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REVIEW ARTICLE

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# Circulating microbiota and metabolites: Insights into cardiovascular diseases

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## Abstract

**Background:** In almost every country, cardiovascular diseases are the major cause of death, which are responsible for 17.7 million deaths worldwide, or 54% of all deaths. However, the latest evidence has shown that non-communicable diseases such as obesity, diabetes, and cardiovascular events are significantly influenced by the blood microbiota and circulating metabolites.

**Methods:** We searched online databases for the most recent related papers through the comprehensive international databases of the Institute of PubMed/ MEDLINE, ISI/WOS, and Scopus up to August 2022, using MESH terms and the related keywords in the English language. Considering the titles and abstracts, unrelated studies were excluded. The full texts of the remained studies were evaluated by authors, independently. Then, the studies' findings were assessed and reported.

**Results:** The study demonstrated that the bacterial profiles of patients with cardiovascular diseases and healthy individuals are significantly different. The diseased patients showed a significantly high abundance of phylum Proteobacteria, an important Proteobacterial component known as lipopolysaccharides that has been linked to the pathogenesis of cardiovascular disease, while phylum Firmicutes were found in healthy individuals. It suggests that Proteobacteria has a direct role in the onset of cardiovascular disease.

**Conclusion:** We focused on the blood bacterial composition and circulating microbial metabolites in their relationship with the etiology and onset of cardiovascular disease. However, the various genera and species in the results reported were not always identical. Therefore, the microbial community structure of blood was more complicated and thus required a more in-depth exploration.

**KEYWORDS**
bacteria, blood microbiota, cardiovascular diseases, circulating metabolites, non-communicable diseases

## 1 | INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of death worldwide.[1] It is predicted that by 2030 the number of deaths due to CVD will increase to 23.3 million per year.[2] For instance, a previous report has shown an estimated 17. 9 million people died due to CVDs in 2019, responsible for almost one-third of all global deaths.[3] Different risk factors such as age, gender, family history, hypertension, diabetes, obesity, smoking, and stress are involved in the attainment of the disease. Rapid changes in the environment and modern lifestyle (e.g., high consumption of meat, a diet rich in lipids, and physical activity) are contributors to underlie the increase in atherosclerosis and myocardial infarction (MI) worldwide.[4] However, the meta-analysis of prospective cohort studies reported no connotation between CVD and dietary saturated fat intake, signifying that other environmental factors are responsible.[5] Moreover, the colonization of specific body sites in contact with the external environment (such as the gastrointestinal tract, skin, and vagina) by microorganisms is both well-described and universally accepted,[6] and the existence of microbial populations in other "classically sterile" locations, including the blood, is a relatively new concept.

The blood that flows perpetually through our veins and arteries performs numerous functions essential to our survival. Besides distributing oxygen, this vast circulatory system facilitates nutrient transport, deters infection, and dispenses heat throughout our bodies.[7] Due to the long-held belief that the bloodstream of healthy individuals is sterile and since the blood is an unfavorable compartment for the microbes due to its bacteriostatic and bactericidal components.[6] Recent studies, particularly cross-sectional studies targeting the 16 S rRNA gene, have revealed a dominant group of blood-borne bacterial phyla (i.e., Proteobacteria, followed by Actinobacteria, Firmicutes, and Bacteroidetes),[8,9] and demonstrate the consistency of the human blood microbiota across time. Moreover, examination of the bacterial taxa reported in these studies reveals similar blood microbiota compositions across the different studies, whereby Proteobacteria dominate (relative abundance values typically ranging from 85% to 90%), and Firmicutes, Actinobacteria, and Bacteroidetes present to a lesser extent.[10–12] This suggests the existence of a core blood microbiome profile that persists independent of the study environment or analytical methodology.

During the last 2 years, numerous studies have been published on blood microbial composition and its association with human health and diseases. For instance, several recent studies demonstrated a significant role of the tissue microbiome in the onset of cancer,[13] other diseases,[14] pre-diabetic and type 2 diabetic patients,[15] COVID-19,[16] metabolic diseases,[17] portal hypertension,[18] polycystic ovary syndrome,[19] myocardial infarction,[20] acute coronary syndrome, and chronic coronary syndrome.[21] Moreover, microbial metabolites present in the gut include amino acid derivatives, hormones, short-chain fatty acids (SCFAs), vitamins, and antioxidants.[22] Such metabolites might be absorbed directly into the circulatory system of the patient. For instance, an association between endotoxemia (elevation of endotoxin from gram-negative bacteria in the blood) and atherosclerosis in a population-based study was discovered several decades ago.[9] More recently, the deleterious effect of a bacterial metabolite, trimethyl amine oxide, on the blood vessel wall was shown in an animal model,[23,24] and the relevance of this finding in humans was suggested in a large prospective population-based study.[25]

The current study aimed to summarize the intricate interplay between blood microbiota, circulating metabolites, and their putative roles in the development and progression of CVD.

## 2 | CIRCULATING MICROBIOTA

Different microbial communities exist within the digestive tract, on the skin surface, and nearly every visible surface of the human body is now well understood, as shown in Figure 1.[26] A rising amount of studies suggests that increased permeability of oral and intestinal epithelial barriers may allow a limited number of microbes to reach the systemic circulation, then they can invade host organs and cause disease.[27,28] Bacteria that exist on or on exposed surfaces or that reach the systemic circulation can directly engage the innate immune system, prompting not only appropriate bactericidal responses but also altering host metabolism and inflammatory pathways connected to CVD.[29] A high-throughput 16 S rRNA gene sequencing has been used as a marker of microbial presence and a way of assessing bacterial diversity since the advent of next-generation sequencing-based technology.[30] Two decades ago a study discovered bacterial 16 S rRNA in healthy human blood, the bacteria's final processes and presence had not been investigated.[31] Although in recent years, a study used quantitative polymerase chain reaction to sequence 16 S rRNA gene V3-V4 hypervariable regions in the blood of 30 young healthy volunteers to investigate the blood microbiome composition in the different fractions. The buffy coat had the highest concentration of bacterial DNA (93.74%), followed by RBCs (6.23%), and plasma (0.03%). In comparison to buffy coats and plasma, the red blood cell fraction had higher bacterial diversity.[11] Unlike the gut taxa Firmicutes and Bacteroidetes, Proteobacteria were found in more than (80%) of blood samples, followed by Actinobacteria (10%) depending on the fraction.[11] These results indicated that the blood microbiota is predominantly translocated from the gastrointestinal tract because most of the above-mentioned bacteria at the phylum level have been discovered in the gut in previous investigations.[32,33] The skin and oral microbiota are more closely linked to the blood microbiota, with research on healthy blood suggesting that more bacterial translocation occurs from these niches rather than the stomach under normal physiology.[7] Microbial DNA is often identified in cellular components in healthy people, and the microbial community composition of the healthy gut and blood showed significant alteration.[1] In the gut, Firmicutes and Bacteroidetes phyla predominated, while Proteobacteria dominated in the blood.[11] Healthy human circulating bacteria are considered dormant since it does not cause issues like sepsis and inflammation; yet, the

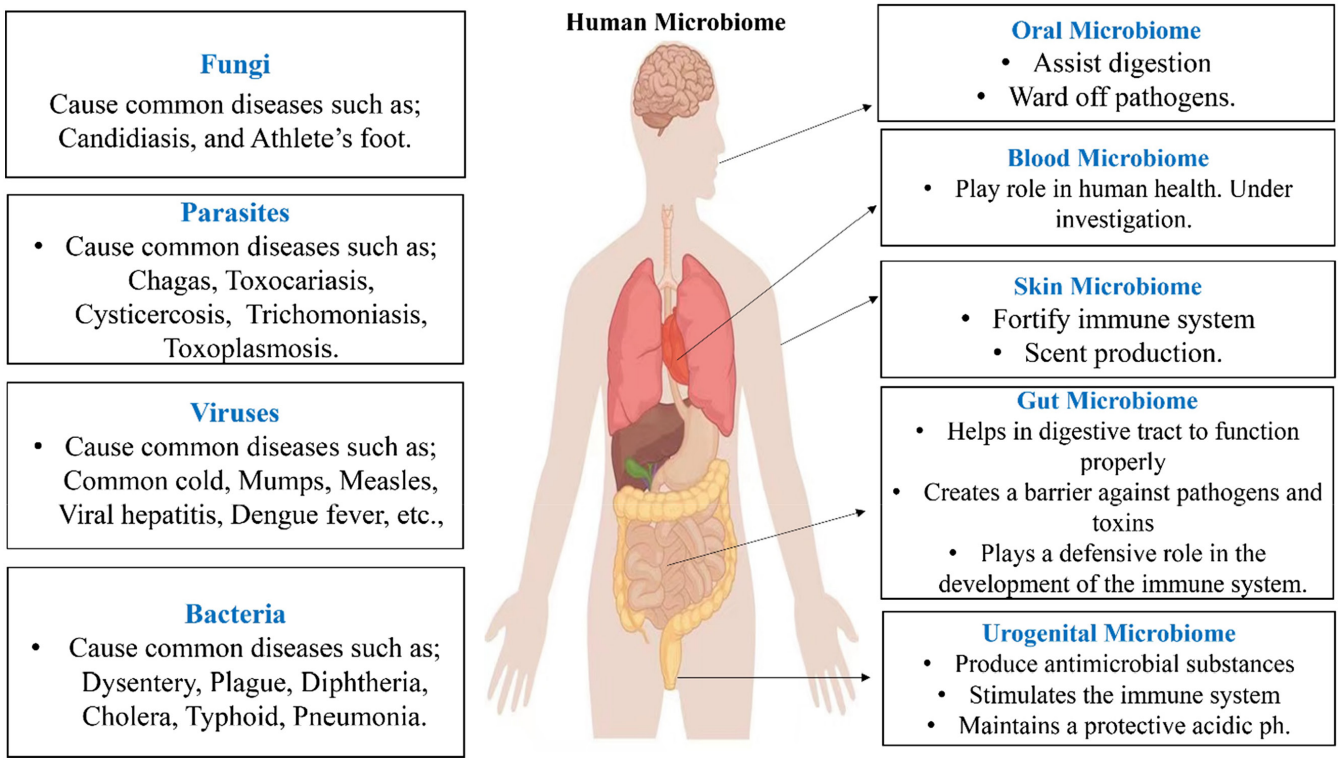


FIGURE 1 The human microbiome and its functions in the human body.

circulating microbiome plays a crucial role in natural physiology and immunology.[34] Furthermore, more research is needed to better understand the role of blood microbiota, physiology, and immunity in healthy individuals.

## 3 | ALTERATION IN BLOOD MICROBIOTA WITH CARDIOVASCULAR DISEASES

The imbalance in host-microbial interactions is known as microbiota dysbiosis. Enhanced disease susceptibility can be directly or indirectly linked to changes in microbial composition or community-derived factors (such as metabolites or genotoxins). The pathophysiological diseases associated with CVDs may develop as a result of these microbiome-based factors activating signaling pathways. Furthermore, it may be possible to target these components for therapeutic purposes (Table 1).

Blood microorganisms were once thought to indicate infection.[21] However, a rising body of evidence suggests that we should re-evaluate blood sterility and embrace a normal microbiome in recent years.[35] Thanks to technological advancements and the spread of novel techniques for analyzing the microbiota, such as 16S rRNA sequencing and metagenome sequencing, recent studies have focused on the identification of bacteria that may be implicated in the genesis of endotoxemia and the development of metabolic disorders.[36] The microbiome has an impact on human health and disease,[37,38] Although each microbial ecology differs, several species are ubiquitous.[38,39] Most of the studies that previously examined the relationship between the microbes and the onset of various diseases focused on the gut microbiota. However, researchers are now investigating links between blood microbiota and human health. The most prevalent bacterial phyla in the gut microbiota of healthy individuals are Proteobacteria, Bacteroidetes, and Firmicutes, while Proteobacteria predominate in the blood microbiota of healthy humans.[7] Although previous studies found that CVD patients had higher levels of circulating microorganisms, with Proteobacteria and Pseudomonadaceae populations significantly higher and Firmicutes Gammaproteobacteria, Bacillales, and Staphylococcaceae populations much lower.[40,41] In addition, congenital heart disease (CHD), valvar heart disease (VHD), and ischemic heart disease (IHD) were all associated with elevated levels of *Staphylococcus* spp., in the blood circulation.[42] The cardiovascular outcomes of individuals with ST-segment elevation myocardial infarction (STEMI) are reportedly influenced by intestinal microbe translocation into the systemic circulation. These STEMI patients have elevated levels of *Lactobacillus*, *Bacteroides*, and *Streptococcus*, potentially due to gut barrier-tight junction abnormalities.[43] Previously the function of microbes in CVD was limited to Myocarditis, endocarditis, pericarditis, and rheumatic carditis are all caused by pathogen infection and species such as cytomegalovirus, *Chlamydia pneumoniae*, herpes simplex virus, *Porphyromonas gingivalis*, *Enterococcus* spp., *Streptococci* spp., *Helicobacter pylori*, and *Trypanosoma cruzi*, which are the main pathogens involved in these infections linked to heart diseases.[44] The spread of these organisms via the bloodstream can cause bacteremia and sepsis, both of which can be fatal.[45] These alterations

**TABLE 1** Altered blood microbial compositions associated with CVDs

| Disease and sample size | Sample | Technique | Disease-associated changes in blood microbial abundance | References |
|---|---|---|---|---|
| 70 ACS, 70 CCS, and 70 Controls | Whole blood | 16S rRNA sequencing | Actinobacteria phylum, and genus *staphylococcus* in the healthy group, phyla Proteobacteria, and Acidobacteria in the ACS group, and phylum Firmicutes, and genus *Lactobacillus* were found in the CCS group | 21 |
| 29 MI patients and 29 controls | Whole blood | 16S rRNA sequencing | Actinobacteria phylum and *Bifidobacterium* genus were significantly increased in MI patients | 20 |
| 20 RA patients | Serum | 16S rRNA sequencing | A high proportion of phylum Proteobacteria, and genus *Serratia*, *Corynebacterium* were observed in RA patients | 53 |
| 103 non-MI and 99 MI patients | Whole blood | 16S rRNA sequencing | In MI patients, the Caulobacterales order and Caulobacteraceae family were observed to be significantly lower | 48 |
| 49 healthy, 100 STEMI, and 50 stable CAD patients | Blood leukocytes | 16S rRNA sequencing | *Streptococcus* spp., *Bacteroides*, and *Lactobacillus* were significantly higher in STEMI patients | 43 |
| 727 incident stroke and 1312 incident CAD patients | Whole blood | 16S rRNA sequencing | Infections were connected to an elevated risk of CVD in both inpatient and outpatient settings | 58 |
| Portal hypertension 58 patients with liver Cirrhosis and 46 control patients | Blood | 16S rRNA sequencing | *Comamonas*, *Cnuella*, *Dialister*, *Escherichia/Shigella*, and *Prevotella* were present in the patient group, whereas *Bradyrhizobium*, *Curvibacter*, *Diaphorobacter*, *Pseudarcicella*, and *Pseudomonas* numbers were decreased | 18 |
| 48 VHD, 35 CHD, 50 IHD, and 45 controls | Whole blood | 16S rDNA analysis | All CVD patients had predominant *Staphylococcus* sp., while VHD patients have more bacteria detected than CHD and IHD patients | 42 |
| 31 CVD patients and 10 controls | Whole blood | 16S rDNA sequencing | In CVD patients, there is a higher in *Pseudomonadaceae* and a lower in *Staphylococcaceae*, Gamma Proteobacteria, and Bacillales | 41 |
| 80 CVD patients and 40 controls | Blood plasma | 16S rDNA sequencing | Actinobacteria predominated in CVD patients, whereas Proteobacteria predominated in healthy | 40 |

**Abbreviations:** (ACS), Acute coronary syndrome; CCS, Chronic coronary syndrome; CKD, Chronic kidney disease; CAD, Coronary artery disease; (MI), Myocardial infarction; PD, Parkinson's disease; PCOS, Polycystic ovary syndrome patients; RA, Rheumatoid arthritis; (STEMI), ST-segment elevation myocardial infarction; (T2DM), Type 2 diabetes mellitus.

in the circulating microorganisms may result in persistent inflammation and infection, which could cause CVDs.

Previously a study performed 16S rRNA sequencing to analyze the microbial communities in whole blood from CVD patients and healthy controls, Proteobacteria were more abundant and Firmicutes were less abundant, but phylum-level bacterial diversity remained the same. The same team found that Bacillales, Staphylococcaceae, and Gammaproteobacteria were significantly underrepresented in shotgun metagenome sequencing, but Pseudomonadaceae was significantly higher prevalent.[41] Another study found that CVD patients had higher levels of proteobacteria/actinobacteria and circulating plasma microbial DNA. Additionally, the author discovered that although eukaryotic viruses were more prevalent in healthy adults, bacteriophages (*Pseudomonas*, *Rhizobium phages*, and *Propionibacterium*) predominated in the circulating virome of CVD patients.[42] In addition, blood cultures from patients with Valvar heart disease, congenital heart disease, and ischemic heart disease showed an increased abundance of bacterial species particularly *Staphylococcus* sp.[40] Pathogen infection was found to be a significant predictor of CVD risk 3 years ago in a study of 1312 incident coronary heart disease patients and 727 incident stroke patients.[45] The most common cause of atherosclerotic plaques, which cause inflammation and CVD, is microbes in the bloodstream.[46] A study discovered microbial translocation from the gut to the blood in individuals with ST-segment elevation myocardial infarction and mice models by changing intestinal permeability, which is made up of tight junction proteins. As a result, gut bacteria such as *Streptococcus*, *Bacteroides*, and *Lactobacillus*

were found in patients with ST-segment elevation myocardial infarction.[47]

In the year 2019, Amar et al. found that cholesterol-degrading bacteria (Aerococcaceae, *Rhodococcus*, *Gordonia*, Norcardiaceae, *Propionibacterium*, and *Chryseobacterium*) are present in the blood of control and MI patients, and the fractions of all of them are remarkably lower in MI patients. The Caulobacterales order and the Caulobacteraceae family were considerably lower in the MI group, and their presence in the blood of patients with myocardial infarction was likely to be negatively correlated with left ventricle ejection fraction.[9] Recently our study also found that members of the phylum Bacteroidetes; class, Bacteroidia; and order Bacteroidales were significantly enriched in healthy controls ($p = 0.05$), while the phylum Actinobacteria; class, Actinobacteria; order, Bifidobacteriales; family, Bifidobacteriaceae; and genus, *Bifidobacterium* were significantly distinct in the myocardial infarction. Our study revealed a significant reduction in alpha diversity (Shannon index, $p = 0.05$), in the MI group when compared to the healthy controls.[20] More recently, we performed another study that confirmed that the patients with myocardial infarction including (acute coronary syndrome patients, and chronic coronary syndrome patients) were distinct blood microbial profiles. Our study observed a significantly higher alpha diversity (Chao1, $p = 0.001$ and Shannon indices, $p = 0.004$) in the acute coronary syndrome group compared with the chronic coronary syndrome and healthy group, although a significantly lower alpha diversity was observed in the chronic coronary syndrome compared to acute coronary syndrome and healthy group, and beta diversity analysis demonstrated a major separation among three groups. Herein, we also found significantly distinct Proteobacteria in acute coronary syndrome patients, which suggests that the proteobacteria might play an important role in the onset of the coronary syndrome.[21] Meanwhile, previously Amar et al reported that an increased Proteobacteria population in CVDs correlated with major factors like age, blood pressure, fibrinogen, and alanine aminotransferase levels.[48] Also, recently several blood microbiota studies found an increased level of Proteobacteria in different chronic diseases. Thus, we conclude that Proteobacteria might play an important role in cardiometabolic diseases. Further studies are needed to investigate the direct role of Proteobacteria in the onset of cardiometabolic diseases.

Besides CVDs the blood microbiome may also have a role in the onset of other inflammatory diseases like Parkinson's disease and Alzheimer's disease.[34,49] Diabetes and cognitive diseases such as Alzheimer's disease and Parkinson's disease are linked. *Porphyromonas gingivalis*, the bacteria that cause chronic periodontitis, were detected in Alzheimer's disease patient's brain tissue, proving that the microorganisms circulated in the bloodstream from the oral cavity to the brain.[50] Blood samples from individuals with Parkinson's disease, Alzheimer's disease, and type 2 diabetes mellitus were examined using correlative light-electron microscopy since bacterial LPS impacts blood coagulation.[51,52] Since it has been suggested that the blood microbiota contributes to the pathophysiology of inflammatory illnesses, antibiotics, including antivirals, have been suggested as therapeutic agents.[49] Another study investigated the circulating microbial DNA patterns of individuals with psoriatic arthritis, ankylosing spondylitis, and rheumatoid arthritis.[53] In a different study that looked at cardiovascular death in patients with ESRD, 16S and ITS rRNA could be found in all but three of the patients' serum samples. Even though there was no significant difference in 16S rRNA levels of alpha diversity between cases and controls, the taxonomic analysis revealed different community profiles between groups, with significantly higher Actinobacteria and fewer Proteobacteria being observed at the phylum level in patients than controls.[54] HIV also increased the number of circulating bacterial proteins. Additionally, HIV boosted the diversity of aerobic bacteria from the Micrococcaceae (Actinobacteria) and Pseudomonadaceae (Proteobacteria) genera, which predominated in the blood.[55] Another study confirmed that the blood microbiome of rosacea patients had larger levels of the bacterial genera *Acidaminococcus* and *Megasphera*, as well as the genera *Rheinheimera*, and *Sphingobium* compared to the gut microbiome. Phylogenetic alpha diversity analysis by Faith revealed some differences between the blood microbiome of rosacea sufferers and controls.[56]

More recently, a study found that the relative abundance of Actinobacteria significantly increased whereas the relative abundance of Proteobacteria, Firmicutes, and Bacteroidetes significantly reduced in the polycystic ovary syndrome group. At various phylogenic stages, the cladogram showed the two populations' different microbiomes. The polycystic ovary syndrome group's Burkholderiaceae, Lachnospiraceae, Bacteroidaceae, Ruminococcaceae, and S24-7 showed substantial declines in LDA, whereas Nocardioidaceae and Oxalobacteraceae showed significant increases. Furthermore, the findings demonstrated that the blood microbiomes of polycystic ovary syndrome patients had significantly lower alpha diversity, different beta diversity, and significant taxonomic alterations as compared to healthy controls.[19] Additionally, the study found that the blood microbiota profile altered with age and that more than 95% of the blood microbiota belongs to the phylum Proteobacteria. The results of the clustering by principal component analysis showed clear patterns for each age group. In the elderly group (those over 60 years old), the class Gammaproteobacteria was found to be significantly higher, although the classes Alphaproteobacteria, Deltaproteobacteria, and Clostridia had significantly lower relative abundances ($p = 0.05$). According to alpha diversity measurements that considered the evenness of microbial taxa, the oldest group had significantly lower microbial taxa diversity than the other groups (Shannon index, $p = 0.002$ for young vs. elderly and middle vs. elderly, Dunn test), and slightly higher richness than the other groups (Chao1 index, $p = 0.033$ for middle verses elderly, Dunn test), there were no significant differences between the two groups.[57] The influence of the gut microbiota on innate immunity and its significant impact on several non-communicable diseases have been demonstrated and examined in recent studies. Consistently, the effect of oral microbiota in the pathogenesis of these diseases has been largely explored. Studies on blood microbiota provide some insights into the mechanism of action of circulating

microbiota and serve as the first line of evidence for the involvement of tissue microbiota in these diseases. Various investigations, which were certainly observational or qualitative, were unable to establish the causality of or the importance of the blood microbiota in the genesis of these diseases. These investigations were also preliminary, and to confirm the existence of a stable blood microbiome in these disorders, the results obtained need to be repeated in a future study with a greater number of samples. Additionally, the bacterial DNA might operate as an uninvolved third party during the illness process. It is crucial to further understand the mechanisms governing the symbiosis of the blood microbiome and the molecular interactions between the host and bacteria.

## 4 | CIRCULATING MICROBIAL METABOLITES

The diversity of functional genes in the microbiota demonstrates that the human microbiota has a higher metabolic capability than human cells, Figure 2. Dietary compounds and other substances are only partially digested by host systems; the remainder is processed by gut bacteria, especially in the anaerobic environment of the large intestine. The human microbiota also metabolizes medications, diverse synthetic compounds, and undigested carbs, lipids, and fiber, and can assess the efficacy or toxicity of these substances.[59,60] The gut microbiota should be considered when developing toxicological risk assessment methodologies for medications and environmental toxins.[61] Our microbial cells metabolize a variety of compounds through proteolysis, reduction, hydrolysis, functional group removal, N-oxide cleavage, denitration, deconjugation, amine formation, amide hydrolysis, thiazole ring-opening, acetylation, and isoxazole scission. Various enzymes are involved, including azoreductases, esterases, lipases, nitroreductases, β-glucuronidases, sulfatases, and β-lyases.[60]

### 4.1 | Trimethylamine N-oxide

Trimethylamine N-oxide (TMAO) has drawn a lot of attention among microbial metabolites as a significant cause of CVDs. Trimethylamine (TMA), which is predominantly produced through bacterial metabolism of dietary choline and phosphatidylcholine, produces TMAO as a byproduct in the liver.[23] Studies on human cohorts and germ-free mice have found a direct correlation between elevated CVD risk and plasma TMAO levels.[62–64] Intestinal microbiota suppression or food supplement depletion can stop TMAO production and lessen atherosclerosis in mice fed choline- and carnitine-rich diets, which resulted in high plasma TMAO levels and the development of atherosclerotic plaques.[23,62] Therefore, circulating TMAO may serve as a helpful CVD diagnostic marker. Increased risks of myocardial infarction may be indicated by elevated TMAO levels,[65] heart failure,[66] peripheral artery disease,[67] stroke[68] along with stable coronary artery disease,[67] independent of the traditional cardiac risk factors.[69] People who use broad-spectrum antibiotics experience gut microbiota depletion and have large decreases in TMAO levels.[63,69] Additionally, people are advised to limit their intake of foods high

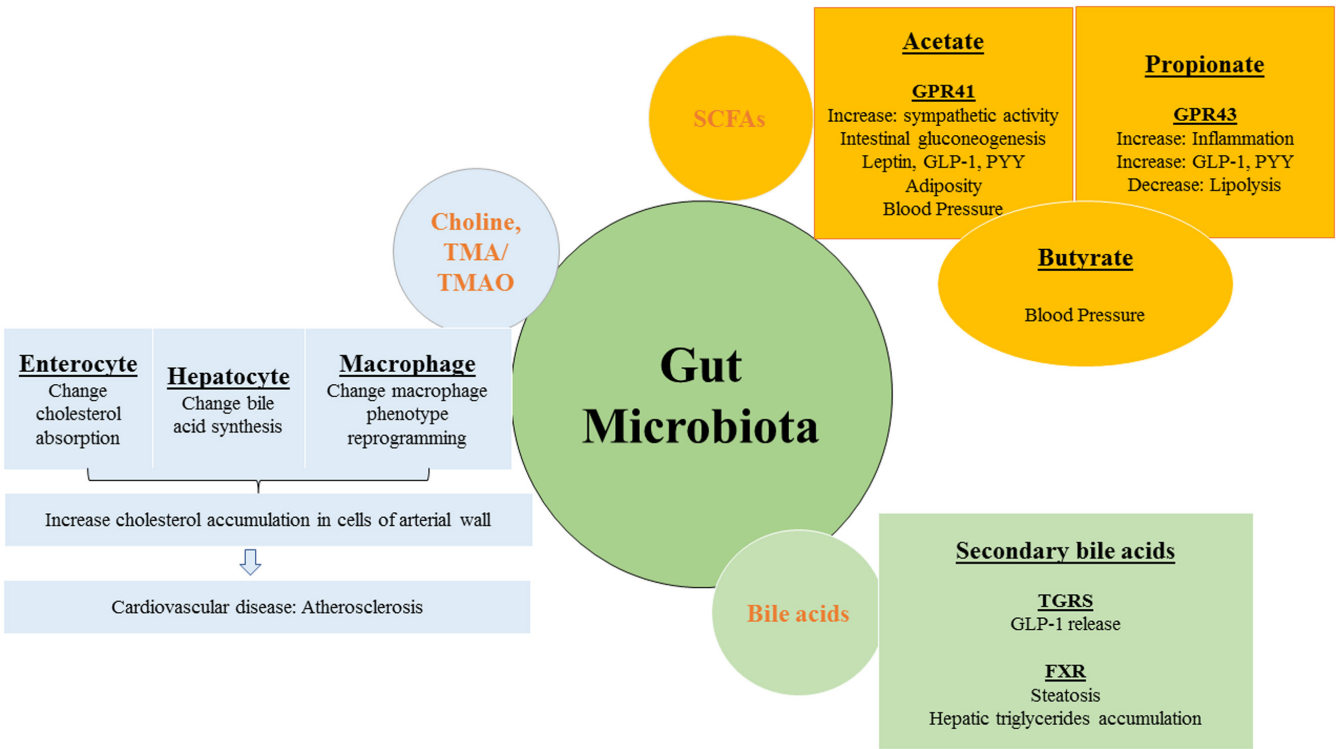


FIGURE 2 The mechanistic view of gut microbiota and circulating metabolites in the etiology and progression of CVD.

in carnitine, choline, and lecithin since diet is a primary source of TMAO[62] to lower the probability of developing a CVD. A previous study found that TMAO has been linked to modifications in plasma lipid, cholesterol, and sterol metabolism.[62] Experimental evidence in animals has shown that TMAO affects vascular dysfunction, inflammatory reactions, and oxidative stress through contributing pathways.[70] The TMAO receptor PERK in the endoplasmic reticulum has been found.[71] It's interesting to note that TMAO has been shown to protect hemodialysis patients with vascular damage, maybe in part because of its inhibitory effects on AGE.[71] It has been noted that several choline analogs lower TMAO levels in the blood. It has been discovered that the natural substance 3,3-dimethyl-1-butanol, which is present in large quantities in red wines, vinegar, and various grape seed oils, inhibits the microbial choline TMA lyase activity. This substance could prevent the formation of atherosclerotic lesions in Apoe−/− mice without affecting blood cholesterol levels.[72] There is evidence that several choline analogs, including fluromethylcholine, chloromethylcholine, bromomethylcholine, and iodomethylcholine, are more effective TMA lyase inhibitors that can lower plasma TMAO levels.[73]

## 4.2 | Short-chain fatty acids

Complex carbohydrates found in dietary fiber cannot be digested by the human intestine to support cell functions. However, fibers can be utilized by the gut bacteria through the fermentation process, which results in the generation of SCFAs.[74] One to six carbon chains can be found in SCFAs, which are saturated fatty acids. Most SCFAs in the human body are found as acetate, propionate, and butyrate.[75] SCFAs have a critical role in the regulation of gluconeogenesis, lipid metabolic pathways, and anti-inflammatory responses. Additionally, these compounds, particularly butyrate, are thought to serve as energy sources for intestinal epithelial cells.[76]

The possible modulatory effects of the microbiota on CVDs are hypothesized to be mediated via SCFAs in systemic circulation. Numerous studies showed an inverse relationship between the operation of the prorenin receptor-mediated intrarenal renin-angiotensin system and the production of sodium butyrate and propionate by the gut microbiota, which helps to lower blood pressure.[77,78] Olfactory receptors 78 (Olfr78) and G protein-coupled receptor 41(GPR41) are two examples of G protein-coupled receptors that G protein-coupled receptors (SCFAs) absorbed into the bloodstream may function through to mediate blood pressure effectors. Both receptors have distinct actions on vascular tone and are found in tiny resistance arteries. GPR41 behaves as a hypotensive protein to widen resistance arteries in an endothelium-dependent manner when stimulated by SCFAs. By using Olfr78, this hypotensive effect can be countered.[79] Under normal circumstances, these two receptors provide the required functional balance to stop excessive blood pressure volatility.[80] Additionally, it has been shown that SCFAs generated from the microbiota have an immunomodulatory effect on reducing oxidative stress and maintaining a healthy immune system. It has been demonstrated that adding 1% butyrate to the diet slows the progression of atherosclerosis due to its anti-inflammatory properties and improved plaque stability.[80] In addition, T cell-dependent protection from cardiac hypertrophy, fibrosis, vascular dysfunction, and hypertension is provided by SCFA propionate.[81]

## 4.3 | Bile acids

The liver makes bile acid from cholesterol, which is then eliminated into the colon via the bile duct.[82] Primary and secondary bile acids are two categories of bile acids.[83] The two main bile acids in humans are cholic acid and chenodeoxycholic acid. They pass through the small intestine after being first excreted into the bile.[84] Bacterial enzymes catabolize bile acids under the influence of bacteria, removing the hydroxyl group to create secondary bile acids such as ursodeoxycholic acid, deoxycholic acid, and lithocholic acid.[85]

Bile acids, however, have also been linked to an increased risk of CVDs.[86] Adults with arrhythmias were more likely to have high taurocholic acid levels, and atrial fibrillation has also been linked to specifically bound bile acid concentrations, such as greater serum levels of non-UDCA bile conjugates.[87] Through FXR-dependent FGFR4 signaling, bile acids may also lower plasma high-density lipoprotein levels and liver paraoxonase-1 expression.[88] Oral metformin has been shown to influence gut microbiota, and bile acid metabolism, and block intestinal FXR signaling. Further investigation revealed that in type 2 diabetics receiving metformin treatment, the prevalence of Bacteroides fragilis was linked with modifications in bile acid metabolites and FXR signaling. Glycoursodeoxycholic acid (GUDCA) also exhibited therapeutic effects on insulin resistance and glucose intolerance.[89] These results suggest that metformin improves metabolic dysfunction, including hyperglycemia, in part through a *Bacteroides fragilis*-GUDCA-intestinal FXR axis. In conclusion, the information at hand suggests that bile acids may contribute to the risk of CVDs, but more research is required.

## 4.4 | Choline metabolites

Three phosphatidylcholine-derived metabolites-choline, TMAO, and betaine were found to be biomarkers for the prediction of CVD risk using untargeted metabolomic screening of a large clinical sample.[69] A choline-rich diet supplement increased the generation of TMAO and increased atherosclerosis in mice, showing that gut flora plays a role in CVD. After these choline metabolites were quantified, TMAO revealed a stronger relationship with an elevated risk of CVD, and taking antibiotics lowered the level of TMAO and prevented subsequent cardiovascular events.[90] The primary microbial metabolite created by the metabolism of L-carnitine, lecithin, choline, and betaine in the gut is trimethylamine (TMA). Flavin-containing monooxygenase 3 is a liver enzyme that oxidizes TMA into TMAO in the liver. In population-based and intervention studies, elevated plasma levels of TMAO have been linked to an increased risk of

T2DM, cardiovascular and cerebrovascular diseases, incident thrombosis risk, carotid intima-media thickness, and STEMI.[91] Recently, a study found that individuals with heart failure had elevated levels of TMAO and indoxyl sulfate and that these levels were related to the prevalence of Escherichia and Shigella spp. in the gut microbiota.[90] To establish TMAO as a therapeutic target in CVD, more studies are needed to elucidate the precise mechanism of action of TMAO on vascular complications.

### 4.5 | Other metabolites

Aromatic amino acids like phenylalanine, tryptophan, and tyrosine might affect immunological, metabolic, and neural responses. Tyrosine, which can be further converted into neurotransmitters, norepinephrine, and adrenaline, is a metabolic precursor for phenylalanine, an important amino acid. Tryptophan is an important amino acid that serves as a precursor to another neurotransmitter, serotonin. A microbiota derivative of tryptophan was dramatically reduced in plasma in people with advanced atherosclerosis.[92] Tyrosine and phenylalanine metabolites in the intestinal microbes have been associated with MI severity in rats, according to a recent study.[93] There needs to be more research into the molecular linkages between these aromatic amino acid metabolites and cardiovascular disorders. The amino acid phenylacetylglutamine is formed when dietary phenylalanine is metabolized to phenylacetic acid and has been linked to CVD, and significant hostile cardiovascular events.[94] Signaling through adrenergic receptors, this gut microbiota-derived chemical enhances platelet activation-related characteristics and thrombosis potential.[95]

## 5 | MICROBIOTA-TARGETED THERAPEUTICS

Modern techniques are being utilized to identify changes in the blood microbiota and circulating metabolites, including metabolomics and next-generation sequencing. The advancement of biosensors and nano-sensors for the detection of specific bacteria or their metabolites will facilitate the incorporation of these biomarkers into routine clinical analysis. The restoration of the blood microbiota and circulating metabolites has been accomplished using a variety of techniques as an alternative therapeutic approach, Figure 3. To repair the intestinal barrier and restore the balance of the blood microbiota, prebiotics, probiotics, and antibiotics have all been used,[23,69,96–99] restoring the diversity of the bacteria in the gut, which may have a big impact on the blood microbiota and its circulating metabolites. Hemodialysis, a treatment for kidney failure that is often used, may be a therapeutic strategy to help diseased people's blood microbiotas repair. Hemodialysis membranes can be made to precisely filter intestinal microbes and their derivatives. For example, utilizing the oral charcoal adsorbent, indoxyl sulfate elimination from patients with advanced renal failure has been clinically performed.[26] Small synthetic chemical compounds are being developed into drugs that target the microbiota, their metabolites, and host mediators; some of these are currently in clinical trials.[99,100]

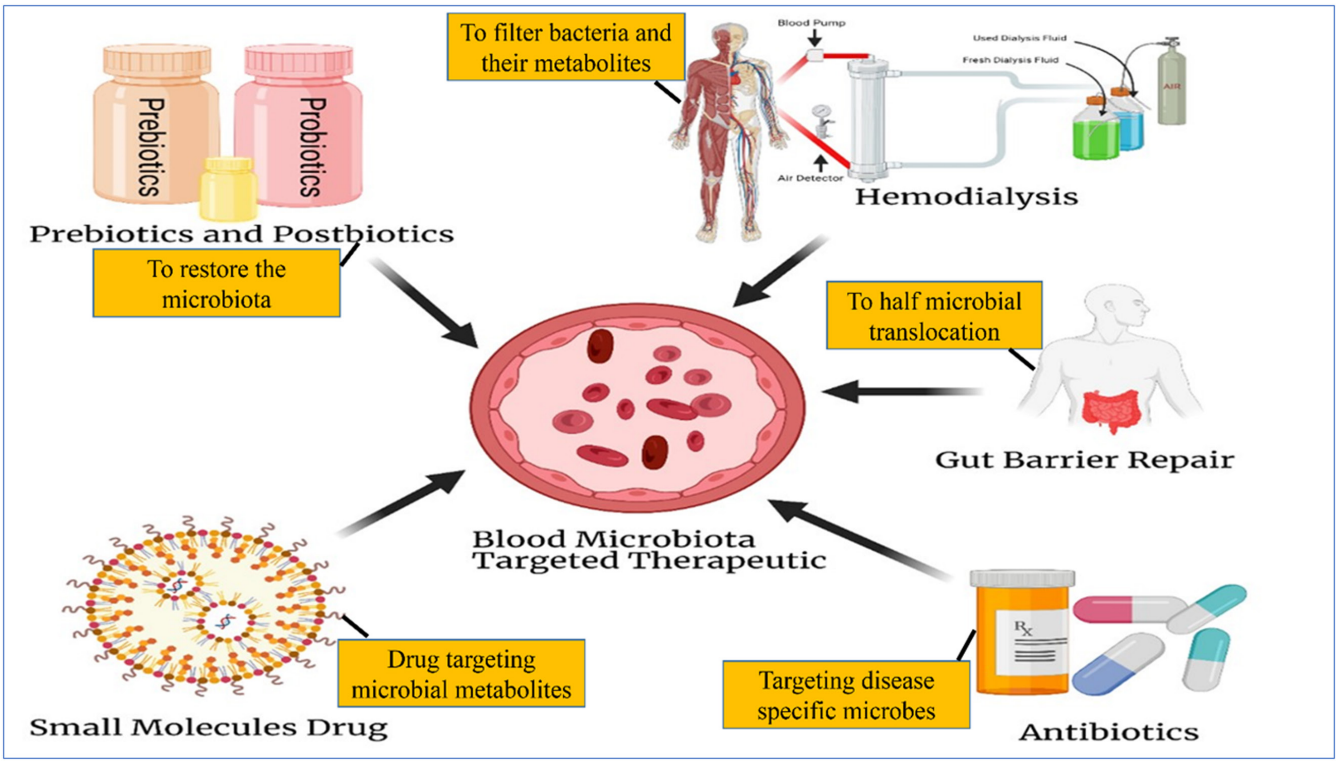


FIGURE 3 Treatment strategies for non-communicable diseases that focus on the blood microbiome.

## 6 | FUTURE PERSPECTIVES AND DIRECTION

Good scientific practice is important in all areas of science. In recent years, this has gained more and more attention, especially considering the scientific reproducibility crises.[101] While most researchers are aware of the issues with good scientific practice, not all these issues are necessarily clear, and the details can be very complicated. For many years, it has been accepted to perform and publish sequencing-based microbiome studies without including proper controls. Although in recent years more scientists realize the necessity of implementing controls, this poses a problem due to the complexity of the field. Another concern is the inability to properly interpret the information gained from controls in microbiome studies. In the microbiological sector, contamination is one of the main problems with 16 S-based categorization, especially when extremely small amounts of bacteria and compounds produced from bacteria are present.[101,102] The presence of environmental pollutants, laboratory reagents, and individuals participating in sample preparation in this situation could have a significant impact on the findings of the investigations and result in incorrect conclusions. It is noteworthy that longitudinal investigations like the one from Turner et al. have demonstrated the significance of the timing of nucleic acid extraction,[103] due to the possibility that various batches of the same extraction kits could identify different contaminants.[104] These illustrations unmistakably show that to account for contaminants and create solid and trustworthy results, microbiological investigations should always contain several negative controls.

It has now become evident that highly diverse microbial communities exist in the systemic circulation of various populations and that both quantitative and compositional changes in the circulating microbiota may contribute to the development and progression of cardiometabolic disease. However, many important questions remain unanswered regarding the nature of circulating microbiota, including the sources, pathophysiological roles, localization across different blood fractions, and distinction from potential contamination, all of which need to be clarified in future in-depth basic, clinical, and population-based research. In an ongoing quest to improve outcomes of patients with cardiometabolic disease, perhaps the time has come to go beyond the "gut feeling" and rigorously incorporate the potential pathophysiological insights gained from the circulating microbiota toward the development of novel biomarkers for diagnosis and prognosis, especially in personalized therapeutic approaches to premature morbidity and mortality in cardiometabolic disease where patterns are emerging.

## 7 | CONCLUSIONS

In this review, we have selected a few of the most recent cross-sectional studies of human cohorts that have made it possible to characterize the alterations in the blood microbiome linked to CVDs and other diseases. The ecological differences (dysbiosis), biological entities ("new species"), and gene-based variations ("biomarkers") related to illness diagnosis and the effects of current treatment therapies on the circulating microbiota have all been the subject of new insights from these investigations. Numerous studies have firmly established the significance of the blood microbiome in the emergence of cardiovascular and other disorders. The "omics"-based techniques are offering new insights into disease pathogenesis despite the complexity of the microbiome and the confounding effects resulting from host genetics, nutrition, medical co-morbidities, and other lifestyle factors. Certain could result in the creation of therapies that could be used to treat and prevent these metabolic illnesses. It is challenging to properly grasp these microbes' involvement in disease development and prevention and to use this information to apply microbiome research to medicine, as many of these microbes are still difficult to cultivate. To effectively complement and communicate the advancements being made by microbial genomics, there is an urgent need for a resurgence of interest in microbial biology. The ability to provide individualized therapeutic and/or preventative interventions, such as "next generation," logically assembled microbial consortia and probiotics, as well as dietary changes for reestablishing a gut microbiota that promotes and sustains health, will be supported by bringing metagenomic data to life, in the form of viable microbes.

### AUTHOR CONTRIBUTIONS

Supervised: An Lizhe and Li Zhiqiang; concept and write-up: Ikram Khan; revised: Zhou Jianye, Zhang Xiao Wei, and Xie Ping; figures preparation: M. Usman, Imran khan, Ikram Ali, and Sarmir Khan; and funding acquisition: An Lizhe and Li Zhiqiang.

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### DATA AVAILABILITY STATEMENT

All processed data used in this study are included in the article.

### CONSENT FOR PUBLICATION

All the authors agree to publish this article.

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