Libyan Journal of Medical and Applied Sciences LJMAS

Volume 1, Issue 1, June-December 2023, Page No: 59-64
Website: https://ljmas.com/index.php/journal/index

Gut Microbiota and its impact on metabolic disorders

Hafiza Arshi Saeed[1], Aqsa Perveen[2], Ayesha Haidar[3], Hafiza Rida Fatima[4], Rameen Atique[5], Maria Aslam[6], Areesha Naveed[7], Javeria Sharif[8], Abdul Samad[9*]

[1, 2,3,4,5,6,7,8] Department of Pathobiology, FVAS, MNS University of Agriculture,25000, Multan Pakistan.

[9] Department of Animal Sciences, Gyeongsang National University, Jinju-si, South Korea.

[*]*Corresponding author: buzdarabdulsamad@gmail.com*

Received: November 12, 2023 Accepted: November 28, 2023 Published: December 10, 2023

Cite this article as: Saeed H, Perveen A, Haider A, Fatima H, Atique R, Aslam M, Naveed A, Sharif J, Abdul S. Gut Microbiota and its impact on metabolic disorders. Libyan Journal of Medical and Applied Sciences (LJMAS). 2023;1(1):59-64.

Abstract:

The numerous communities of microorganisms in the gut, called the human gut microbiota, living in the gastrointestinal tract, perform a critical role in maintaining metabolic balance. Interacting with the host's diet and physiology, this complicated environment of bacteria, viruses, fungi, and archaea considerably affects diverse metabolic processes. Imbalances in gut microbiota composition, called dysbiosis, have been related to the onset of metabolic issues like obesity, type 2 diabetes, and cardiovascular disease. Several mechanisms underline the gut microbiota's contribution to metabolic health, which includes the fermentation of dietary fibres to provide short-chain fatty acids, modulation of energy extraction from food, regulation of gut barrier function, and effect on host immune responses. Moreover, the intestine microbiota can affect systemic metabolism by affecting adipose tissue function, insulin sensitivity, and lipid metabolism. Recent research underscores the potential of therapeutic techniques for modulating the gut microbiota to cope with metabolic disorders. Probiotics, prebiotics, and dietary interventions to foster a healthy gut microbiota composition have proven to enhance metabolic parameters in animal and human studies. Exploring the complicated relationship between gut microbiota and metabolic disorders offers promising avenues for growing novel therapeutic approaches. This review highlights the role of gut microbiota and its role in gut and metabolic diseases; further studies are vital to get to the bottom of the particular mechanisms through which the gut microbiota affects metabolic health to identify personalized techniques for manipulating the microbiota to prevent and deal with metabolic disorders.

Keywords: Gut Microbiota, Functions, Microbiome, Gastrointestinal Track, Symbiosis, Dysbiosis, Host Interactions, Obesity, Cardiovascular Disease.

ميكروبات الأمعاء وتأثيرها على الاضطرابات الأيضية

حفيظة أرشي سعيد[1]، الأقصى بروين[2]، عائشة حيدر[3]، حفيظة رضا فاطمة[4]، رامين عتيق[5]، ماريا أسلم[6]، أريشا نفيد[7]، جافريا شريف[8]، عبد الصمد[9*]

[1،2،3،4،5،6،7،8] قسم علم الأحياء المرضي، جامعة MNS للزراعة، ملتان، باكستان.

[9] قسم علوم الحيوان، جامعة جيونج سانج الوطنية، جينجو سي، كوريا الجنوبية.

الملخص

تلعب المجتمعات العديدة من الكائنات الحية الدقيقة في القناة الهضمية، والتي تسمى ميكروبات الأمعاء البشرية، والتي تعيش في الجهاز الهضمي، دورًا حاسمًا في الحفاظ على التوازن الأيضي. من خلال التفاعل مع النظام الغذائي للمضيف وعلم وظائف الأعضاء، تؤثر هذه البيئة المعقدة من البكتيريا والفيروسات والفطريات والعتائق بشكل كبير على عمليات التمثيل الغذائي المتنوعة. ارتبطت الاختلالات في تكوين الكائنات الحية الدقيقة في الأمعاء، والتي تسمى ديسبيوسيس، ببداية مشاكل

التمثيل الغذائي مثل السمنة، ومرض السكري من النوع 2، وأمراض القلب والأوعية الدموية. تؤكد العديد من الآليات على مساهمة الكائنات الحية الدقيقة في الأمعاء في الصحة الأيضية، والتي تشمل تخمير الألياف الغذائية لتوفير الأحماض الدهنية قصيرة السلسلة، وتعديل استخلاص الطاقة من الطعام، وتنظيم وظيفة حاجز الأمعاء، والتأثير على الاستجابات المناعية للمضيف. علاوة على ذلك، يمكن أن تؤثر الكائنات الحية الدقيقة في الأمعاء على عملية التمثيل الغذائي الجهازي من خلال التأثير على وظيفة الأنسجة الدهنية، وحساسية الأنسولين، واستقلاب الدهون. تؤكد الأبحاث الحديثة على إمكانات التقنيات العلاجية لتعديل الكائنات الحية الدقيقة في الأمعاء للتعامل مع الاضطرابات الأيضية. أثبتت البروبيوتيك والبريبايوتكس والتدخلات الغذائية لتعزيز تكوين الكائنات الحية الدقيقة في الأمعاء أنها تعزز المعلمات الأيضية في الدراسات الحيوانية والبشرية. إن استكشاف العلاقة المعقدة بين الكائنات الحية الدقيقة في الأمعاء والاضطرابات الأيضية يوفر طرقًا واعدة لتطوير أساليب علاجية جديدة. تسلط هذه المراجعة الضوء على دور الكائنات الحية الدقيقة في الأمعاء ودورها في أمراض الأمعاء والتمثيل الغذائي. يعد إجراء المزيد من الدراسات أمرًا حيويًا للوصول إلى الجزء السفلي من الآليات المحددة التي تؤثر من خلالها الكائنات الحية الدقيقة في الأمعاء على الصحة الأيضية لتحديد التقنيات الشخصية لمعالجة الكائنات الحية الدقيقة لمنع الاضطرابات الأيضية والتعامل معها.

الكلمات المفتاحية: ميكروبات الأمعاء، وظائفها، الميكروبيوم، مسار الجهاز الهضمي، التكافل، ديسبيوسيس، تفاعلات المضيف، السمنة، أمراض القلب والأوعية الدموية.

Introduction

The human gastrointestinal (GI) tract is a significant interface, spanning 250–400 m2, connecting the host with environmental elements and antigens. Over a mean lifetime, about 60 tonnes of food traverse the human GI tract, followed by an array of environmental microorganisms that pose a significant risk to gut integrity. The assembly of bacteria, archaea, and eukarya residing in the GI tract is called the 'gut microbiota,' having co-evolved with the host over millennia to set up a complex and mutually helpful relationship. The populace of microorganisms in the GI tract is expected to surpass 10^14, comprising approximately ten times more bacterial cells than human cells and over one hundred times the genomic content (microbiome) compared to the human genome (Duan et al., 2019). This abundance of bacterial cells has characterized the host and its resident microorganisms as a ‘superorganism.

The human is a complex superorganism, functioning in concord with trillions of symbiotic bacteria and eukaryotic cells. Together, they form a "holobiont." their collective genome is called the "hologenome." Changes in the host genome and the microbiome can introduce variation to the hologenome, keeping the holobiont's adaptability. The completion of the Human Genome Project in 2001 highlighted the need to recognize the synergistic activities among humans and microbes for a complete grasp of biology. Initiatives just like the Human Microbiome Project (HMP) and the Metagenome of Human Intestinal Tract (MetaHIT) have exposed new insights into host–microbe interactions at main colonization sites: oral, gut, vagina, and skin. The human gut microbiota has captivated microbiologists because of its enormous medical implications.

The dynamic nature of the gut microbial community is characterized by variations for successful colonization inside the gastrointestinal tract. Essential trends consist of owning numerous enzymes to utilize available nutrients successfully, the presence of particular cell-surface molecular styles facilitating attachment to the suitable habitat, evasion capabilities towards bacteriophages, and resilience to the host’s reactive immune system. Microbes must undergo both physical and chemical stresses in the gut to proliferate hastily and avoid being washed out. Moreover, they need to survive dry and toxic conditions in the outside environment when transitioning to new hosts. The relationship between host and microbes is generally commensal, with one partner benefiting simultaneously as the opposite seems unaffected instead of mutualistic. Homeostasis in the gut microbiota is maintained through feedback mechanisms, wherein exemplary feedback can disrupt microbial community cooperation, leading to a potential runaway effect. An instance is a collaboration among two species, with upsurges in one promoting increases in the other, disrupting general community variety and abundance inside particular ecological niches (Festi et al., 2014).

The complicated method of setting up and colonizing gut microbiota includes several interactions among microbes and between microbes and the host. The critical basis for keeping lifelong health is laid at some point in infancy and early childhood when a healthy gut microbial ecology is established. While the uterine environment is historically believed to be sterile, unborn babies are on the start line of this dynamic microbial journey. Significant gut microbial composition and feature variations have

been reported among U.S. and Malawian/Amerindian populations (Jandhyala et al., 2015) In a rural African village, children exhibited a significantly higher abundance of Bactericides and a decreased abundance of Firmicutes in their fecal microbiota compared to European (Italy) children (Gurung et al., 2020). The gut microbiota is diagnosed as a pivotal "organ" influencing host biology, indicating that any compromise in the gut microbiota will have systemic results in the complete body (Adak & Khan, 2019). Dysbiosis in the gut microbiota is thought to be connected with improving and developing diverse human diseases.

In the realm of immunology, the host immune system perceives microorganisms as potential threats and orchestrates responses to eliminate them. However, many gut bacteria are non-pathogenic, accomplishing a symbiotic coexistence with enterocytes. These gut commensals play a pivotal role in activities including nutrient and drug metabolism, safeguarding against the colonization of dangerous microorganisms, and fortifying the integrity of the intestinal barrier. Simultaneously, the immune system has gone through coevolution to establish a harmonious partnership with the beneficial microbiota, successfully balancing its role in fighting invasive pathogens.

Methods to Study Gut Microbiota:

Examining the gut microbiota includes collecting stool samples from people and isolating DNA from the stool. Employing traditional culture-based techniques to isolate, identify, and enumerate the large array of gastrointestinal microorganisms poses a challenging endeavor. Initially, scientists could only isolate 10%-25% of the microbiota using culture-based strategies, generally because of the predominance of anaerobic microorganisms in the gut (Burcelin et al., 2012). Subsequent improvements in anaerobic culturing strategies allowed for the identity of dominant genera such as Bacteroides, Clostridium, and Bifidobacterium. However, those methods have drawbacks, which include the complexity of studying the cultural traits of numerous colonies on a Petri plate and the time-consuming nature of the process.

The gut microbiota research has advanced with the arrival of high-throughput gene sequencing technology, concerning two primary phases: (1) sequencing the bacterial gene through 16S rRNA and (2) conducting bioinformatics analysis. Concurrently, the sector of gut microbiota studies is witnessing speedy growth in metabolomics, which assesses small molecules connected to the complicated interaction between host-bacterial metabolism, holding importance for health and disease. The amalgamation of information from the gut microbiota and the metabolome presently serves as the most compelling evidence, providing insights into the nearest associations with both health and disease states.

Interaction of Gut Microbiota and its Host:

Establishing and maintaining favorable interactions between the host and its related microbiota is crucial for maintaining host health. While preceding research generally focused on the gut microbiota in the context of inflammatory diseases, it has now become evident that this microbial community performs a valuable role in normal homeostasis. It actively modulates the host's immune system and affects diverse host development and physiology factors, including organ development, morphogenesis, and metabolism. Despite the restricted understanding of the molecular mechanisms underlying host-microorganism interactions, current studies shed light on the critical signalling pathways concerned with the cross-species homeostatic regulation between the gut microbiota and its host.

The gastrointestinal tract hosts numerous microbiota that interact with the host, impacting gut physiology and extraintestinal functions. Energy metabolism plays a pivotal role in this interaction, with the gut microbiota receiving energy from the host for growth and reciprocally providing the host with metabolites like short-chain fatty acids, amino acids, bile acids, ClpB, and LPS. Research shows a significant effect of gut microbiomes on human and animal eating behavior. Eating disorders, including anorexia nervosa (AN) and bulimia nervosa (BN), correlate with distinct changes in gut microbiota composition (Sommer et al., 2013). For instance, AN patient show decreased faecal microbial diversity, while BN patients display an elevated abundance of bacterial ClpB protein. These microbial changes impact appetite and feeding behaviour, as proven through a piglet model associating lysine restriction-induced microbial communities with altered satiety hormones and increased feed intake. The complicated relationship between gut microbiota, appetite regulation, and energy metabolism is highlighted in a review (Graf et al., 2015) presenting an integrative homeostatic model. Dietary

interventions emerge as influential in shaping gut microbial communities and metabolism, potentially overshadowing the role of host genetics in improving metabolic diseases.

Impact on Metabolic Disorders:

The intricate interaction between gut microbiota and metabolic syndrome has been substantiated through substantial research on both human subjects and animal models. Dietary selections significantly affect the composition and features of the gut microbiome. This intricate intestinal ecosystem plays a pivotal role in modulating diverse host metabolism factors, including energy absorption, gut motility, appetite, and the regulation of glucose and lipid metabolism. Disruptions in the delicate equilibrium between gut microbes and the host's immune system can probably lead to the translocation of bacterial fragments, fostering the emergence of "metabolic endotoxemia" associated with systemic inflammation and insulin resistance. Weight loss caused by dietary changes or bariatric surgical procedures induces notable shifts in gut microbial composition, impacting the efficacy of treatment strategies. Strategic manipulation of the gut microbiota, done through the management of prebiotics or probiotics, holds promise in mitigating low-grade inflammation, fortifying gut barrier integrity, and ultimately fostering metabolic stability and weight loss (Li et al., 2016).

The metabolic syndrome is characterized by a blend of physiological, biochemical, clinical, and metabolic elements interlinked with an increased risk of cardiovascular diseases and type 2 diabetes mellitus. Key features, as described by the International Diabetes Federation (IDF), embody elevated blood pressure, dyslipidemia (notably increased triglycerides and reduced high-density lipoprotein cholesterol), raised fasting glucose, and central obesity. Global occurrence varies widely, spanning from less than 10% to 84%, contingent on geographical factors, the demographic composition of the studied population, and the standards used for definition (Han et al., 2021). Despite this variability, the syndrome's significant economic and social burden continues to escalate, prompting extensive clinical research aimed at unraveling the intricate pathogenesis of metabolic disorders.

Recent findings suggest the potential involvement of gut microbiota as a pathogenic factor influencing host metabolic equilibrium and associated disorders. Indeed, gut microbiota exhibits numerous beneficial properties that significantly impact human physiology and pathology. These include the modulation of host nutrition and energy extraction through the production of vitamins and fermentation of indigestible food components, the effect on intestinal epithelial homeostasis, participation in the improvement of the host immune system, safety towards pathogens, and involvement in drug metabolism (Thursby & Juge, 2017)

Gut Microbiota and Obesity:

It has been demonstrated that dysbiosis of the intestinal microbiota is strongly associated with obesity. Numerous microorganisms of the intestines have been linked to obesity. Modulating circadian rhythms, promoting fat storage, facilitating chronic inflammation, and increasing central appetite contribute to the development and occurrence of obesity. The induction of obesity by the intestinal microbiota remains an area that requires additional research owing to its intricate and varied nature. A confluence of environmental and genetic influences causes obesity. Future research will center on functional group studies of ecological significance to identify potential pathogenic members of the gut microbiota associated with obesity, data analysis utilizing larger samples to elucidate the mechanism underlying the association between the gut microbiota and obesity, and targeted microbiota management for individuals who are obese. (Liu et al., 2021)

Gut Microbiota and Cardiovascular Diseases:

Atherosclerosis is recognized as an inflammatory condition, with rising evidence suggesting a potential autoimmune connection. Infections play a significant role in fueling inflammation within the body, offering a plausible link to atherosclerosis. Numerous microorganisms, including Chlamydophila pneumoniae, Porphyromonas gingivalis, Helicobacter pylori, Influenza A virus, Hepatitis C virus, cytomegalovirus, and human immunodeficiency virus, have been linked to an elevated risk of cardiovascular diseases. The involvement of infections in atherosclerosis stems from two main pathways: direct infection of the blood vessel wall, making it at risk of plaque formation, and oblique impact from infections at distant sites, triggering systemic immune responses that influence plaque growth through pro-inflammatory mediators. Moreover, dysbiosis in the gut contributes to the

production of atherosclerotic metabolites such as trimethylamine N-oxide (TMAO) and may alter bile acid metabolism (Rahman et al., 2022; Novakovic et al., 2020)

Direct Infection:
Observations display the presence of bacterial DNA from more than 50 species within atherosclerotic plaques. The Proteobacteria phylum, notably represented by Chryseomonas and Helicobacter genera, emerges as the most abundant in these plaques. Concurrently, the Firmicutes phylum, encompassing Anaeroglobus, Clostridium, Eubacterium, Lactobacillales, and Roseburia genera, generally living in the oral and gut cavities, is also diagnosed in atherosclerotic plaques. Beyond this, versions in the gut microbiota among individuals with atherosclerotic cardiovascular disease extend to Lactobacillales, Collinsella (found in stenotic atherosclerotic plaques in the carotid artery leading to cerebrovascular events), Enterobacteriaceae, and Streptococcus spp. Notably, there is a proposition that specific gut microbiota, especially Bacteroides, Clostridium, and Lactobacillales, could potentially serve as diagnostic markers for individuals stricken with coronary artery disease (vogat et al., 2017) . This emphasizes the complicated interaction between bacterial diversity and atherosclerotic processes.

Indirect Infection:
Microorganisms play a pivotal role in fostering atherosclerosis by inducing the production of inflammatory cytokines and triggering acute-phase reactants, thereby intensifying chronic inflammation inside atheromatous plaques. In murine models, the management of antibiotics has proven the capability to adjust the gut microbiome, eventually impacting carbohydrate and lipid metabolism. While earlier investigations into the involvement of pathogens in atherosclerotic plaque development targeted individual microorganisms, modern knowledge emphasizes the importance of the overall microbiome. Recent research recognizes that the cumulative number of microorganisms an individual is colonized or infected with, termed "pathogen burden" or "infectious burden," correlates more closely with atherogenesis. This evolving perspective underscores the complexity of microbial interactions in influencing the dynamics of atherosclerosis.

Gut Microbiota and Diabetes:
Gut Microbiota is involved in Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). Type 1 diabetes is characterized as an autoimmune disorder due to the targeted destruction of insulin-producing pancreatic beta cells by T lymphocytes. This method results in an irreversible insulin deficiency over time. Early intervention with immunosuppressive agents has proven promise in stopping this destruction. The immune reaction is directed at antigens like Glutamic Acid Decarboxylase (GAD)-64 proteins. T lymphocytes infiltrate pancreatic islets, causing insulitis and progressively eliminating insulin-secreting cells. The mystery lies in understanding the mechanisms preventing the proper destruction of autoreactive T lymphocytes or allowing the autoimmune reaction due to self-antigens misrecognition, such as GAD64. Impaired maturation of T lymphocytes can be a contributing factor. Recent findings highlight gastrointestinal microbiota's crucial role in protecting against or triggering type 1 diabetes. Animal models like non-obese diabetic (NOD) mice and BB rats mimic this autoimmune method, offering insights into the disease's dynamics.
Recent reviews have explored diverse molecular mechanisms through which gut microbiota contributes to metabolic diseases and type 2 diabetes (T2D). The microbiota impacts inflammation, interacts with nutritional components, modulates gut permeability, and affects glucose, lipid metabolism, insulin sensitivity, and overall energy balance in the host. Notably, Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia, and Roseburia genera show off negative associations with T2D, while Ruminococcus, Fusobacterium, and Blautia genera display fine associations. The Lactobacillus genus, although frequently identified, offers inconsistent outcomes throughout studies. Interestingly, typically advised microbial community metrics like diversity indexes and the Bacteroidetes/Firmicutes ratio do not constantly correlate with T2D (Blandino et al., 2016).

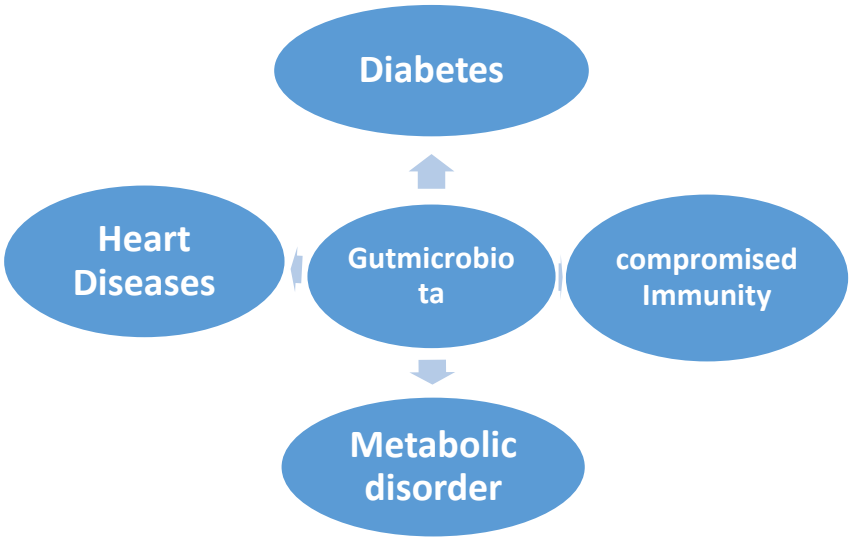

Figure 1 *This shows the effect of misbalancing of gut Microbiota*

Conclusion:

In summary, a scientific review found differences in gut microbiota between individuals with IBS and healthy controls, as indicated by 16S rRNA-targeted sequencing. Patients with IBS exhibited decreased α-diversity in both fecal and mucosal samples. Consistently, changes included increased Firmicutes, faded Bacteroidetes, elevated F/B ratio at the phylum level, in conjunction with heightened Clostridia and Clostridiales, and decreased Bacteroidia and Bacteroidales at lower taxonomic levels in fecal microbiota. Ultimately, achieving consensus is essential concerning whether a universally 'healthy' composition exists for the intestinal microbiota and, if so, how this equilibrium may be attained. Another perspective indicates that an individual's optimal microbiota may hinge on their lifestyle. Given bacteria's ability to hastily adapt metabolically to numerous conditions, it will become evident that microbiota composition, while crucial, may be overshadowed by the importance of gene expression profiles and functionality. Consequently, comparing solely microbiota composition proves insufficient, as similar combinations of bacterial phylotypes may yield distinct valuable properties. These concerns underscore that we are still in the early stages of comprehending these intricate matters, underscoring the need for further well-controlled human intervention studies.

References

1. Adak, A., & Khan, M. R. (2019). An insight into gut microbiota and its functionalities. Cellular and Molecular Life Sciences, 76, 473-493.
2. Blandino, G., Inturri, R., Lazzara, F., Di Rosa, M., & Malaguarnera, L. (2016). Impact of gut microbiota on diabetes mellitus. Diabetes & metabolism, 42(5), 303-315.
3. Burcelin, R., Serino, M., Chabo, C., Blasco-Baque, V., & Amar, J. (2011). Gut microbiota and diabetes: from pathogenesis to therapeutic perspective. Acta diabetologica, 48, 257-273.
4. Duan, R., Zhu, S., Wang, B., & Duan, L. (2019). Alterations of gut microbiota in patients with irritable bowel syndrome based on 16S rRNA-targeted sequencing: a systematic review. Clinical and translational gastroenterology, 10(2).
5. Festi, D., Schiumerini, R., Eusebi, L. H., Marasco, G., Taddia, M., & Colecchia, A. (2014). Gut microbiota and metabolic syndrome. World journal of gastroenterology: WJG, 20(43), 16079.
6. Graf, D., Di Cagno, R., Fåk, F., Flint, H. J., Nyman, M., Saarela, M., & Watzl, B. (2015). Contribution of diet to the composition of the human gut microbiota. Microbial ecology in health and disease, 26(1), 26164.
7. Gurung, M., Li, Z., You, H., Rodrigues, R., Jump, D. B., Morgun, A., & Shulzhenko, N. (2020). Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine, 51.
8. Han, H., Yi, B., Zhong, R., Wang, M., Zhang, S., Ma, J., ... & Zhang, H. (2021). From gut microbiota to host appetite: gut microbiota-derived metabolites as key regulators. Microbiome, 9(1), 1–16.
9. Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Reddy, D. N. (2015). Role of the normal gut microbiota. World journal of gastroenterology: WJG, 21(29), 8787.

10. Li, D., Wang, P., Wang, P., Hu, X., & Chen, F. (2016). The gut microbiota: A treasure for human health. Biotechnology Advances, 34(7), 1210-1224.
11. Liu, B. N., Liu, X. T., Liang, Z. H., & Wang, J. H. (2021). Gut microbiota in obesity. World journal of gastroenterology, 27(25), 3837.
12. Novakovic, M., Rout, A., Kingsley, T., Kirchoff, R., Singh, A., Verma, V., ... & Chaudhary, R. (2020). Role of gut microbiota in cardiovascular diseases. World journal of cardiology, 12(4), 110.
13. Rahman, M. M., Islam, F., Or-Rashid, M. H., Mamun, A. A., Rahaman, M. S., Islam, M. M., ... & Cavalu, S. (2022). The gut microbiota (microbiome) in cardiovascular disease and its therapeutic regulation. Frontiers in Cellular and Infection Microbiology, 12, 903570.
14. Sommer, F., & Bäckhed, F. (2013). The gut microbiota—masters of host development and physiology. Nature Reviews Microbiology, 11(4), 227-238.
15. Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. Biochemical Journal, 474(11), 1823-1836.
16. Vogt, S. L., & Finlay, B. B. (2017). Gut microbiota-mediated protection against diarrheal infections. Journal of travel medicine, 24(suppl_1), S39-S43.