	0	0	0	0	339	5656	91	0	3
20	JQ336417	NA	0	0	0	0	0	0	1	8131	1	0	0	0	1	0	0	0	0	0	0	0	0	0
21	GU101283	FJ651876	9	934	369	39	1	40	258	3358	171	171	255	1866	177	65	14	461	1	141	508	143	76	450
22	FJ676460	AY985876	0	12	4	1	0	1	0	27	6	2	0	0	48	0	0	0	6	763	874	778	5522	720
23	DQ824470	EF402178	24	0	8	7	0	2	3	8	0	100	0	78	185	255	3	26	3	3802	0	3	0	45
24	KC001106	KC001109	29	34	6	10	35	10	0	19	3	30	0	395	351	19	79	5	1009	572	18	213	201	183
25	HQ807765	HQ807761	0	3	4	1	1	0	0	10	3	0	0	10	54	292	0	333	0	45	1	111	175	6
26	FJ509421	FJ509416	3791	192	71	641	5309	30	23	68	9	939	6	3858	397	2991	9	680	2038	500	1300	481	19	110
27	EU764978	EU765104	26	1284	192	115	2	6	28	1705	137	13	20	2313	62	96	70	624	0	473	487	205	11	75
28	JQ186794	KF230857	0	0	1	1	2	0	0	3590	1744	0	610	1599	447	129	0	0	1	21	2151	38	8	1505
29	HQ788531	FJ505077	2769	75	41	2439	7697	9	0	12	0	827	6	4305	158	5	2	16	0	18	215	127	155	36
30	GQ451200	GQ451224	0	0	1083	0	0	0	26	8896	2	16	1	0	306	0	0	221	0	0	4	0	0	0
31	HQ806054	HQ805743	0	0	0	0	0	0	83	16472	2	0	0	0	5	0	0	0	0	0	0	0	0	0
32	FJ650796	HQ751170	86	230	97	1073	4	7	19	441	81	5	19	281	54	868	153	380	1	7	35	12	4	127

	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX
1	p15	p16	p17	p18	p19	p2	p20	p3	p4	p5	p6	p7	p8	p9
2	0	1862	2342	3751	310	5381	1774	192	207	2423	26513	1606	4509	6358
3	0	0	0	0	0	193	0	0	0	0	0	0	0	0
4	0	229	63	359	6	74	379	20	1	460	1425	400	192	955
5	0	555	538	35	5	107	222	696	9	3837	107	258	650	159
6	0	1185	142	5069	4	4591	867	1708	51	1194	225	1863	0	1
7	0	352	108	39	4	642	398	7	3	117	5	324	1180	241
8	59	166	5	94	4	8	38	22	15	1505	17	45	312	3
9	529	1600	1063	734	376	304	1451	2503	52	1496	563	713	942	731
10	0	4	1	2	0	92	1	8	1	38	0	80	7	25
11	1354	286	376	253	95	60	153	94	1809	2991	184	122	621	743
12	0	0	0	0	0	5	0	0	0	0	0	0	0	0
13	1	58	183	34	13	95	5	27	22	13	45	87	53	31
14	0	0	0	0	0	0	0	0	0	0	0	3	0	0
15	0	123	1465	11	364	25	443	7	0	642	1909	77	638	146
16	0	0	81	7	1	4	16	1502	54	0	521	44	0	0
17	0	0	0	0	0	692	1	1	0	31	2	140	1	0
18	0	28	0	0	0	160	31	4	0	9	2	12	72	24
19	0	0	2	3	61	900	25	5	1	1	8	2469	64	2
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	133	93	51	76	43	161	74	63	592	31	170	381	88
22	0	4	316	29	9	31	107	7856	394	114	1849	310	0	3
23	3622	0	4	0	0	0	599	2	2691	3	1	30	127	0
24	943	10	324	888	204	47	241	416	187	6778	62	34	12	17
25	5	0	778	426	105	11	8	243	12	7842	98	2	7	0
26	1294	0	16	20	175	29	126	31	311	3643	428	313	53	53
27	0	119	31	180	32	19	59	16	0	238	19	153	399	54
28	0	2065	428	1190	0	27	18	6	6	499	7	564	0	1
29	484	0	27	1	1	0	5	294	405	87	63	66	12	0
30	0	0	0	0	0	140	14	0	0	0	0	8	0	0
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	14	62	27	85	195	2	86	4	1	189	19	16	16	66

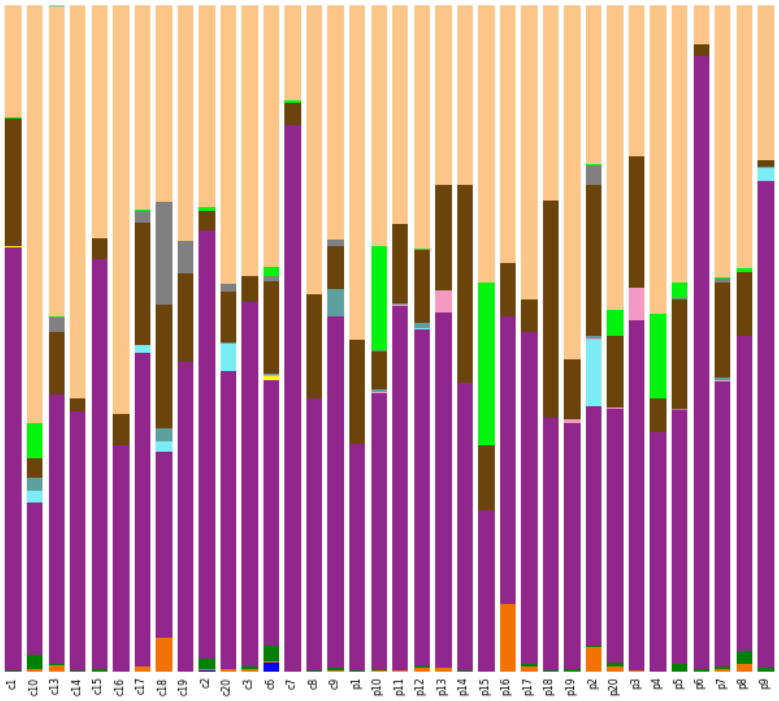
Appendix. (4) results of Community richness and diversity of microbiome in diabetes mellitus type 2 and control,

Community richness & diversity

SampleName	OTUs	Chao1	Shannon	Gini-Simpson	Good's Coverage
c1	123	136.57	3.621678	0.866788772	0.998928877
c10	307	345.15	6.282865	0.975420147	0.999497937
c13	317	377.27	5.734748	0.951692153	0.99828156
c14	175	217.5	4.615556	0.918701435	0.99746064
c15	141	182.05	2.647029	0.753196981	0.998148062
c16	171	214.05	4.401326	0.927475055	0.997125453
c17	261	299.9	5.159269	0.935159696	0.99761952
c18	603	644	5.6325	0.960695433	0.999899943
c19	182	210.5	4.201519	0.890998139	0.998248529
c2	216	229.5	5.173939	0.952140998	0.998766357
c20	173	225	4.886776	0.925521877	0.997507322
c3	313	352	5.632113	0.956464213	0.999635408
c6	358	419	6.244372	0.974847516	0.997313459
c7	188	210.88	2.981327	0.752837262	0.999597178
c8	152	173.08	4.40446	0.905262272	0.998632499
c9	221	242.94	4.869114	0.919994153	0.9995926
p1	73	112.43	3.494682	0.864043222	0.997651893
p10	249	282	4.8509	0.917618908	0.998280014
p11	249	284.06	5.061186	0.93529823	0.99939127
p12	258	320.67	5.764789	0.960787328	0.996693759
p13	242	281.2	4.302245	0.855433572	0.996915523
p14	191	222.07	4.986793	0.940818901	0.99877531
p15	97	108	4.279741	0.904812695	0.999193548
p16	256	289.48	5.499085	0.959238392	0.998462597
p17	252	308.4	5.456344	0.952752818	0.997307757
p18	173	195.14	4.26387	0.884492634	0.998452012
p19	126	149.25	5.035568	0.947449501	0.990803916
p2	256	279.38	4.843387	0.919038977	0.998815166
p20	277	351.39	5.519932	0.953247524	0.996265823
p3	255	281.86	4.138126	0.865525501	0.998054554
p4	154	217	4.761283	0.9321297	0.998286775
p5	266	297.5	5.182981	0.943026774	0.999344811

p6	200	241.78	2.353428	0.512122664	0.998745
p7	274	305.54	5.63006	0.95754153	0.998064577
p8	220	257.06	5.30384	0.936837957	0.998332716
p9	128	170.86	3.732106	0.805364549	0.99837282

Appendix (5) Percentages of bacteria in diabetes mellitus type 2 patients and control.



		Total	c1	c10	c13	c14	c15	c16	c17	c18	c19	c2	c20	c3	c6	c7	c8	c9	p1	p10	p11	p12	p13	p14	p15	p16	p17	p18	p19	p2	p20	p3	p4	p5	p6	p7	p8	p9
Legend	Taxonomy	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
	Archaea;__Euryarchaeota	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	Bacteria;Other	0.1%	0.00%	0.00%	0.1%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.3%	0.00%	0.00%	1.5%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Bacteria;__	0.7%	0.0%	0.4%	0.9%	0.0%	0.0%	0.0%	0.7%	5.0%	0.0%	0.2%	0.3%	0.3%	0.2%	0.0%	0.0%	0.2%	0.0%	0.3%	0.1%	0.5%	0.5%	0.1%	0.0%	10.1%	0.8%	0.0%	0.0%	3.8%	0.8%	0.1%	0.0%	0.0%	0.0%	0.5%	1.1%	0.1%
Bacteria;__Actinobacteria	0.4%	0.2%	2.1%	0.1%	0.1%	0.4%	0.0%	0.1%	0.0%	0.0%	1.5%	0.0%	0.5%	2.2%	0.1%	0.2%	0.3%	0.1%	0.1%	0.2%	0.4%	0.1%	0.0%	0.1%	0.0%	0.3%	0.2%	0.3%	0.1%	0.6%	0.1%	0.0%	1.3%	0.4%	0.3%	1.9%	0.4%
Bacteria;__Bacteroidetes	46.9%	63.5%	23.0%	40.5%	38.9%	61.5%	33.9%	47.1%	28.0%	46.5%	64.3%	44.7%	54.6%	39.9%	81.9%	40.8%	52.7%	34.0%	41.4%	54.7%	50.5%	53.3%	43.2%	24.1%	43.2%	49.9%	37.9%	37.0%	35.9%	38.1%	52.6%	35.9%	37.9%	91.9%	42.8%	47.3%	73.2%
Bacteria;__Deinococcus-Thermus	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteria;__Elusimicrobia	0.6%	0.0%	1.6%	0.0%	0.0%	0.0%	0.0%	1.1%	1.5%	0.0%	0.0%	4.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.9%
Bacteria;__Fusobacteria	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.1%	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	3.3%	0.0%	0.0%	0.0%	0.1%	0.0%	0.7%	0.0%	0.0%	4.8%	0.1%	0.0%	0.1%	0.1%	0.0%	0.0%
Bacteria;__Lentisphaerae	0.3%	0.0%	1.9%	0.0%	0.0%	0.0%	0.0%	0.1%	1.9%	0.0%	0.0%	0.3%	0.0%	0.1%	0.0%	0.0%	4.3%	0.0%	0.5%	0.2%	0.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.2%	0.0%	0.4%	0.0%	0.2%
Bacteria;__Proteobacteria	11.1%	19.3%	3.0%	9.2%	1.9%	3.2%	4.6%	18.3%	18.5%	13.3%	2.9%	7.6%	3.9%	14.0%	3.3%	15.6%	6.4%	15.7%	5.7%	11.9%	11.0%	15.8%	29.7%	9.8%	8.0%	4.9%	32.6%	8.8%	22.7%	10.8%	19.7%	4.9%	16.5%	1.8%	14.2%	9.6%	1.1%
Bacteria;__Tenericutes	0.9%	0.0%	0.1%	2.3%	0.0%	0.0%	0.0%	1.9%	15.4%	4.8%	0.0%	1.1%	0.0%	0.7%	0.0%	0.0%	0.9%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.0%	0.0%	0.0%	0.1%	0.0%	0.7%	0.0%	0.0%
Bacteria;__Verrucomicrobia	1.9%	0.1%	5.1%	0.2%	0.1%	0.0%	0.0%	0.0%	0.2%	0.0%	0.6%	0.0%	0.1%	1.5%	0.3%	0.0%	0.0%	0.0%	15.5%	0.0%	0.0%	0.0%	0.2%	24.3%	0.0%	0.0%	0.0%	0.0%	0.1%	3.8%	0.1%	12.8%	2.4%	0.0%	0.1%	0.6%	0.0%
Bacteria;__Candidatus Saccharibacteria	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteria;__Cyanobacteria/Chloroplast	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bacteria;__Firmicutes	36.8%	16.8%	62.8%	46.5%	59.0%	34.9%	61.3%	30.6%	29.3%	35.4%	30.2%	41.8%	40.6%	39.1%	14.3%	43.3%	35.2%	50.1%	36.2%	32.8%	36.6%	26.9%	26.8%	41.7%	38.6%	44.0%	29.2%	53.2%	23.9%	45.7%	22.6%	46.2%	41.6%	5.8%	40.9%	39.4%	23.1%
Unassigned;Other	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

الخلاصة:

داء السكري من النوع الثاني (T2D) هو مرض استقلابي مزمن متعدد الأعضاء ومتعدد العوامل. يتميز بعدم قدرة الجسم على امتصاص الجلوكوز (ارتفاع السكر في الدم) نتيجة لعيوب في إفراز الأنسولين أو عمل الأنسولين (مقاومة الأنسولين) أو مزيج من الاثنين معًا. الاستعدادات البيئية والوراثية هي السبب الرئيسي للاصابة بمرض السكري النوع الثاني . حيث تم دراسة العلاقة بين ميكروبيوم الأمعاء وتطور وشدة مرض السكري بشكل كامل. قارنت دراستنا الحالية بين الميكروبات المعوية لمرضى السكري من النوع 2 والأفراد غير المصابين (مجموعة المقارنة)، للكشف عن أكثر أنواع البكتيريا وفرة. تم جمع (36) عينة براز من المصابين بمرض السكري النوع الثاني(20) عينة وكانت اعمارهم تتراوح بين (30-39و40-50)، والاصحاء (16) عينة وكانت اعمارهم تتراوح بين (30-39و40-50) الذين راجعوا مختبر العمارة الاهلي في محافظة ميسان، للمدة من سبتمبر 2021 إلى فبراير 2022. وجدت النتائج أن هناك أنواع عديدة من البكتيريا في أمعاء الإنسان مثل *Firmicutes* و*Bacteriodetes* و*Verrucomicrobia*, و*Protobacteria*,و *Lentisphaerae* و *Elusimicrobia* و *Actinobacteria* , *Tenericutes* و *Fusobacteria* وأوضحت النتائج أن المرض يمكن أن يحدث عن طريق الانواع البكتيرية حيث اظهرت النتائج أن (2.9%) *Verrucomicrobia* و *Proteobacteria* (12.7%) , (*Fusobacteri*)%(0.47% في المرضى أعطت أعلى النسب في داء السكري من النوع الثاني مقارنة بالاصحاء.(0.5و9.06و0)على التوالي بينما *Firmicutes* (36.78%) و*Bacteroidetes* (44.89%) و*Tenericutes*(0.195%) و *Actinobacteria*(0.34%) في المرضى أعطت أقل النسب في داء السكري من النوع الثاني مقارنة مع الاصحاء (39.9% و47.6% و1.7%و0.48%) على التوالي.