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CURRENT OPINION

Bacteria in the brain: do they have a role in the pathogenesis of Alzheimer's disease?

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Purpose of review
Worldwide efforts continue to unravel the complex pathological pathways that lead to Alzheimer's disease. The gut–brain–microbiome axis, a communication pathway between the gut, brain and microorganisms, is emerging as a potential mechanism involved in Alzheimer's disease pathogenesis. While the gut microbiome's role in Alzheimer's disease has gained significant attention, the brain microbiome remains relatively unexplored. This review summarizes the latest research on the brain microbiome in Alzheimer's disease.

Recent findings
In the past 4 years, four out of five studies have found bacteria, such as *Streptococcus pneumoniae*, in postmortem samples of both control and Alzheimer's disease brains, supporting the idea that the brain is not a sterile environment. Two studies report the overabundance of several bacterial phyla, including *Proteobacteria and Actinomycetes,* in postmortem Alzheimer's disease brains versus controls. One study reported the presence of *Borrelia burgdorferi* in a subset of Alzheimer's disease cases compared to controls.

Summary
Limitations and challenges persist in studying the brain microbiome, including the lack of standardized assays and data analysis methods, small sample sizes, and inconsistent use of controls for environmental microbial contamination during sample processing. Well designed studies that employ reproducible and rigorous methods are required to elucidate whether microbes are involved in the pathogenesis of Alzheimer's disease.

Keywords
Alzheimer's disease, bacteria, brain, dementia, microbiome, microbiota, neuroinflammation

INTRODUCTION

Alzheimer's disease is a fatal, progressive neurodegenerative disease and the most common form of dementia [1]. It is estimated that approximately 55 million people live with dementia worldwide, with global numbers projected to soar to 135 million in 2050 [2,3]. The socioeconomic burden of dementia is growing, as our global population ages [4]. Alzheimer's disease is characterized by the slow loss of memory and other cognitive functions [5]. There are still no effective preventive and treatment strategies for Alzheimer's disease, and no cure, largely because the disease pathogenesis has not been fully elucidated.

The human microbiota is a complex and dynamic community of microorganisms, including bacteria, fungi and viruses, which inhabit our body, and, together, with their collective genomes are referred to as the microbiome [6–8]. The microbiome has been linked to nearly all aspects of health and disease [9], including Alzheimer's disease [10]. Although researchers have made significant strides in understanding the gut–brain axis and the gut microbiome in Alzheimer's disease [11], few research studies have examined the brain microbiome and its role in the pathogenesis of Alzheimer's disease.

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KEY POINTS

- Emerging evidence suggests bacteria are present in the brain of both Alzheimer's disease cases and neurological controls.
- Some evidence that certain bacteria are more abundant in Alzheimer's disease brains compared to controls.
- There is a lack of consistency and details of sample collection and research methods across studies.
- Consensus methodology is needed to standardize the study of the human brain microbiome.

The brain is assumed to be sterile, or microbe-free, because of its protective blood–brain barrier (BBB). However, recent reports have challenged this assumption [12▪▪]. Studies have shown that bacteria-derived extracellular DNA and lipopolysaccharides can compromise the integrity of the BBB, causing a leaky BBB that allows microbes to cross into the brain [13,14]. Other proposed routes of microbe entry into the brain, are via the olfactory system, trigeminal nerves and ganglia, the lungs and the vagus nerves from the gut [15▪▪].

The infection-Alzheimer's disease theory posits that microbial infection plays a causal role in Alzheimer's disease [10,16–18]. Several reports have shown an association between bacterial infection and Alzheimer's disease [19–21]. Animal studies have provided further insights, with research showing injecting Herpes Simplex Virus-1 (HSV-1) into the brains of transgenic mice overexpressing beta-amyloid accelerates amyloid protein deposition [22]. In addition, amyloid oligomers have been shown to inhibit HSV-1 replication *in vitro*, leading to the hypothesis that amyloid proteins might be produced as a defensive response to infection [22]. This suggests a potential mechanism by which viral infections could contribute to Alzheimer's disease development. However, the infection-Alzheimer's disease theory remains contentious, as a direct causal association has not been conclusively demonstrated [23]. Nonetheless, neuroborreliosis and neurosyphilis – both of which affect the central nervous system and can lead to secondary dementia – provide proof of concept that infections might play a role in the development of dementia [24–27].

Identifying bacteria can be undertaken in several culture-free ways, including quantitative polymerase chain reaction (qPCR) and next-generation sequencing of the bacterial 16S ribosomal RNA (16S rRNA). There are limitations to both methods; qPCR amplifies sequences using preselected targeted primers, thereby limiting the detection to the targeted bacteria. In contrast, 16S rRNA sequencing can identify specific bacterial species and/or bacterial families but does not comprehensively screen the microbiome [27]. 16S rRNA sequencing is the most widely used method, as it is well established, cost-effective and quick [28]. However, it is prone to PCR biases and artefacts, such as off-target amplification, which should be controlled for [29,30▪]. This method primarily amplifies the bacterial 16S rRNA gene, which is 1500 base pairs long, and contains variable regions ('V' regions) against which the primers are designed. The choice of primers determines the bacterial species that can be identified [31].

This review summarizes the key findings from human studies conducted over the past 4 years specifically focusing on research examining the presence and/or abundance of bacteria within Alzheimer's disease brain tissue. It also outlines the limitations of these studies and provides recommendations for advancing research in this area.

RECENT STUDIES EXPLORING THE BRAIN MICROBIOME IN ALZHEIMER'S DISEASE

Only five studies have analysed the relationships between Alzheimer's disease and the presence of brain microbiota in the last 4 years (Table 1). All studies used postmortem brain tissue samples collected through different brain banks, and experiments were performed in different laboratories (four studies from the UK and USA [20,32–34] and one from Korea [35]). One study [20] pooled data from multiple sources (Mount Sinai Brain Bank, Edinburgh Brain Bank, Miami and Rockefeller studies) and reported consistent findings, indicating that samples collected through different biobanks may not impact study outcomes. All studies compared Alzheimer's disease cases (4–48 cases) with controls (3–31 cases) and analysed tissue from at least two Alzheimer's disease-affected brain regions (e.g. frontal cortex, hippocampus [32]). Additional regions that are less affected in Alzheimer's disease were sometimes included as internal controls (e.g. occipital cortex, hypothalamus [35]). A summary of the study outcomes and methodologies is provided in Table 1.

Senejani *et al.* used qPCR alone and used a targeted approach to detect *Borrelia burgdorferi* specifically [27], the bacterium responsible for Lyme disease (neuroborreliosis) [26], which has been linked to Alzheimer's disease [32]. Three other studies [20,33–35] used 16S rRNA sequencing but different primers to identify different bacterial species,

Table 1. Human brain microbiome studies in Alzheimer's disease, 2021–present

First author, year	Sample size		Brain regions	Microbiome method	Bioinformatic tools	Bacteria identified	Main findings
	AD	Ctrl					
Ko *et. al.*, 2024 [35]	4	3	Frontal cortex, hippocampus, hypothalamus,	16S rRNA[a]	VSEARCH, PERMANOVA[e]	Proteobacteria, firmicutes, actinobacteria, bacteroidetes[c]	No taxa difference between AD and controls
Mone *et. al.*, 2023 [33]	16	16	Entorhinal cortex, frontal lobe, temporal lobe	16S rRNA[a]	MCSMRT, CCS[e]	*Acinetobacter, Comamonas, Nitrospora, Methylobacterium*[c]	No taxa difference between AD and controls
Hu *et. al.*, 2023 [20]	48	31	Not provided	Meta-analysis of RNAseq data from four studies	eTOL, Morpheus[e]	Not provided	No taxa difference between AD and controls
Hu *et. al.*, 2023 [20]	6	3	Amygdala, cingulate cortex, hippocampus, hypothalamus	Metagenomics[d]	eTOL, Morpheus[e]	*Streptococcus, Staphylococcus, Sphingomonas, Ralstonia*[c]	No taxa difference between AD and controls. but trend of overabundance in AD–*Streptococcus, Staphylococcus* and *Sphingomonas*
Senejani *et. al.*, 2022 [32]	6	10	Hippocampus, frontal lobe	16S rRNA[a], IHC[b]	Not provided	*Borrelia burgdorferi*	*Borrelia* observed only in AD hippocampus (2/6 cases)
Emery *et. al.*, 2022 [34]	22	31	Temporal cortex, entorhinal cortex, hippocampus, locus coerulus, pre-frontal cortex, substantia nigra	16S rRNA[a], qPCR	VSEARCH, QIIME, UPARSE[e]	*Corynebacterium, Propionibacterium, Cutibacterium, Actinobacteria*[c]	No taxa difference between AD and controls. Overabundance of *Streptococcus* and other oral bacteria for AD versus controls.

[a]16S rRNA sequencing.
[b]IHC, immunohistochemistry.
[c]Main bacterial species identified, for other identified species please see the reference.
[d]Deep sequencing (NovaSeq 6000).
[e]Not an exhaustive list of all bioinformatic tools, please see individual papers.

applying a more holistic approach to exploring the microbiome rather than targeting specific bacterial species [33–35]. One study performed a meta-analysis using RNA sequencing data and in an extension of this work using a set of new samples, employed one of the most up-to-date techniques, metagenomics, to study the brain microbiota in greater detail at the species level [20].

Only a single study observed differences between bacteria in Alzheimer's disease and control brain tissue. However, this study only examined for the presence of *B. burgdorferi*, with two out of six Alzheimer's disease cases testing positive, while no *B. burgdorferi* was detected in the controls ($n = 10$) [32]. This study incorporated controls at most steps of the experimental process and included a positive control for *B. burgdorferi* (cultured strain B31), negative controls for DNA extraction (no tissue), along with no-template controls in the PCR (NTCs, no DNA), which are crucial to test for environmental or procedural contamination. They further demonstrated co-localization of *B. burgdorferi* with an amyloid marker using immunohistochemistry but utilized only a single sample, nevertheless suggesting a potential role in the abnormal aggregation of amyloid beta that characterizes Alzheimer's disease. The authors acknowledged the limitations of their study, noting the small sample size and the fact that *B. burgdorferi* was not consistently found in all Alzheimer's disease cases [32].

All other studies found no difference in the taxa of bacteria between Alzheimer's disease and controls [20,33–35]. Indeed, Hu and colleagues combined data from four sources (Alzheimer's disease: 48; control: 31) using meta-analysis and found that microbial profiles were conserved in both control and Alzheimer's disease brain tissue, but no specific Alzheimer's disease microbe was identified. Unfortunately, the samples were pooled for analysis meaning regional variations could not be determined. In an extension of their work, Hu and colleagues analysed four brain regions (amygdala, cingulate cortex, hippocampus, hypothalamus) from six Alzheimer's disease cases and three controls using metagenomics and found no differences in taxa detected. However, analysis of bacterial read

counts revealed a trend for overabundance of bacteria (*Streptococcus, Staphylococcus/Bacillus and Sphingomonas*) in Alzheimer's disease cases versus controls (highest in cingulate cortex) [20]. Significant differences could not be determined because of the low sample size and the considerable inter-regional and inter-case variation observed. The authors noted the need for replication of their work in larger, more diverse cohorts. In a separate study, Emery and colleagues using 16S rRNA sequencing and a relatively large sample size (Table 1), also provide evidence supporting bacterial overabundance in multiple brain regions in Alzheimer's disease, with reported increases in *Streptococcus* and other oral bacteria [34]. This result provides additional evidence supporting the premise that periodontal disease is linked to increased Alzheimer's disease risk [36–38], although a causal role is yet to be determined.

Although carried out on a limited number of cases, the metagenomics approach used by Hu *et al.* [20] offers advantages over 16S rRNA sequencing, as it allows for a more comprehensive assessment of the microbiome and provides greater resolution down to the species or even strain level. This technique captures all microbial DNA, eliminating the PCR bias inherent in 16S rRNA sequencing, which can skew the amplification of certain bacterial taxa over others. However, metagenomics comes with its challenges; it is more expensive, the data analysis can be complex, and the results can be confounded by the incorporation of host DNA contamination within the reads [39–41]. As a result, this approach requires the inclusion of robust controls at every stage of the experimental process, from sample collection to sequencing and data analysis, to ensure the accuracy and reliability of the findings.

Previous studies have faced significant problems relating to potential contamination of samples, as it can be introduced at every step of the process, including tissue collection, DNA extraction and analysis [42,43]. The inclusion of appropriate controls during each of these steps represents best practice, and whilst all studies reviewed included some controls (e.g. blank DNA extraction controls [33,35], NTC [34,35]), full details of the experimental and procedural controls were lacking, indicating the need for publishing these details in their entirety, allowing ease of replication by others. Postprocessing bioinformatics can also identify and remove common environmental contaminants if they are present across all samples [44], although deciphering changes in low biomass microbes from such contaminants is a challenge facing all areas of microbiome research [35].

All studies employed the R programming language, but utilized different packages (e.g. FactoMineR, ggplot2, vegan) for bioinformatic analysis [34,35]. Microbial profiling was carried out using a range of software tools, including VSEARCH, HMMER, QIIME, UPARSE and MCSMRT (see Table 1) [33–35]. This variability in bioinformatic pipelines is potentially problematic, as different tools and approaches applied to the same raw data can yield divergent results [45], making it difficult to draw firm conclusions and to compare findings across studies.

DISCUSSION

In this brief review, we examined the last 4 years of published human brain microbiome Alzheimer's disease research. Four out of the five studies reviewed reported no difference in bacterial species between Alzheimer's disease cases and controls [20,33–35], although changes in bacterial abundance were observed in two studies [20,34]. Furthermore, one research group demonstrated a specific bacterium (*B. burgdorferi*) in a subset of Alzheimer's disease brains that colocalized with amyloidosis in the brain, a hallmark feature of Alzheimer's disease, suggesting the microbiome may play a role in disease pathogenesis [32]. As acknowledged by the study authors, all of these studies are somewhat preliminary, and the findings cannot be used to infer causation.

We recognize several notable strengths in some of these recent studies, including pooling datasets [20], comprehensive bioinformatic analysis [20,33] and the use of modern metagenomics techniques [20]. However, not all studies provide sufficient details about their methods, including specifics of their experimental and procedural controls. Additionally, important information regarding bioinformatic analysis, such as whether low biomass microbes – potential environmental contaminants – were excluded, is lacking.

The limited sensitivity of traditional techniques makes it challenging to detect low-biomass microorganisms from the overwhelming signals of the host brain tissue (e.g. host genome) [30▪,44,46▪]. However, newer techniques, such as metagenomics, metaproteomics and metabolomics [47–49], although expensive, offer significant advantages over conventional techniques, such as 16S RNA sequencing [50–52]. These advanced approaches provide greater sensitivity and utilize unbiased, nontargeted strategies to identify bacteria with higher resolution, enabling more detailed studies of microbiome structure, function and bacterial interactions [53–55]. Although these cutting-edge techniques are becoming standard for analysing the gut microbiome, they have yet to be widely applied to research on the brain microbiome.

Lathe *et al.* recently proposed an international effort to address many of the issues faced by the brain microbiome research community, including appropriate tissue collection methods, optimal sample

extraction, controls, sequencing and bioinformatic methods. The aim of this initiative (Alzheimer's Pathobiome Initiative) is to develop a consensus protocol for investigating the brain microbiome, which can be used by all research groups in the future [15▪▪]. This initiative promises to assist the field in improving methodologies for examining the brain microbiome in the future.

CONCLUSION

In conclusion, recent evidence over the past 4 years lends support to the existence of a brain microbiota. However, this short review was limited to examining bacteria only and hence did not explore potential associations between other microorganisms, such as viruses and fungi with Alzheimer's disease.

Variability in study design, methodologies, and sample sizes across the recent studies makes it challenging to draw definitive conclusions about whether there are significant differences in the brain microbiota of Alzheimer's disease cases compared to controls. As research in this area progresses, it will be crucial to conduct further studies with larger sample sizes, sensitive and standardized methodologies and to comprehensively survey the entire microbiome (bacteria, fungi, viruses). This approach would also facilitate investigating potential interactions between different microbes and Alzheimer's disease. Analysis of tissue from various disease stages and in variably affected brain regions would also help to determine whether the microbiota is affected early in the disease. These steps are essential for gaining a more comprehensive understanding of the potential role of the brain microbiome in Alzheimer's disease.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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