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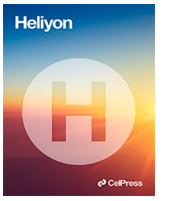

Review article

Microbiome signatures in ischemic stroke: A systematic review

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ABSTRACT

Microbial structural changes and dysfunction play an important role in the development of cerebral ischemia. We searched PubMed, Embase, Web of Science, and Cochrane Library and conducted a systematic review to assess the relationship between the human microbiome and ischemic stroke. A total of 24 studies were included, and the intestinal bacterial communities detected in both stroke and healthy people were dominated by 4 main phyla, including Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. Significant diversity (alpha and beta) in patients with ischemic versus nonischemic stroke was observed in nine out of 18 studies, and 3 studies showed that the severity of ischemic stroke affected microbial diversity. The imbalance of bacteria that produce short-chain fatty acids (SCFAs) changes the bacterial metabolic pathway, and disorders in the level of bacterial metabolites (trimethylamine N-oxide TMAO) lead to significant changes in intestinal flora function, which may aggravate the severity of stroke and affect its prognosis. Further studies are needed to explore the relationship between the microbiome and ischemic stroke.

1. Introduction

According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD 2019), stroke is still the second leading cause of death and the third leading cause of disability [1]. Stroke includes ischemic stroke and hemorrhagic stroke [2]. Ischemic stroke is the most common, accounting for 70 %–80 % of the total number of strokes [3]. The pathogenesis and risk factors for ischemic stroke are intricate and complex, and clinical prevention and treatment tasks are difficult [4].

The central nervous system, microbiome and gut constitute a bidirectional enterobrain communication system called the microbiota-gut-brain axis (MGBA), which plays an important role in brain physiology, behavior, and cognitive function [5,6]. Recent studies have shown that intestinal flora dysbacteriosis is closely related to the occurrence and prognosis of ischemic stroke [5]. However, there are various deviations in clinical studies, such as different regions, sample sources, and the lack of universally recognized and unified standards for microbial identification. These limitations have led to inconsistent research results related to microorganisms in ischemic stroke; for example, for *Roseburia*, which is a SCFA-producing bacterium, studies have shown that the

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relative abundance in the intestines of ischemic stroke patients is reduced [7], and there are also conclusions that the relative abundance is increased [8,9].

In addition to intestinal microbes, oral pathogens are involved in the pathogenesis of ischemic stroke through the microbiota–oral–brain axis [10]. One investigation has shown that the periodontitis salivary microbiota increases IL-17A-producing immune cells in the gut and migrates to the brain, triggering an early severe cascade of reactions that aggravates the severity of stroke and neuroinflammation in mice [11]. *Streptococcus* is not only a common bacteria in the oral cavity but also the most frequent bacteria in cerebral thrombus aspirates [12], and it may cause or contribute to platelet aggregation in the coronary artery [13]. The role of oral pathogens in stroke is expected to be determined from the perspective of the brain-oral-microbial axis.

In this study, the differences in microbiome composition in ischemic stroke patients were evaluated through meta-analysis, and the differences in microbiome composition and diversity (α and β diversity), Linear discriminant analysis Effect Size (LEfSe), and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional analysis were evaluated by systematic review.

2. Materials and methods

2.1. Registration and search strategy

The systematic review protocol was developed using PRISMA 2020 guideline (Checklist S1) [14]. We registered the review in PROSPERO (CRD42023395058) (https://www.crd.york.ac.uk/prospero/). Cochrane Library, Web of Science, PubMed, Embase database were searched in December 2022. There was no definite time limit for the retrieval literature. The retrieval included the combination of MeSH terms and free text, and the MESH terms included “Microbiota”, “cerebral infarction” and “ischemic stroke” to retrieve articles related to stroke and microbiome (Table S1 for search strategy).

2.2. Inclusion and exclusion criteria

Inclusion Criteria: (1)the objects were definitely diagnosed with ischemic stroke, and their ages were over 18 years old; (2)types of outcomes included detection of microorganisms; (3)studies published in any country with human subjects; (4)case–control study; cross-sectional study; retrospective and prospective cohort study; randomized and nonrandomized controlled trials (RCT); exploratory observational studies.

Exclusion Criteria: animal research; abstracts only; review; a letter to the editor; case reports or case series.

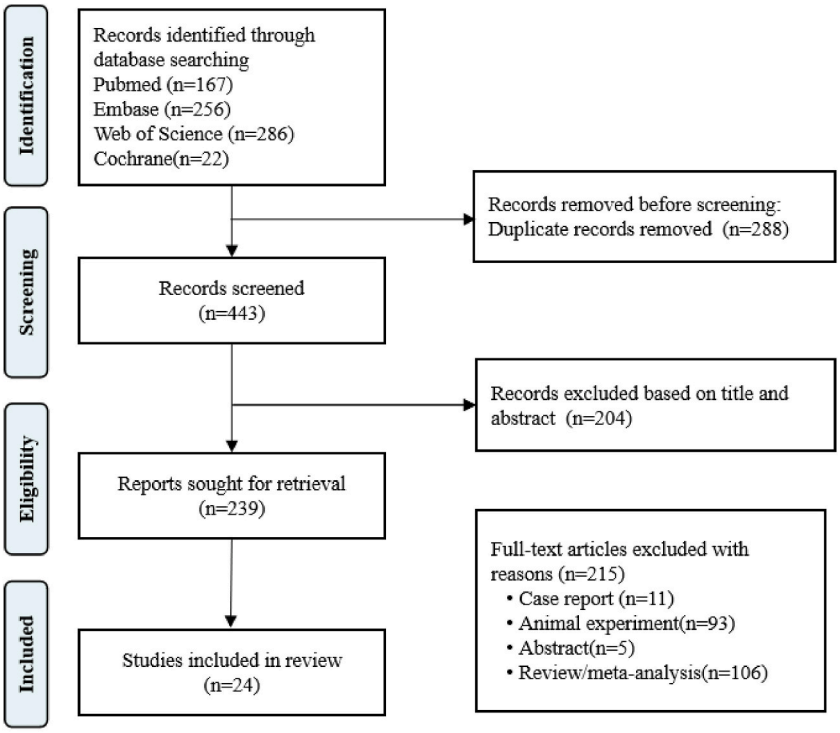

Figure 1. Description of the selection of the included studies following a PRISMA flflow diagram.

Fig. 1. Description of the selection of the included studies following a PRISMA flflow diagram.

Table 1
Characteristics of the included studies.

Study ID	Country	Study Design	Study Period	Total Number (Case/Control)		Intervention	Age (Case/Control)		Gender (Male/ Female)	Disease Type	Sample Collection	Method to Determine Microbiota	Sequencing Platform
Zhao et al., 2022	China	CCS	2017/ 5–2018/1	30	30	—	[#]60.75 ± 3.19	[#]62 ± 3.43	39/21	IS	stool	metagenomics	Illumina HiSeq
Walker et al., 2022	USA	OS	2020/ 1–2020/10	4	—	—	[#]62 ± 18.8		4/0	AIS	thrombus	16S rRNA V1–V9region	Illumina MiSeq
Liao et al., 2022	China	CS	2019/ 6–2020/6	(LAA) = 59 (CE) = 45	—	—	65 ± 3.98		60/44	LVO	Thrombus plasma fecal oral	16S rRNA V3–V4 region Bacterial culture FISH	Illumina HiSeq 2500
Guo et al., 2022	China	CCS	2017/ 1–2018/12	ITCM = 26	WM = 23	THD; WM	ITCM:[#]62.11 ± 15.32	WM:[#]56.22 ± 8.73	30/19	AIS with phlegm-heat syndrome	stool	16S rRNA V3–V4 region	Illumina MiSeq PE300
Zhang et al., 2021	China	CCS	—	CI = 28 OSAHS + CI = 28	30	—	NR	NR	43/43	OSAHS complicated by IS	stool	16S rRNA V4 region	—
Vajpeyee et al., 2021	India	OS	2018/ 4–2019/3	4	—	—	[b]51 ± 13.6		4/0	AIS	thrombus	metagenomics	—
Tan et al., 2021	China	CS	2017/ 6–2018/1	Mild = 78 Moderate = 47 Severe = 15	92	—	Mild:[a]59 (19) Moderate:[a]59 (20) Severe:*66 (28)	[a]60 (13)	146/86	AIS	stool	16S rRNA V4 region	Illumina HiSeq 2500
Sun et al., 2021	China	CS	2018/ 3–2019/6	mRS 0–2 (n = 105) mRS 3–6 (n = 27)	—	—	[#]67.5 ± 7.90		89/43	AIS	stool	16S rRNA V3–V4 region	Illumina HiSeq 2500
Haak et al., 2021	Netherlands	CCS	2010/ 7–2014/3; 2017/ 10–2018/3	349; IS = 287 TIA = 25 HS = 37	51	—	[#]71.5 ± 3.09	[#]71 ± 1.78	223/177	IS and HS	stool	16S rRNA V3–V4 region	Illumina MiSeq
Guo et al., 2021	China	CCS	2018/ 6–2019/6	THD = 26	WN = 23	THD; WN	THD:[#]61.6 ± 15.4	WN:[#]56.2 ± 8.7	30/19	AIS	stool	16S rRNA V3–V4 region	Illumina MiSeq PE300
Gu et al., 2021	China	CS	2018/ 5–2019/6	minor = 68 non-minor = 67	—	—	minor:[#]65.0 ± 9.2 non-minor:[b]71.0 ± 10.4		91/44	AIS	stool	16S rRNA V3–V4 region	Illumina Miseq
Wang et al., 2021	China	CCS	2014/ 2–2016/2	15	15	—	NR	NR	NR	AIS	stool	16S rRNA V4 region	Illumina Iseq100
Chang et al., 2021	Korea	CCS	2018/ 1–2019/12	mRS <3 (n = 159) mRS ≥3 (n = 39)	200	—	[b]63.7 ± 12.5	[b]63.5 ± 12.5	233/165	AIS	blood	16S rRNA V3–V4 region	Illumina Miseq
Xiang et al., 2020	China	CCS	—	LI = 10 AI = 10 PI = 10	16	—	LI:[b]72.5 ± 10.34 AI:[b]73 ± 9.05	[b]71 ± 5.65	21/25	AIS	stool	16S rDNA V3 region	—

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Table 1 (*continued*)

Study ID	Country	Study Design	Study Period	Total Number (Case/Control)		Intervention	Age (Case/Control)		Gender (Male/ Female)	Disease Type	Sample Collection	Method to Determine Microbiota	Sequencing Platform
							PI:[b]72.25 ± 7.44						
Li et al., 2020	China	CCS	—	79	98	—	[#]66.61 ± 12.07	[b]64.01 ± 10.44	107/70	IS	stool	16S rRNA not reported	Illumina Miseq
Cieplik et al., 2020	Germany	CS	2018/ 2–2018/7	IS = 49 SP = 8	SM = 42	—	IS:[b]69.33 ± 14.51 SP:[b]79.03 ± 12.51	SM:[b]65.37 ± 20.72	57/42	IS	tongue dorsa subgingival plaque	MALDI-TOF MS 16S rRNA V1–V3 region	Ion Torrent
Xia et al., 2019	China	CCS	2014/ 2–2016/2; 2017/ 1–2017/12	ACS = 72 PCS = 32	90	—	[a]59.38 (12.61)	[a]56.62 (8.16)	151/43	AIS	stool	16S rRNA V4 region	Illumina Miseq (PE 150)
Patrakka et al., 2019	Finland	OS	2013/ 11–2017/1	75	—	—	[b]66.9 ± 12.4		52/23	AIS	Thrombus blood	qPCR	—
Li et al., 2019	China	CCS	—	30	30	—	[#]60.47 ± 10.57	[b]64.17 ± 12.67	39/21	IS	stool	16S rRNA V1–V2 region	Illumina MiSeq
Huang et al., 2019	China	CCS	2018/ 2–2018/5	31	9	—	[b]64 ± 13.15	[b]61 ± 5.35	28/12	AIS	stool	16S rRNA V4 region	Illumina Miseq (PE 150)
Wang et al., 2018	China	CCS	2015/ 5–2017/3	10	10	—	[b]62.25 ± 4.01		10/10	IS	stool	16S rRNA V4 region	Illumina HiSeq 2500 (PE250)
Yamashiro et al., 2017	Japan	CCS	2014/ 4–2015/3; 2014/ 6–2015/2	41	40	—	[b]65.4 ± 14.1	[b]67.4 ± 8.9	55/26	IS	Stool blood	16S and 23S rRNA-targeted quantitative reverse transcription (qRT)-PCR	—
Ji et al., 2017	China	CCS	2015/5-2015-10	10	10	—	[b]65.9 ± 9.38		11/9	IS	stool	16S rDNA V4 region	Illumina MiSeq
Yin et al., 2015	China	CCS	2014/ 2–2015/2	322	231	—	[a]61 (19)	[a]56 (11)	350/203	IS and TIA	Stool plasma	16S rRNA V4 region	Illumina Miseq (PE 150)

NIHSS, National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; CCS, case-control study; CS, cohort study; OS, observational study.

LAA, The stroke etiology was large-artery atherosclerosis; CE, cardioembolism; OSAHS, obstructive sleep apnea hypopnea syndrome; HS, hemorrhagic stroke; ITCM, Tanhuo decoction (THD) + Western medicine (WM); WM, Western medicine; AIS, acute ischemic stroke; LVO, stroke patients with large vessel occlusion; SP, stroke-associated pneumonia, patients diagnosed with stroke who developed pneumonia; SM, stroke mimic, patients with stroke-like symptoms who were not diagnosed with stroke; IS, ischemic stroke; TIA, transient ischemic attack; LI, lacunar infarction; AI, non-lacunar acute ischemic infarction; PI, post-ischemic stroke patients who had undergone 15 days of treatment after acute ischemic stroke; ACS, anterior circulation stroke; PCS, posterior circulation stroke.

[a] Data represent median (interquartile range).

[b] Data represent mean ± standard deviation; NR, not report.

Table 2
Description of microbial alpha and beta diversity.

Study ID	Software	Alpha							Beta			
		Alpha Richness and Diversity	Shannon	Simpson	Chao1	Observed_species	ACE	PD whole tree	Beta Diversity	PCA	PCoA	Others
Zhao et al. 2022	R	higher IS than HC	⊙	—	⊕	⊕	⊕	—	no difference between IS and HC	—	⊙	—
Liao et al. 2022	VSEARCH	thrombus samples significantly differed from that of the fecal and oral samples but was similar to that of the plasma samples	⊕	—	—	—	—	—	thrombus samples was obviously distinct from that of fecal, oral, and plasma samples	—	⊕	—
Guo et al. 2022	QIIME	higher ITCM than WN	⊕	—	—	—	—	—	NR	—	—	—
Tan et al. 2021	QIIME	NR	—	—	—	—	—	—	intestinal microbiota distinguished AIS patients from HC, AIS patients with higher stroke severity exhibited significantly greater distances from HC	—	⊕	—
Sun et al. 2021	Mothur	mRS 3–6 group had less diversity than the mRS 0–2 group	⊕	⊕	⊙	⊙	⊙	—	obvious dissimilarity between the mRS 0–2 and mRS 3–6	—	⊕	—
Haak et al. 2021	phyloseq package	IS and HS was lower than HC	⊕	⊕	—	⊕	—	—	Difference between IS/HS and HC	—	⊕	—
Guo et al. 2021	QIIME 2	higher THD than WN	⊕	—	⊕	—	—	—	NR	—	—	—
Gu et al. 2021	Mothur	no difference between minor and non-minor stroke	⊙	⊙	⊙	⊙	⊙	—	minor stroke was different from non-minor stroke	—	⊕	—
Wang et al. 2021	R	no difference between IS and HC	⊙	—	—	—	—	—	difference between IS and HC	⊕	—	—
Chang et al. 2021	QIIME	NR	—	—	—	—	—	—	difference between AIS and HC	⊕	—	—
Xiang et al. 2020	Mothur	**1.**the chao1 richness index of the AI group differed significantly from those of the LI, PI, and HC	—	—	⊕	—	—	—	**1.**AI microbiome differed significantly from those of the LI and PI groups, and was comparable to HC	⊕	⊕	—
		2.the Observed_species index of the AI group differed significantly from those of the LI and PI groups	—	—	—	⊕	—	—	**2.**ANOSIM indicated that the microbiotic structure differed significantly among the groups	—	—	⊕
		3.the Shannon and Simpson indices of the PI group differed significantly from those of the AI and HC	⊕	⊕	—	—	—	—	**3.**significant differences between AI and the other three groups	—	—	⊕
		4.the ACE index of the AI group differed significantly from those of the LI, PI, and HC	—	—	—	—	⊕	—				
Li et al. 2020	Usearch	no different between IS and HC	⊙	⊙	⊙	—	⊙	—	no difference between IS and HC	—	⊙	—
Cieplik et al. 2020	phyloseq package	no different IS, SP and SM at BL	—	—	—	—	—	—	**1.**no difference between SM and SP at BL	—	⊙	—
									2.no difference between SM and IS	—	⊙	—

(*continued on next page*)

Table 2 (*continued*)

Study ID	Software	Alpha						Beta			
		Alpha Richness and Diversity	Shannon	Simpson	Chao1	Observed_species	ACE	PD whole tree	Beta Diversity	PCA	PCoA	Others
Li et al. 2019	QIIME	no difference between IS and HC	⊙	⊙	⊙	—	⊙	—	no difference between IS and HC	—	⊙	⊙
Huang et al. 2019	QIIME	no difference between AIS and HC	⊙	⊙	⊙	⊙	⊙	—	no difference between AIS and HC	—	—	⊙
Wang et al. 2018	QIIME	IS was lower than HC	—	—	⊕	—	—	—	significant difference IS and HC	—	⊕	—
Ji et al. 2017	QIIME	NR	—	—	—	—	—	—	significant difference between the IS and HC	⊕	⊕	—
Yin et al. 2015	QIIME	**1.**higher IS/TIA than HC	⊕	—	⊕	⊕	—	⊕	**1.**difference between IS/TIA and HC	—	⊕	—
		2.no difference between samples collected within 24 h and within 48 h in IS and TIA	⊙	—	—	—	—	⊙	**2.**no difference between samples collected within 24 h and within 48 h in IS and TIA	—	⊙	—
		3.no difference between the IS and TIA	⊙	—	—	—	—	⊙	**3.**no difference between the IS and TIA	—	⊙	—
		4.no difference between the initial stroke patients and recurrent stroke patients	⊙	—	—	—	—	⊙	**4.**no difference between the initial stroke patients and recurrent stroke patients	—	⊙	—
		5.severe stroke patients (NIHSS score >4) was higher than that of mild stroke patients (NIHSS score ≤4)	⊙	—	—	—	—	⊕	**5.**difference between severe stroke patients (NIHSS score >4) and mild stroke patients (NIHSS score ≤4)	—	⊕	—

IS, ischemic stroke; HC, healthy groups; HS, hemorrhagic stroke; TIA, transient ischemic attack; NR, not report; NIHSS, National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; SP, stroke-associated pneumonia, patients diagnosed with stroke who developed pneumonia; SM, stroke mimic, patients with stroke-like symptoms who were not diagnosed with stroke; BL, baseline, within 24 h of inpatient; LI, lacunar infarction; AI, non-lacunar acute ischemic infarction; PI, post-ischemic stroke patients who had undergone 15 days of treatment after acute ischemic stroke; TIA, transient ischemic attack; THD, Tanhuo decoction; WN, Western medicine; ITCM, Tanhuo decoction (THD) + Western medicine (WM); NR, not report; ⊕ represented $P < 0.05$, ⊙ represented $P > 0.05$.

2.3. Data selection and extraction

Two authors (ZW and TRB) independently selected the studies of titles and abstracts and reviewed the full texts and supplementary materials. Any discrepancies were resolved by consensus and arbitration by a third author (YLH). The researchers designed the data extraction table and extracted the data independently. For each included study, the following information was extracted: author, year, country, study design, study period, total number, intervention methods, age, gender, disease type, sample collection, method to determine microbiota and sequencing platform, microbial diversity, LEfSe and KEGG functional analysis results of ischemic stroke patients and non ischemic stroke population.

2.4. Quality Assessment

Newcastle–Ottawa Scale (NOS) scales were used to evaluation criteria for cohort studies and case–control studies, divided into 3 domains (population selection, comparability, exposure evaluation or outcome evaluation), 8 items, using a star system with a full score of 9 stars [15]. The risk of bias was then considered as high (<5 points), moderate (6–7), or low (8–9) [16]. The observational study was evaluated by Agency for Health care Research and Quality (AHRQ) bias risk assessment tool, and the risk of bias was evaluated from five domains (selection bias, implementation bias, follow-up bias, measurement bias, and reporting bias). Randomized controlled trial used the Outcomes Cochrane Collaboration tool for assessing risk of bias [17].

2.5. Outcomes

The differences in alpha and beta diversity between ischemic stroke and nonischemic stroke population; the difference of LEfSe between ischemic stroke and nonischemic stroke population; the difference of KEGG functional analysis between ischemic stroke and nonischemic stroke population.

2.6. Data synthesis

2.6.1. Qualitative synthesis

The included studies are summarized in the table, including the basic characteristics of the included manuscripts, Alpha diversity, beta diversity, LEfSe, KEGG functional analysis results.

3. Results

3.1. Search flow

The detailed retrieval process is shown in the PRISMA flowchart (Fig. 1). A total of 731 literatures were searched, and after deleting duplicates, 443 literatures were left. According to the inclusion and exclusion criteria, 24 studies were finally included.

3.2. Study characteristics

Table 1 describes the characteristics of the included studies. The studies were published between 2015 and 2022 and had 16 case–control studies, 5 cohort studies, and 3 observational studies. One study was from North America (USA, n = 1) and 22 studies from Asian countries (China, n = 17; India, n = 1; Japan, n = 1; South Korea, n = 1), 3 studies from Europe (Finland, n = 1; Germany, n = 1; Netherlands, n = 1). 19 studies collected stool samples, 4 studies collected thrombus, 5 studies collected blood samples, and two collected oral samples. The most widely used method for determining the microbiome is 16S rRNA (n = 18), with different amplification regions such as V3–V4 (n = 7), V4 (n = 8), V1–V9 (n = 1), V3 (n = 1), V1–V2 (n = 1), V1–V3 (n = 1), and one study did not mention the amplified region. In addition, there are techniques such as PCR, MALDI-TOF MS, FISH technologies to identify microorganisms. The most widely used sequencing platforms are Illumina MiSeq and Illumina HiSeq, with only one study using the Ion Torrent sequencing platform.

One study included both ischemic and hemorrhagic stroke patients [18], with inconsistent changes in gut microbiota composition in both types of stroke. Two studies from the same team evaluated difference of intestinal microbiome before and after treatment using a combination of traditional Chinese and Western medicine for acute ischemic stroke [19,20].

One study, involving both human and animal subjects, compared the gut microbiotics of acute ischemic stroke patients and healthy people, simulated the stroke dysbiosis index (SDI) and clinical validation, and established mouse models to examine neurobehavioral and assess the effects of dysbiosis after stroke [21].

Four studies analyzed microorganisms in thrombotic samples [22–25] and found oral pathogens in thrombus, particularly cariogenic *Staphylococcus*, *Streptococcus*, and *Lactobacillus* [22]. Two studies analyzed microbes in oral samples [23,26]. One study focused on comparing differences in thrombus, plasma, oral, and stool samples; bacterial composition of thrombus was similar to plasma samples, but different from oral and fecal samples, where 2.3 % of thrombotic microbial communities came from oral [23].

3.3. Alpha and beta diversity

A total of 18 studies analyzed microbial diversity and richness (Table 2). The alpha and beta diversity was analyzed, Shannon (n = 12), Simpson (n = 7), chao1 (n = 10), Observed_species (n = 7), ACE (n = 8), PD whole tree (n = 1), PCA (n = 4), PCOA (n = 15), ANOSIM (n = 2), PLSDA (n = 2). Mainstream analysis software was QIIME, and a few studies have used Mothur, VSEARCH, R and Usearch. In terms of alpha diversity, two studies showed that the microbial diversity of ischemic stroke patients was higher than that of

Table 3
Description of Linear Discriminant Analysis Effect Size. IS, ischemic stroke; LAA, The stroke etiology was large-artery atherosclerosis; CE, cardioembolism; NIHSS, National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; LI, lacunar infarction; AI, non-lacunar acute ischemic infarction; PI, post-ischemic stroke patients who had undergone 15 days of treatment after acute ischemic stroke; OSAHS, obstructive sleep apnea hypopnea syndrome; IS, ischemic stroke; TIA, transient ischemic attack; AE, adverse event.

Study ID	LDA Score Threshold	Case(abundance)				Control(abundance)
Zhao et al. 2022	>2.0	**IS** *g_Oscillibacter, f_Oscillospiraceae, s_Oscillibacter sp.CAG:241, c_Alphaproteobacteria, s_Clostridium sp.CAG:226, s_Oscillibacter sp. ER4, s_Azospirillum sp. CAG:239, g_Acetobacter, f_Coriobacteriaceae, s_Acetobacter sp.CAG:267, s_Bacteroides caccae, g_Odoribacter, g_Collinsella, s_Prevotella stercorea CAG:629, s_Odoribacter planchnicus, s_Akkermansia, muciniphila CAG:154, g_Desulfovibrio, s_Dorea sp. CAG:105, s_Bacteroides oleiciplenus, s_Alistipes senegalensis, c_Opitutae, f_Puniceicoccaceae, o_Puniceicoccales, g_Coraliomargarita, s_Coraliomargarita sp.CAG:312, s_Desulfovibrio piger, s_Alistipes shahii, s_Ruminococcus sp.CAG:488, f_Pasteurellaceae, o_Pasteurellales, s_Candidatus Alistipes marseilloanorexicus, s_Eubacterium limosum, s_Prevotella amnii, s_Clostridium sp.CAG:715, s_Clostridium sp.CAG:1024*				*f_Rhodospirillaceae, g_Azospirillum, s_Bacteroides caecimuris, s_Barnesiella intestinihominis, s_Eubacterium sp.CAG:841, s_Acetobacter sp. CAG:977, s_Azospirillum sp. CAG:260*
Liao et al. 2022	—	**LAA** *Chryseobadterium, Lactobacillaceae, Lactobacillus, Burkholderiales, incertae sedis, Aquabacterium, Corynebacterium, Corynebacterlaceae*	**CE** *Negativicutes, Selendmonadales, Veillonellaceae, Veillonella*	**with_AE** *Serratia, Enterobacteriales, Enterobacteriaceae, Acinetobacter, Moraxellaceae, Pseudomonadales*	**survival** *Burkholderiales incertae sedis, Aquabacterium, Rahnella, Variovorax*	**without_AE** *Sphingobacteriia, Sphingpbacteriales, Pedobacter, Sphingobacteriaceae, Comamonadaceae, Comamonas* **death** *Moraxellaceae, Pseudomonadales, Acinetobacter*
Zhang et al. 2021	—	**IS** *Coriobacteriales, Vagococcus, Sphingobacteriales, Adlercreutzia*		**OSAHS + IS** *Bifidobacterium, Parascardovia, Metascardovia, Anaerostipes caccae*		*Actinobacteria*
Tan et al. 2021	=2.5	**mild** *Porphyromonadaceae, Mycoplasmataceae, Phyllobacteriaceae, Carnobacteriaceae, Staphylococcaceae*	**moderate** *Enterobacteriaceae, Enterococcaceae, Erysipelotrichaceae, Methanobacteriaceae, Corynebacteriaceae, Eubacteriaceae*	**severe** *Lactobacillaceae, Helicobacteraceae, Bradyrhizobiaceae, Deferribacteraceae, Micrococcaceae*		*Bacteroidaceae, Prevotellaceae, Lachnospiraceae, Alcaligenaceae, Moraxellaceae, Pasteurellaceae*
Sun et al. 2021	>2.0	**mRS3-6** *g_Enterococcus, f_Enterococcaceae, g_ErysipelotricHaceae_incertae_sedis, s_Clostridium_innocuum, s_uncultured_bacterium, g_Eggerthella, s_Eggerthella_lenta, s_uncultured_bacterium, g_Corynebacterium, s_Peptococcus_sp_oral_taxon_167*		**mRS0-2** *g_Butyricicoccus, s_uncultured_organism, f_Sutterellaceae, f_Desulfovibrionaceae, o_Desulfovibrionales, c_Deltaproteobacterla, g_Faecalibacterium, s_uncultured_organism, o_Selenomonadales, c_Negativicutes*		—
Gu et al. 2021	>2.0	**minor stroke** *s_uncultured organism, g_Bilophila, g_Roseburia, o_Selenomonadales, c_Negativicutes, f_Bacteroidaceae, g_Bacterpides, P_Bacteroidetes, c_Bacterodidia, o_Bacteroidales*		**non-minor stroke** *c_Bacilli, f_Enterococcaceae, g_Enterococcus, g_Erysipelotrichaceae incertae sedis, s_Clostridium innocuum, g_Anaerovorax, f_Corynebacteriaceae, s_Corynebacterium tuberculostearicum, P_Cyanobacteria Chloroplast, c_Chloroplast*		—
Wang et al. 2021	—	*Actinomyces, Actinomycetaceae, Dorea, Roseburia, Actinomycetales, Peptostreptococcaceae, Atopobium, _Ruminococcus_, Coriobacteriales Coriobacteriia, Coriobacteriaceae Clostridiaceae Bifidobacterium, SMB53, Bifidobacteriaceae Bifidobacteriales Actinobacterla, Blautia, Actinobacteria*				*Bacteroidales, Bacteroidia, Bacteroidetes, Bacteroidaceae, Bacteroides, Dethiosulfovibrionacea, Pyramidobacter, Oxalobacteraceae, Oxalobacter, Megasphaera*
Xiang et al. 2020	—	**AI** **Family:** *Weeksellaceae, Bacillaceae, Paenibaciiaceae, Brucellaceae, Xanthomnadaceae* **Genus:** *Bacteroides, Ruminococcaceae, Bifidobacterium, Blautia, Enterobacteriaceae, Lachnospiraceae, Coriobacteriaceae, Prevotella*	**LI** **Family:** *Bacteroidaceae, Erysipelotrichaceae* **Genus:** *Bacteroides, Enterobacteriaceae, Phascolarctobacterium, Megasphaera, Lachnospiraceae, Acidaminococcus, Lachnospira, Parabacteroides*	**PI** **Family:** *Methanobacteriaceae, Sphingomonadaceae, Pseudomonadaceae, Verrucomicrobiaceae* **Genus:** *Enterobacteriaceae, Enterococcus, Bacteroides, Veillonella, Ruminococcaceae, Akkermansia, Lactobacillus, Lachnospiraceae*		**Family:** *Lactobacillaceae* **Genus:** *Lachnospiraceae, Bacteroides, Ruminococcaceae, Prevotella, Blautia, Enterobacteriaceae, Bifidobacterium, Ruminococcus*

Li et al. 2020	>2.4	**IS** *Lactococcus, Lactobacillus*			*Faecalibacterium, Subdoligranulum, Roseburia, Lachnoclostridium, Butyricicoccus, Eubacterium_rectale_group*
Cieplik et al. 2020	—	**Stroke-associated pneumonia group** *Parvimonas_micra, Fusobacterium_nucleatum_CC53, Treponema_denticola_ATCC_35404, Eikenella_corrodens_CC92I, Fusobacterium_hwasookii_ChDC_F128*		**Stroke group** *Neisseria_elongata_subsp_glycolytica_ATCC_29315, Prevotella_sp_, Neisseria_sp_HMSC065C04, Porphyromonas_sp_oral _clone_CW034 _Porphyromonas_sp_oral_clone_DP023, _Neisseria_sp_HMSC067G12, Neisseria_flavescens, Neisseria_subflava_NJ9703, Neisseria_elongata*	**Stroke mimic group** *Streptococcus_sp_HPH0090, Streptococcus_parasanguinis_CC87K, Streptococcus_sp_HMSC065C01, Actinomyces_sp_ph3*
Li et al. 2019	=2	**IS** *g_Odoribacter, c_Verrucomicrobiae, f_Verrucomicrobiaceae, g_Akkermansia, p_Verrucomicrobia, o_Verrucomicrobiales, g_Ruminococcaceae_UCG_005, g_norank_f_Flavobacteriaceae, p_Parcubacteria, g_norank_p_Parcubacteria, g_Victivallis, c_norank_p_Parcubacteria, o_norank_p_Parcubacteria*	**NIHSS≤4** *g_Enterobacter, g_Pyramidobacter, p_Synergistetes, f_Synergistaceae, o_Synergistales, c_Synergistia, g_Lachnospiraceae_UCG 001*	**NIHSS>4** *g_Ruminococcaceae_UCG_002, f_Christensenellaceae, g_Christensenellaceae_R_7_group, c_Actinobacteria, p_Actinobacteria, g_Ruminococcaceae_UCG_005, g_norank_f_Ruminococcaceae, g_norank_f_Alcaligenaceae, f_Peptostreptococcaceae, g_Comamonas, g_Erysipelatoclostridium, g_Tyzzerella_4, g_Adlercreutzia, f_norank_p_Saccharibacteria, c_cnorank_p_Saccharibacteria, g_Marinobacter, f_Alteromonadaceae, o_Alteromonadales, o_norank_p_Saccharibacteria, o_Oceanospirillales, g_Flavonifractor, g_Gordonibacter, g_Ruminococcus_gauvreauii_group, g_RB41, g_norank_p_Saccharibacteria, p_Saccharibacteria, g_Family_XIII_UCG _001, o_Blastocatellales, p_Acidobacteria, f_Blastocatellaceae_Subgroup_4_, c_Acidobacteria*	*g_Anaerostipes, f_Clostridiaceae_1, g_Ruminiclostridium _5*
Yin et al. 2015	=3	**TIA** *Proteobacteria, Porphyromonadaceae, Parabacteroides, Enterobacter Rikenellaceae, Alistipes, Megasphaera, Deltaproteobacteria, Desulfovibrionales, Oscillibacter, Desulfovibrionaceae, Desulfovibrio, Lactobacillus, Lactobacillaceae, Eubacteriaceae, Eubacterium, Synergistaceae, Synergistetes, Synergistales, Synergistia, Subdoligranulum*	**NIHSS≤4** *Burkholderiales, Bacteroidetes, Bacteroidia, Bacteroidales, Bacteroidaceae, Bacteroides*	**NIHSS>4** *Escherichia/Shigella, Gammaproteobacteria, Enterobacteriales, Enterobacteriaceae, Proteobacteria, Guggenheimella, IncertaeSedisXII, Paralactobacillus, Peptococcus, Cronobacter, Mobiluncus, Oscillibacter*	*Anaerosporobacter, Shewanella, Shewanellaceae, Paraprevotella, Faecalibacterium, Prevotella, Prevotellaceae, Bacteroidetes, Bacteroidales, Bacteroidia*

healthy controls [27,28], three studies showed that the microbial diversity of ischemic strokes was lower than nonstroke patients [9, 18,29], and five studies showed no significant difference [7,26,30–32]. The severity of ischemic stroke affects the diversity of gut microbes; one study showed that patients with severe stroke was lower microbial diversity than mild stroke [33], while another study found the opposite conclusion [28] and another showed no difference [8]. Seven studies showed significant differences between patients with ischemic stroke and those without ischemic stroke [9,18,28–30,34,35] and five studies showed no difference [7,26,27, 31,32] at beta diversity. Four studies also showed differences in beta diversity between mild and severe ischemic stroke [8,28,33,36].

The alpha and beta diversity varied among stroke types (lacunar infarction, nonlacunar acute ischemic infarction, and patients treated for 15 days after acute ischemic stroke) [29]. Patients with acute ischemic stroke had poor prognostic outcomes and were characterized by decreased alpha diversity and SCFA-producing bacteria (e.g., *Bacteroidaceae*, *Ruminococcaceae*, and *Faecalibacterium*) and increased pathogenic bacteria (e.g., *Enterococcus* and *Eggerthella*) [33]. The Shannon index of thrombus and plasma was similar in ischemic stroke patients, but different from that of stool and oral samples, the Beta diversity of these samples was significantly different [23]. The alpha diversity of intestinal microbes in patients who received traditional Chinese medicine and Western medicine was significantly higher than that in the Western medicine treatment group [20].

3.4. Linear discriminant analysis Effect Size

LefSE was used in twelve studies to assess the significantly altered bacterial between groups (Table 3). The relative abundance of SCFA-producing bacteria (e.g., *Roseburia*, *Bacteroides*, *Enterococcaceae*, *Lachnospiraceae*, *Faecalibacterium*, *Blautia*, *Fusicatenibacter*, *Ruminococcus*, *Romboutsia*, *Prevotella*) was reduced in patients with ischemic stroke [30,33,36]. zhao and Li, et al. showed that SCFA bacteria were enriched in the gut of patients with ischemic stroke (e.g., *Odoribacter*, *Akkermansia*, and *Ruminococcus*, *Victivallis*) [27, 32], which was inconsistent with previous results. In addition, the abundance of SCFA-producing bacteria in patients with severe ischemic stroke [8,33] and poor functional outcome [36] was low, and the composition of intestinal flora are related to the severity and

functional outcome of stroke.

Chryseobacterium and *Lactobacillaceae* was enriched in large atherosclerosis (LAA) stroke, and *Veillonellaceae* family in the phylum *Firmicutes* was enriched in cardioembolic (CE) stroke [23]. Levels of *Bacteroidaceae* and *Erysipelotrichaceae* were enriched in lacunar cerebral infarction, opportunistic pathogens of the *Bacteroidaceae* and *Bifidobacterium* were more abundant in the nonlacunar cerebral infarction, and after 15 days of treatment for acute ischemic cerebral infarction, *lactobacillus* levels were highest [29]. *Bifidobacterium* were enriched in (obstructive sleep apnea hypopnea syndrome) OSAHS complicated by cerebral infarction [37]. Species enrichment in *Neisseria*, *porphyromonas* and *Prevotella* was characteristic of pneumonia combined with stroke [26]. These studies have shown that opportunistic pathogens may be involved in thrombosis development, and the abundance and enrichment of bacterial species contribute to the diagnosis of stroke type.

3.5. KEGG functional analysis

Functional analysis was performed in five studies (Table 4). Zhao et al. showed that in the KEGG pathway enrichment analysis at level 3, there were 14 metabolic pathways showing significant differences between ischemic stroke and control group ($P < 0.05$) [27]. Stroke patients with poor and good outcomes showed significant differences in 34 metabolization-related pathways [33], and the two studies focused on amino acid synthesis and metabolism, including alanine, aspartic and glutamic.

Based on 2 level KEGG, human disease-related modules in patient with ischemic stroke increased, including infectious diseases, metabolism, genetic information processing, and membrane transport, and signal transduction were all highly abundant, 3 level KEGG.

Table 4
Description of KEGG functional analysis.

Study ID	KEGG	Case		Control
Zhao et al. 2022	3 level	Tropane, piperidine and pyridine alkaloid biosynthesis; Butanoate metabolism; Carbon fixation in photosynthetic organisms; Chlorocyclohexane and chlorobenzene degradation; Influenza A; Isoquinoline alkaloid biosynthesis; Novobiocin biosynthesis; Pantothenate and CoA biosynthesis; Protein processing in endoplasmic reticulum		Biosynthesis of vancomycin group antibiotics; Galactose metabolism; Polyketide sugar unit biosynthesis; Starch and sucrose metabolism; Sulfur metabolism
Sun et al. 2021	2 level 3 level	**mRS0-2, 2 level** Metabolism of cofactors and vitamins; Folding, sorting and degradation; Immune system; Metabolic diseases; Amino acid metabolism; Endocrine system; Environmental adaptation; Digestive system; Enzyme families; Cell growth and death; Replication and repain; Energy metabolism mRS0-2, 3 level One carbon pool by folate; Drug metabolism - other enzymes; Carbon fixation in photosynthetic organisms; Zeatin biosynthesis; D-Glutamine and D-glutamate metabolism; Alanine, aspartate and glutamate metabolism; Histidine metabolism; Lipid biosynthesis proteins; Peptidases Tropane, piperidine and pyridine alkaloid; Biosynthesis; Novobiocin biosynthesis; Amino acid related enzymes; Isoquinoline alkaloid biosynthesis; Vitamin B6 metabolism, Carbon fixation pathways in prokaryotes	**mRS3-6, 2 level** Membrane transport; Xenobiotics biodegradation and metabolism; metabolism; transcription; Infectious diseases mRS0-2, 3 level Chlorocyclohexane and chlorobenzene; Degradation; Styrene degradation; Pyruvate metabolism; Dioxin degradation; Ascorbate and aldarate metabolism; Chloroalkane and chloroalkene degradation; Selenocompound metabolism; Inositol phosphate metabolism; Tetracycline biosynthesis; Xylene degradation; Carotenoid biosynthesis; Penicillin and cephalosporin biosynthesis; Drug metabolism - cytochrome P450; Aminobenzoate degradation Protein kinases; Fructose and mannose metabolism; Limonene and pinene degradation; Glycerolipid metabolism; Tryptophan metabolism	—
Xiang et al. 2020	—	**pathways were highly enriched in both AI patients and healthy controls** Folate biosynthesis; Photosynthesis; Peroxisomal action; Citrate cycle (TCA cycle); Galactose metabolism; Prenyltransferase; Other glycan degradation; Amino sugar and nucleotide sugar metabolism; Fructose and mannose metabolism		—
Li et al. 2020	2 level	Immune system diseases; Poorly characterized; Infectious diseases; Neurodegenerative diseases; Metabolism; Xenobiotics biodegradation and metabolism; Genetic information processing; Membrane transport; Signal transduction; Cancers; Metabolism of other amino acids		Environmental adaptation; Immune system; Nervous system; Endocrine system; Biosynthesis of other secondary metabolites; Amino acid metabolism; Metabolism of cofactors and vitamins; Cell motility; Energy metabolism; Replication and repair; Cell growth and death; Translation; Transcription; Cardiovascular diseases
Huang et al. 2019	—	Methane metabolism; Lipopolysaccharide biosynthesis proteins; Secretion system; Flagellar assembly	—	—

mRS, Modified Rankin Scale; AI, non-lacunar acute ischemic infarction.

The expression level of spore function genes in butyrate-producing bacteria was significantly reduced, while the lactic acid bacteria-related phototransferase genes were significantly increased ($P < 0.0001$) [7]. Nine functional KEGG pathways and 12 COG categories were also found to be highly enriched in the nonlacunar cerebral infarction and healthy control groups [29]. There were also some pathways, including transposase (and inactivated derivatives), predicted Rossmann fold nucleotide-binding protein, ribonucleotide reductase alpha subunit, and L-asparaginase/archaeal Glu-tRNAGln amidotransferase subunit D were significantly more abundant in the lacunar infarction [29]. Acute ischemic stroke is mainly concentrated in methane metabolism and lipopolysaccharide synthesis pathways [31].

3.6. Risk of bias assessment

Quality Assessment of studies is described in Supplement Table S2. Among the 5 cohort studies, two studies scored 8 points. The other studies were of moderate quality: two scored 7 points, and one scored 6 points. Most of the studies did not clearly describe the "Demonstration that outcomes of interest were not present at start of study" section. Among the 16 identified case control studies, only four studies were of high quality: one scored 9 points, three studies scored 8 points; 8 studies were of moderate quality: five scored 7 points, and three scored 6 points; other studies were of lower quality: four scored 5 points. "Comparability of Cases and Controls on the Basis of the Design or Analysis" and "Non-Responserate" were not clearly identified in most studies. Three observational studies had a higher risk of bias.

4. Discussion

In this study, we assessed the characteristics between the microbiome and ischemic stroke through a systematic review. We conclude that gut microbial dysbiosis could be a risk factor for patients with ischemic stroke and is also associated with severity and prognosis.

The intestinal bacterial communities detected in both stroke and healthy people were dominated by 4 main phyla, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* [8,28,29,32–34]. The dominant families that accounted for more than 80 % of the total bacteria were *Bacteroidaceae*, *Ruminococcaceae*, *Enterobacteriaceae*, *Prevotellaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Porphyromonadaceae*, *Verrucomicrobiaceae*, *Rikenellaceae*, *Alcaligenaceae*, *Fusobacteriaceae*, and *Clostridiaceae* [33,36]. The genus-level characterization is more complex, mainly *Bacteroides*, *Prevotella*, *Faecalibacterium*, *Escherichia/Shigella*, and *Roseburia* [28,32]. Among the intestinal flora of ischemic stroke patients, the relative abundance of Proteobacteria increased significantly [27,32,35,37], and the relative abundances of Firmicutes and Bacteroidetes decreased [7,18,29,34]. *Bacteroidetes*, as a common bacteria in adult intestinal flora, contains a large number of intestinal resident bacteria that are beneficial or harmless to the host, and a decrease in its relative abundance represents a change in normal intestinal structure [7,21]. *Proteobacteria* accounts for a relatively small proportion, but it is unstable among them [38]. Disorders of the intestinal microbiome are usually caused by a continuous increase in *Proteobacteria*, and the excessive proliferation of *Proteobacteria* is considered a biological marker of intestinal flora disorder and a potential diagnostic marker [39].

This systematic review shows that in most of the included studies, alpha and beta diversity in both groups were different, proving that the intestinal microflora composition and relative abundance were different between stroke and healthy people and that stroke type was related to severity and outcome. The intestinal microbiota of ischemic stroke patients is significantly disordered [28,32,36], mainly concentrated in SCFA-producing bacteria (Roseburia, *Bacteroides*, *Lachnospiraceae*, *Faecalibacterium*, *Blautia* [36], *Odoribacter*, *Akkermansia*, *Ruminococcus* [27], *Parabacteroides*, *Coprococcus,* and *Prevotella* [33]). SCFAs, which are produced by intestinal microbial fermentation of unabsorbed carbohydrates and dietary fibers in the cecum and colon [40], affect the central nervous system [41] and directly or indirectly regulate brain function through immune, endocrine, vagus, and other humoral pathways [42]. Further analysis showed that decreased levels of SCFAs, which worsened stroke severity [18,33], particularly acetate, propionate, and butyrate, were significantly associated with an increased risk of 90 days of dysfunctional outcomes [8,18,33,36]. Compared with other SCFAs, butyrate showed the highest negative correlation with ischemic stroke [43], and butyric acid significantly enhanced the alpha diversity of the gut microbiota and reduced the level of pathogenic bacteria such as *Bacteroides* [43]. *Clostridium butyricum* has been used to modulate the gut microbiota to improve cognitive function and reduce neuronal damage in mice subjected to ischemia/reperfusion [44]. Therefore, the use of probiotics to modulate gut microbiota may be a potential treatment for ischemic stroke [45]. In addition, trimethylamine N-oxide (TMAO) is a microbio-derived metabolite that can be synthesized from dietary choline [46]. Studies have shown that serum levels of TMAO are an independent risk factor for adverse cardiovascular events [47] and that elevated levels of TMAO are associated with an increased risk of stroke [48], but the results are also controversial; stroke patients experience a decrease in blood TMAO levels [28], and the risk of ischemic stroke is gradually reduced by increasing TMAO concentrations [49]. These inconsistent results may be related to differences in disease type, sample type, ethnicity, etc. Of course, targeted inhibition of microbial generation of TMAO will reduce the formation of blood clots in the body [50] to prevent the occurrence of cardiovascular adverse events.

Disorders of the intestinal flora in ischemic stroke patients have led to significant changes in the function of the intestinal flora, involving circulatory, cardiovascular diseases, nervous system, infectious diseases, immune system, amino acid metabolism, glucose metabolism, lipid metabolism, nucleotide metabolism, metabolism of Cofactors and Vitamins, energy metabolism, signal transduction, transcription, signaling molecules and interactions, and other related pathways [7,27,29,31,33]. *Oscillibacter*, *Clostridium*, *Ruminococcus* and *Clostridium* in the gut of patients with ischemic stroke are positively associated with the KEGG pathway of cardiovascular disease (CVD) and major cardiovascular adverse events (myocardial infarction, stroke, or death) [7,27]. In the healthy control group,

the intestinal resident bacteria or beneficial bacteria (*Bacaeroides*, *Prevotella*, *Lachnospiraceae*, butyrate-producing bacteria) were negatively correlated with KRGG pathway in ischemic stroke[12]. Microorganisms influence stroke outcomes by regulating immune response and related pathways in the metabolic system [29]. The abundance of SCFAs producing bacteria (*Bacteroides*, *Roseburia* and *Parabacteroides*, *Ruminococcus* and *Coprococcus*) were increased in the gut microbiota of patients with ischemic stroke with good outcomes, and the corresponding KEGG pathways upregulation include amino acid metabolism, cofactor and vitamin metabolism, enzyme family, replication and repair [33].

Among these changes in the KEGG pathway, we also noted changes in the abundance associated with the infectious disease pathway in patients with severe ischemic stroke [7,33], including tetracycline biosynthesis, penicillin and cephalosporin biosynthesis. Poststroke infection, especially stroke-associated pneumonia, is the most common complication in patients with stroke [51] Changes in these pathways may be associated with an increased incidence of infection after ischemic stroke. Only one study on ischemic stroke-related pneumonia included in this review did not include KEGG functional analysis, but it is certain that the occurrence of pneumonia is related to microbial imbalance, and the carrying of pneumonia-related pathogens or dysbiosis in the oral microbiota may lead to the development of stroke-related pneumonia [26].

The combination of traditional Chinese and Western medicine in the treatment of ischemic stroke has achieved good results, which are reflected in better clinical outcomes, increased microbial diversity, increased abundance of beneficial bacteria, and improved composition of intestinal microorganisms compared with healthy people [19]. In terms of microbial-level mechanisms, the traditional Chinese medicine ingredients berberine and rhubarb may mediate host metabolism and immunity by regulating some bacteria, including *Coprococcus*, *Dorea*, *Clostridium*, *Parabacteroides*, and *Phascolarctobacterium*, reducing the levels of lipopolysaccharide and TMAO and inhibiting the deterioration of acute ischemic stroke [20]. However, due to the limited number of studies, the effect of traditional Chinese medicine on gut microbes still needs to be verified in a large number of clinical samples.

Streptococcus was found in the thrombus by PCR, mainly the *S mitis* group DNA [25], and the relative abundance could not be determined. The relative abundance of *Streptococcus* was investigated by next-generation sequencing and was very low, approximately 1.1 % [23]. The bacteria in thrombi were enriched in Proteobacteria, with a relative abundance of 73.3 %, probably originating mainly from plasma [23]. Different types of ischemic stroke have different dominant microflora; the dominant bacteria in cardioembolic stroke patients is the *Veillonellaceae* family, while the *Chryseobacterium* and *Lactobacillaceae* families are dominant in large-artery atherosclerosis stroke [23]. Another study showed that in addition to *Lactobacillus* and *Staphylococcus*, thrombosis also included *Pseudomonas putida*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, and *Finegoldia magna* [24]. Due to the small number of thrombus samples, the microorganisms in the thrombus sample need to be further clarified.

Ischemic stroke-associated pneumonia, some changes took place over time, with the relative abundance of *Streptococcus*, *Neisseria*, *Prevotella*, *Rothia*, and *Haemophilus* decreasing by >20 %, and *Staphylococcus*, *Klebsiella*, and *Candida* increasing by >20 % each. Dysbiosis in the oral microbiota or carrying pneumonia-associated pathogens may contribute to the development of stroke-associated pneumonia [26], so patients with ischemic stroke should also pay attention to oral health. DNA from Streptococcus (representative oral microorganisms) can be detected in 79 % of thrombus samples [25], and opportunistic pathogenic bacteria in the oral cavity may enter the bloodstream through the damaged oral mucosa, participating in thrombosis, so regular dental care is necessary [23]. There was also an increase in the incidence of stroke-associated pneumonia in patients with poor oral hygiene [26]. Implementation of oral hygiene programmes significantly reduces the incidence of stroke-related pneumonia in people with acute stroke [52]. However, due to the limited number of studies, the relationship between oral microflora and ischemic stroke cannot be described at present.

5. Limitations

Overall, only observational studies were included in this study, which may be affected by mixed factors. The results of this study could not be included in the meta-analysis due to the variation in outcomes and lack of raw data related to the microorganism. It was not possible to further explore whether the different sequencing platforms affected the results. Biological information processing methods such as microbial sequencing technology, sequencing platform and sample collection method and time were also responsible for the discrepancy in results [7]. The sample size of the included studies was small, with the largest number being 349 stroke cases, and large sample sizes and high-quality trials are needed to verify the relationship between ischemic stroke and the microbiome in the future. In addition, ischemic stroke was classified into more detailed categories, such as lacunar cerebral infarction, nonlacunar cerebral infarction, acute ischemic stroke, aortic atherosclerotic ischemic stroke, large vessel occlusive stroke, and cardioembolism. The criteria for defining stroke severity are also different; Tan et al. defined NHISS<5 as mild stroke [36], Gu et al. defined NHISS≤3 as mild stroke [8], and some studies use MRS scores to judge stroke severity [33,34]. Therefore, further harmonization of microbiological testing standards or experimental methods is needed to narrow down research differences.

6. Conclusion

In this study, we observed that there were differences in the composition of the gut microbiome of populations with ischemic stroke and nonischemic stroke, resulting in changes in the function of the intestinal flora, which may aggravate ischemic stroke and affect prognosis.

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Ethical statement

The ethical statement is not applicable in this study as this is a review paper, and we are using secondary published information.

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Wei Zhang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing - original draft. **Rongbing Tang:** Investigation, Methodology, Writing - review & editing. **Yanfei Yin:** Formal analysis, Writing - original draft. **Jialong Chen:** Investigation, Writing - review & editing. **Lihe Yao:** Conceptualization, Investigation, Supervision, Writing - review & editing. **Bin Liu:** Conceptualization, Investigation, Methodology, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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