

The gut microbiome in drug-resistant epilepsy

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Abstract

Drug-resistant epileptic patients make up approximately one-third of the global epilepsy population. The pathophysiology of drug resistance has not been fully elucidated; however, current evidence suggests intestinal dysbiosis, as a possible etiopathogenic factor. Ketogenic diet, whose effect is considered to be mediated by alteration of gut microbiota synthesis, has long been administered in patients with medically refractory seizures, with positive outcomes. In this review, we present data derived from clinical studies regarding alterations of gut microbiome profile in drug-resistant epileptic patients. We further attempt to describe the mechanisms through which the gut microbiome modification methods (including ketogenic diet, pre- or probiotic administration) improve drug-resistant epilepsy, by reporting findings from preclinical and clinical studies. A comprehensive search of the published literature on the PubMed, Embase, and Web of science databases was performed. Overall, the role of gut microbiome in drug-resistant epilepsy is an area which shows promise for the development of targeted therapeutic interventions. More research is required to confirm the results from preliminary studies, as well as safety and effectiveness of altering gut bacterial composition, through the above-mentioned methods.

KEYWORDS

antibiotics, gut microbiota, intestinal dysbiosis, ketogenic diet, probiotics, refractory epilepsy

1 | INTRODUCTION

The term *microbiota-gut-brain axis* describes the complex bidirectional communication pathways linking the CNS with gut bacteria. This system has become a focus of neuroscience lately, as evidence provided by animal and human studies has documented the crucial role of the microbiota in regulating CNS homeostasis, cognitive development, and behavior. Over the last decade, by virtue of technological advances in the field of biomedicine, gut dysbiosis has been associated with the genesis or progression of a variety of neurological

disorders,¹ such as autism,² multiple sclerosis,³ Parkinson's disease,^{4,5} and Alzheimer's disease.⁶ Recently, changes in the composition or function of gut microbiome have been linked to epilepsy and in particular to its medically refractory form⁷ Figure 1.

Epilepsy is a chronic neurological disorder “characterized by an enduring predisposition to generate seizures”,⁸ with an estimated global prevalence of more than 50 million affected individuals and an annual incidence of 2.4 million patients.⁹ An estimated 35% of epilepsy cases are directly linked to a genetic background whereas in the remaining

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cases, genetic risk in combination with acquired and environmental factors is considered to both contribute to epileptogenesis. Environmental causes may include cerebral insults such as trauma, tumors, strokes, traumatic brain injury, or infections.¹⁰ Classic epilepsy therapy involves antiepileptic medications, epilepsy surgery, vagus nerve stimulator (VNS), and ketogenic diet.

Despite recent advances in the discovery of new therapeutic drug agents for epilepsy, it is estimated that approximately one out of three patients with epilepsy is refractory to treatment. According to the definition by the International League Against Epilepsy (ILAE), *drug-resistant epilepsy (DRE)* implies that a patient has failed to achieve sustained seizure freedom after adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schemes, whether as monotherapies or through a combination of drugs.¹¹ Despite intensive research in the field, no advances in the effective therapeutic management of DRE have been made.¹²

Currently, there is emerging evidence that alterations in gut microbiota composition and/or function exist in patients with DRE.¹³ Studies aiming to reveal gut dysbiosis in epilepsy are conducted in both pre- and clinical levels. Although research in the field had been halted for many years due to technological limitations, rapidly advancing sequence-based screening and the development of metagenomics and metabolomics^{14–16} that have flourished during the last decades have contributed greatly toward a better understanding of the role of gut dysbiosis in epilepsy.

The possibility for modifying gut microbiota using supplements like prebiotics, probiotics (collectively called

Key Points

- Approximately 1/3 of the world epilepsy population suffers from drug-resistant epilepsy
- Intestinal dysbiosis challenges brain homeostasis and seems to be present in patients with drug-resistant epilepsy
- The mechanism of action of the ketogenic diet might be mediated by alterations in the intestinal microbiota composition
- Overall, current evidence supports modification of gut microbiome, as a therapeutic target for drug-resistant epilepsy, but more research is necessary

symbiotics), or diets (eg, ketogenic diet) as add-on with antiepileptic drugs or alone has been keenly investigated during the last years.¹⁷ We hereby present a review of available literature concerning evidence for intestinal dysbiosis in DRE. It outlines the gut-brain axis connection; evidence of the link between microbiome, epileptogenesis, DRE, and treatment outcomes; and proposed pathophysiological mechanisms and treatment studies. To our knowledge, there is only one published review with focus on intestinal dysbiosis and DRE.¹⁸ In this article, Holmes et al thoroughly review all existing literature regarding the gut microbiome's effect on treatments of patients with DRE (diets,

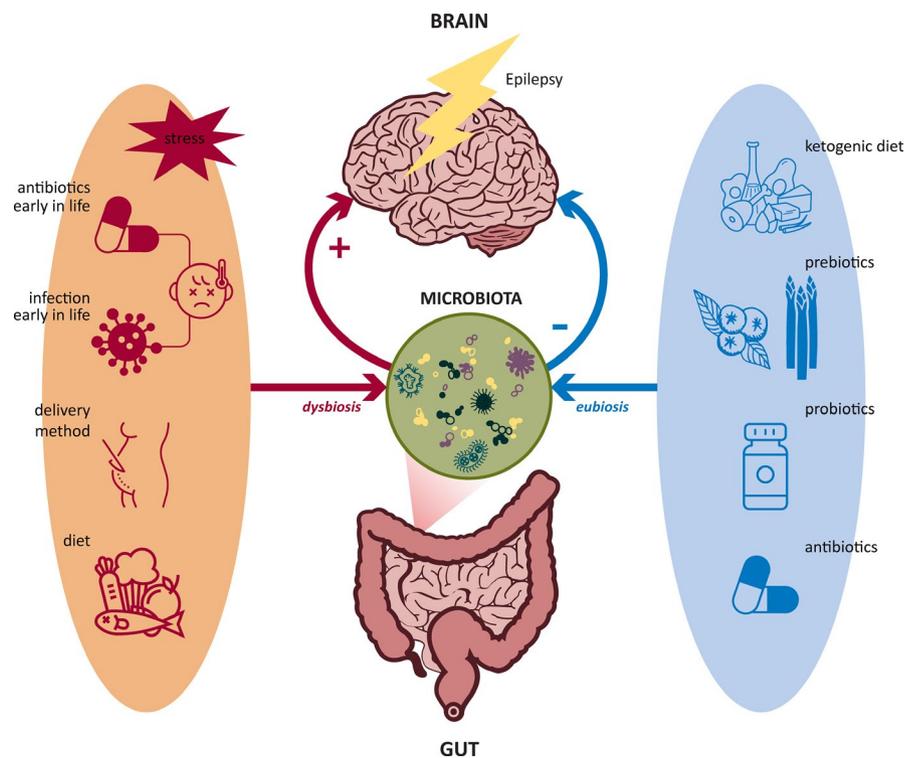


FIGURE 1 The gut microbiome effects on epilepsy are driven by environmental factors. On the right column (painted in red), we present the factors considered to provoke intestinal dysbiosis and indirectly induce epileptogenesis. On the left column, we present the factors that are assumed to enhance intestinal eubiosis. These are considered as potential therapeutic targets in drug-resistant epilepsy.

pre-,probiotics, antibiotics, antiepileptic medications). In this review, we have added a section reporting findings of all clinical studies that have examined the intestinal microbiota composition in DRE cohorts.

2 | MICROBIOTA-GUT-BRAIN AXIS AND ITS ASSOCIATION WITH NEUROLOGICAL DISEASE

The term “gut-brain axis” refers to the bidirectional interactions between the gastrointestinal (GI) and the central nervous system (CNS). The CNS regulates the function and integrity of the GI tract through the neuroendocrine hypothalamic-pituitary-adrenal axis (HPA) and through the autonomic nervous system.¹⁹ These pathways regulate the permeability of the intestinal barrier, the motility of the GI tract, its secretory activity, and the composition of the intestinal microbiome. At the same time, the GI system can affect brain function and behavior by promoting synaptogenesis, activation of stress response, synthesis of neurotransmitters in the CNS, and blood-brain barrier (BBB) integrity. This symbiotic relationship between the brain and gut is mediated by the activity of enteric microbial populations, collectively known as “gut microbiota.”²⁰ Recent discoveries have led to the emerging concept of the “microbiota-gut-brain (MGB) axis” which has attracted the interest of neuroscientists, during the last decades.^{21–23}

The MGB axis collectively describes a network that includes gut microbiota and their metabolic products, enteric nervous system, autonomic nervous system (sympathetic and parasympathetic branches), neuroendocrine system, neural immune system, and central nervous system.^{19,24}

Gastrointestinal microbiota colonize the GI tract and comprise trillions of microorganisms of about 1000 different species, including bacteria, fungi, yeasts, archaea, protozoa, and viruses. Bacteria are the most numerous species among commensal microbes (3.8×10^{13} bacteria), and Firmicutes and Bacteroides are the most prevalent bacterial phyla among them.²⁵ While the composition of the core gut microbiota is formed during the first year of life, dynamic changes may occur during the course of human development, driven by various stressors such as infection, drug, illness, and diet.¹⁹ Gut microbiome plays a crucial role in the development and maturation of the gut immune system, resistance to pathogens, carbohydrate metabolism, and metabolic homeostasis.²⁶ It exerts its protective actions for the host through its byproducts (eg, short-chain fatty acids [SCFAs], cytokines, hormones, neurotransmitters) and neural activity (eg, ANS signaling modulation).²⁵

The gut microbiome has been shown to affect CNS physiology and neurotransmission, through the neural network, neuroendocrine system, and metabolic and immune

system.^{19,27} Gut microbial disbalance, namely dysbiosis, describes alterations in the taxonomic or functional profile of intestinal microbial communities relative to the community observed in healthy subjects.

3 | CAN THE MICROBIOME AFFECT SEIZURES? PRESUMPTIVE MECHANISMS THROUGH WHICH THE MICROBIOTA MODULATE SEIZURE SUSCEPTIBILITY

Animal and human studies have brought up evidence supporting dysbiosis as a causative factor of epilepsy, most often of a refractory form. There are five possible communication routes between gut microbiota and the brain. These include neural connections, the neuroendocrine HPA axis, biosynthesis of neurotransmitters by intestinal bacteria, the intestinal immune system, and lastly, interconnection between intestinal mucosal barrier and blood-brain barrier.^{19,24}

At the same time, there are several pathophysiological pathways currently under research, possibly linking gut dysbiosis with epileptogenesis.^{28–32} The first mechanism involves the direct effect on neural networks through the *release of neurotransmitters* (specifically serotonin [5-HT], γ -aminobutyric acid [GABA] and glutamate), or their precursors (tryptophan and its metabolites) by gut bacteria.^{32,33} This affects the balance between excitation and inhibition within the CNS. The second mechanism involves *neural signaling*.^{29,31} Under homeostatic conditions, gut bacteria stimulate afferent neurons of the enteric nervous system (ENS) following stimulation via the vagus nerve that induces antiinflammatory activity. A disturbance in normal bacteria abundance or function might compromise this balance toward excess inflammation.³⁴ Furthermore, the *decrease in normal biosynthesis of short-chain fatty acids* (butyric, propionic, acetic acids, etc) that possess a known antiinflammatory action, by the gut microbiota,³⁵ is a potent pathogenetic mechanism of inducing secondary neuroinflammation that can trigger seizures. In addition, *changes in the gut microbiome (composition or functional profile)* may act by disrupting the HPA axis and normal response to stress³⁶ or by modulating the levels of brain-derived neurotrophic factor (BDNF) and therefore influence seizure mechanisms. Gut dysbiosis might also interfere with the *endocannabinoid system*,^{37,38} possibly linked to the pathogenesis of epilepsy. Finally, the occurrence of seizures, as well as epileptogenesis, might reflect a *“leakage” in the integrity of the barrier system* (intestinal mucosal barrier and blood-brain barrier) which is known to be affected by the gut microbiota. Changes in gut microbiome profile may lead to excess in lipopolysaccharide (LPS)-producing bacteria, that cause increased permeability of the intestinal immune barrier (by increased LPS levels).^{34,39} LPS

effect on seizure induction is mediated through upregulation of brain cytokines IL-1 β , TNF, and COX-2.⁴⁰ Consequently, peripheral produced inflammatory factors may act on the blood-brain barrier, increasing its permeability, and allowing pro-inflammatory mediators to enter the CNS Figure 2.

All in all, there seems to be various pathways of the MGB axis involved in the pathogenesis and maintenance of epileptic activity. Potential modifiers of these disturbances should aim to restore gut dysbiosis. Those include diet modifications, antibiotic, pre- or probiotic supplements and are currently intensively investigated, as they might represent potential therapeutic approaches for treating epilepsy, in the future.

4 | WHAT STUDIES SUGGEST A ROLE FOR MICROBIOTA IN DRE?

4.1 | Bacterial composition studies in DRE cohorts

It has been suggested that intestinal dysbiosis may contribute to the mechanism of DRE.⁴¹ A few recent, observational, or interventional clinical studies have searched for potential alterations in fecal microbiota profiles between DRE patients and healthy individuals, and/or drug-sensitive epileptic (DSE) patients. Their principal findings will be reviewed below.

Peng et al studied a cohort of 42 patients with DRE, 49 with DSE, and 65 matched healthy controls who were members of the patient families. Individuals from different groups were matched for age, sex, exposure to medication and included only subjects who had not received antibiotics or probiotics

within the past 3 months and who had no other chronic disease except for epilepsy. Fecal samples were analyzed with the method of 16S rDNA sequencing, to map the participants' gut microbiome composition. The results demonstrated important fecal microbiota alterations for the group of DRE patients as compared to both the two other groups, mainly concerning the increased abundance of rare flora, observed in the first group. (a) More precisely, the refractory epilepsy group microbiome synthesis presented increased α -diversity, which was more prevalent for the subgroup of those with four or more seizures per year than for those with less than four seizures per year. (b) When compared with the controls, the refractory group also revealed elevated levels of Firmicutes and decreased levels of Bacteroides. (c) More specifically, selected species from the phylum Firmicutes that were found to be increased included *Roseburia*, *Coprococcus*, *Ruminococcus*, and *Coprobacillus*. Comparison between DRE and DSE epilepsy groups showed relative abundance of *Methanobrevibacter*, *Fusobacterium*, *Neisseria*, and *Akkermansia* within the first group. Interestingly, the analysis of gut microbiome profile showed no important differences between DSE patients and healthy controls.⁴²

Another study with similar structure was recently published, by Gong et al⁴³ The researchers recruited a total of 55 patients with epilepsy (30 with DRE and 25 with DSE) and 46 controls, who were healthy spouses of the patients. Inclusion and exclusion criteria were similar to the above-mentioned study by Peng et al Gut microbiome profiling was performed through 16S rDNA sequencing of stool samples. The authors reported substantial alterations of gut microbiota composition in the disease group including decreased α -diversity,

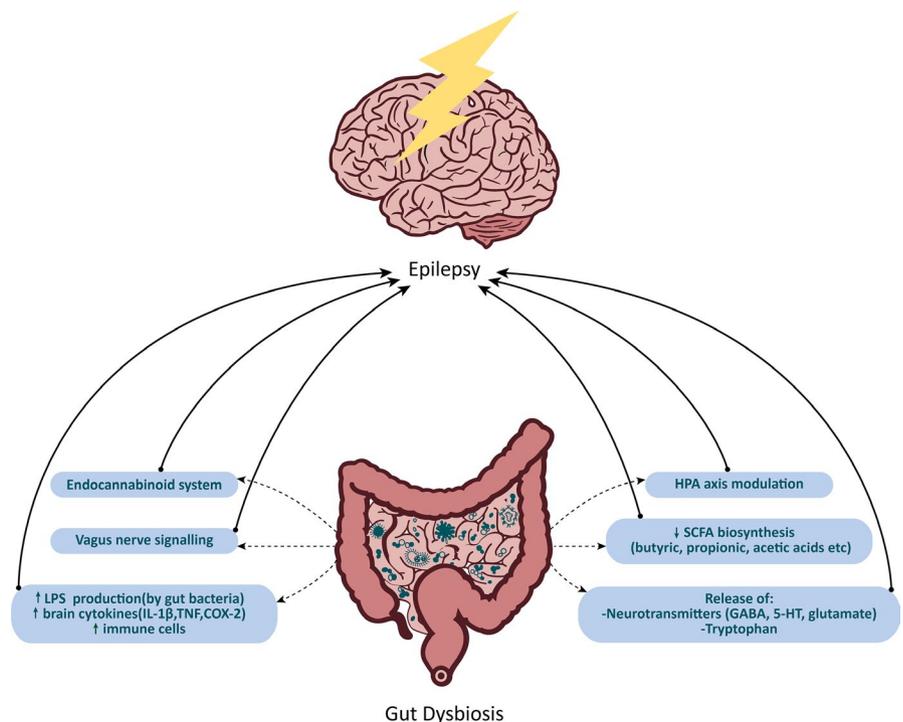


FIGURE 2 Presumptive mechanisms through which the gut microbiota modulate seizure susceptibility

changes at the phylum level (increases in Actinobacteria and Verrucomicrobia and decreases in Proteobacteria) and at the genus level (increases in *Prevotella_9*, *Blautia*, *Bifidobacterium*). Of note, the results of subgroup analyses showed statistically important differences between the DRE, DSE, and control groups. Among patients with epilepsy, differences in the gut microbiota composition were reported depending on clinical phenotype (DRE or DSE), with DRE patients exhibiting enrichment of bacterial taxa in Actinobacteria, Verrucomicrobia, and Nitrospirae and the genera *Blautia*, *Bifidobacterium*, *Subdoligranulum*, *Dialister*, and *Anaerostipes*.⁴³

Another cohort of pediatric population included 14 infants with medically refractory epilepsy and 30 age-matched healthy controls, whose microbiota profiling was done by 16S rDNA sequencing of stool samples. The calculation of α -diversity with the Shannon index for evenness revealed no statistically important difference between the two groups. After the principal component analysis, however, differences in fecal-microbial diversity among groups were assessed. More specifically, in the patient group they noted an increased relative abundance of Firmicutes and Proteobacteria when compared to the healthy control group; there was also a reduction in Bacteroidetes and Actinobacteria, similar to the findings by Peng et al. A potential confounder of this study that

should be noted is the absence of household controls, the inclusion of which could limit the effect of baseline diet differences between participants.

This confounder was eliminated in the study by Lindefeldt et al⁴⁵ that studied 12 children with DRE, aged 2-17 years and 11 healthy parents, as controls. In this study, fecal microbiome profiling was realized by shotgun metagenomic sequencing. In the patient group, fecal microbiota samples demonstrated slightly reduced α -diversity, measured by the Shannon index, in comparison with healthy controls. Furthermore, the microbiomes of children with refractory epilepsy displayed larger variation, whereas those of healthy parents exhibited clear clustering, along the first principal component analysis of taxonomic and functional profiles. Here, taxonomic analysis revealed decreased relative abundances of Bacteroidetes and Proteobacteria and increased relative abundances of Firmicutes and Actinobacteria in the microbiota from medically refractory epileptic children compared to control parents. Functional profiling also demonstrated differences between the two groups, with patients' microbiomes hosting diminished gene content for genes known to regulate the acetyl-CoA pathway (β -hydroxybutyryl-CoA dehydrogenase and crotonase), compared to healthy controls' microbiomes. In view of the lack of age-matched controls, these findings should be interpreted with caution, since gut

TABLE 1 Alterations of gut microbiome in drug-resistant epilepsy, clinical studies

Reference	Study design	Age group	Methodology	Reported changes in microbiota composition of drug-resistant patients
Xie et al, 2017 ⁴⁴	Retrospective cohort study	Children. Patients (n = 14) and healthy infants (n = 30), mean age 1.95 (\pm 3.10) years	Fecal samples, 16s ribosomal DNA sequencing	\downarrow α -diversity, \uparrow Firmicutes and Proteobacteria (Cronobacter) \downarrow Actinobacteria (Bifidobacterium) and Bacteroidetes (Bacteroides, Prevotella)
Peng et al, 2018 ⁴²	Retrospective cohort study	Adults. Drug-resistant patients (n = 42) and drug-sensitive patients (n = 49), and healthy controls from the same families (n = 65)	Fecal samples, 16s ribosomal DNA sequencing	\uparrow α -diversity, more prevalent for the subgroup of those with four or more seizures per year than for those with less than four seizures per year. \uparrow Firmicutes (Roseburia, Coprococcus, Ruminococcus, Coprobacillus) and \downarrow Bacteroides. No important differences between DSE patients and healthy controls.
Lindefeldt et al, 2019 ⁴⁵	Prospective observational study	Children and adolescents. Patients (n = 20) and healthy parents as controls (n = 11), age: 2-17 years	Fecal samples, Shotgun metagenomic sequencing	not significant differences in α -diversity, \uparrow Firmicutes and Actinobacteria \downarrow Proteobacteria and Bacteroidetes
Gong et al, 2020 ⁴³	Retrospective cohort study	Teenagers and adults, 15-60 years. 1. Exploration cohort: Drug-resistant patients (n = 40) and drug-sensitive patients (n = 20), and healthy controls, spouses (n = 46) 2. Validation cohort: patients (n = 13) and controls (n = 10)	Fecal samples, 16s ribosomal DNA sequencing	\downarrow α -diversity, phylum level: \uparrow Actinobacteria and Verrucomicrobia, \downarrow Proteobacteria / genus level: \uparrow Prevotella_9, Blautia, Bifidobacterium. Comparison between DRE and DSE: DRE patients \uparrow taxa Actinobacteria, Verrucomicrobia, and Nitrospirae, \uparrow genera Blautia, Bifidobacterium, Subdoligranulum, Dialister, and Anaerostipes.

Abbreviation: DNA, deoxyribonucleic acid.

microbiome synthesis is largely known to display age dependency⁴⁵ Table 1.

In the studies by Xie et al and Lindefeldt et al,^{44–45} an interventional branch with administration of ketogenic diet was performed, following the initial analysis. The corresponding findings are described below (ketogenic diet in DRE).

4.2 | Treatment studies in DRE

The notion that the gut microbiome influences seizure activity may be clarified through microbiota manipulation with probiotics, prebiotics, antibiotics, or diet, measuring clinical outcomes in prospective cohorts of patients with epilepsy.¹⁷ There are limited publications covering this topic.

4.3 | Ketogenic diet studies

4.3.1 | Ketogenic diet in DRE

The ketogenic diet (KD) is a nonpharmacologic therapeutic intervention, used in the management of medically refractory epilepsy since the 1920s,⁴⁶ mostly in pediatric populations. It proposes high consumption of fat, in addition to sufficient protein and very restricted carbohydrate daily intake (the classic KD consists of a ratio between 2:1 and 4:1 of fat to protein and carbohydrates combined). Its hallmark feature is the production of ketone bodies (β -hydroxybutyrate, acetoacetate, and acetone) in the liver from the dietary fat, a biochemical procedure called ketosis.^{47,48} Ketones substitute glucose as an energy substrate for ATP synthesis in body cells including brain cells. This systemic metabolic shift is considered to elucidate biochemical, metabolic, and hormonal changes that may ameliorate severity and reduce the frequency of epileptic seizures, although the exact mechanisms through which it exerts these actions remain unclear.^{7,49} KD is the therapy of choice for pyruvate dehydrogenase deficiency and for GLUT1 deficiency syndrome.⁵⁰ Various clinical studies and meta-analyses have reported significantly positive results of the KD also in the management of DRE, mainly in children, but also in adults.^{44–45,51–54} Furthermore, it has been applied in various other types of childhood epilepsy and epileptic encephalopathies and has also been tried in other neurological disorders.^{55–58}

4.3.2 | Proposed mechanisms behind the therapeutic effects of the KD in DRE

Following the high effectiveness of the KD in the therapy of hard-to-treat epilepsies, there has been substantial

scientific interest in defining the underlying pathways through which it exerts this action. It is highly probable that KD exerts its anticonvulsant effect through numerous mechanisms, which may vary with different types of epilepsy.⁴⁹ The most widely described mechanisms include an increase in the expression of energy metabolism genes, a direct effect of polyunsaturated fatty acids on ion channels and neurotransmitters control. In addition to these, upregulation of neuroprotective factors such as calbindin, restriction of apoptotic factors like caspase 3, enhanced mitochondrial biogenesis, increased production of the inhibitory factor adenosine^{47,49} and reduction of pro-inflammatory cytokines have been described.⁵⁹ Lately, it has been suggested that the intestinal microbiota may interfere with the KD mechanism of action. We will further discuss the role of gut microbiota on the antiepileptic action of KD. The current evidence suggests that the antiepileptic effect of the KD may be attributed to changes in the gut microbiota.^{44–45,60–64}

4.3.3 | Preclinical studies

Hampton proposes that intestinal microbes mediate the inhibitory impact on seizure activity, achieved by the KD.⁶⁰ In a pivotal study of 2018, Olson et al⁶¹ studied two mouse models (one with 6-Hz induced seizures and the other with spontaneous tonic-clonic seizures) and showed that KD alters the gut microbiota and that this dietary modification was found to be protective against seizure activity in refractory epilepsy. More precisely, they noted that the KD reduced the microbial alpha diversity while simultaneously resulting in an increase in the abundance of *Akkermansia muciniphila* and *Parabacteroides* spp. Those mice that were treated with antibiotics were resistant to the anticonvulsant effect of KD. Only after colonization with both *Akkermansia muciniphila* and *Parabacteroides* spp did they regain the ability to benefit from the antiepileptic effect of KD. The authors described how microbiota transformation led to alterations in the colonic luminal, serum, and hippocampal metabolome, mainly associated with amino acid metabolism. The decline in peripheral subsets of ketogenic gamma-glutamylated amino acids that serve as substrates for the synthesis of γ -aminobutyric acid (GABA) led to the consequent increase in hippocampal GABA inhibitory neurotransmitter, relative to glutamate excitatory neurotransmitter levels, in KD-fed mice compared with controls. These modifications in neurotransmitters' expression were correlated with the antiseizure effect of the KD.⁶¹ More extensive research is required to ascertain whether similar biological pathways' modifications (alterations in amino acids and neurotransmitters' expression) take place in humans on a ketogenic diet.

4.3.4 | Clinical studies

A few studies in humans have also reported evidence about alterations of gut microbiota in patients with epilepsy after KD, although their results are not unanimous.^{44–45,62–64} A case-control study by Xie et al⁴⁴ which included 14 DRE children who received KD and 30 healthy controls showed vast differences of the gut microbiome compositions between the two groups. Also, in the group of the patients, serial examinations of intestinal microflora revealed changes in microbiota populations along the course of the treatment. The authors identified a reduction in pathogenic Proteobacteria (specifically Cronobacter) and an increase in the beneficial Bacteroidetes (specifically Bifidobacterium, Bacteroides, and Prevotella) within one week of commencing the ketogenic diet. These changes to the intestinal microflora were considered to correlate with suppression of seizure activity.⁴⁴ Zhang et al prospectively analyzed gut microbiota changes in 20 children with DRE after a 6-month treatment with KD. The authors reported different patterns of modification of intestinal microflora as well as different levels of response in terms of antiseizure result, in the above group of patients. In general, the microflora profiles of the studied population showed reduced levels of Firmicutes and Actinobacteria and increased levels of Bacteroidetes, after the treatment. A subgroup analysis revealed that those with no response to treatment (<50% reduction of seizures) had a corresponding increase in the levels of Alistipes, Clostridiales, Lachnospiraceae, Ruminococcaceae, and Rikenellaceae compared to the responders. This study raised an important issue about the varying efficacy of the KD in drug-resistant epilepsy that requires investigation by future studies.⁶²

Another case-control study included 12 children with DRE and analyzed the fecal-microbial composition of the cohort before and after a 3-month treatment with the KD. After the treatment, there was a reduction in the relative abundance of *Dialister*, *Actinobacteria*, *Bifidobacteria*, and *Eubacterium rectale* with a simultaneous increase in the relative abundance of *Escherichia coli*. Lindefeldt et al⁴⁵ mentioned that the above findings raise important concerns about the general health of patients receiving KD, since they are indicative of a decrease in relative abundance of health-promoting, fiber-consuming bacteria, in response to the diet.

In a similar three-month prospective study of Tagliabue et al, DRE children with GLUT1 deficiency syndrome demonstrated a marked increase in levels of *Desulfovibrio* spp., but showed no changes in Bacteroides and Firmicutes following the KD.⁶³ Last, Ramm-Petersen et al⁶⁴ found elevated levels of *Desulfovibrio* spp. in the intestines of six GLUT1 deficiency patients after 3 months of KD. This

species has been linked to increased intestinal inflammation following ingestion of animal fat.^{63,65}

4.4 | Fecal transplant studies

In the literature, there is only one case report correlating gut microbiome modulation to difficult-to-treat epilepsy. The authors reported on a patient with a long history of epilepsy and concomitant Crohn's disease, who became seizure-free after fecal microbiota transplantation (FMT), and surprisingly retained remission even after the cessation of the antiepileptic regimen. This article brought up an innovative potential mechanism involved in epileptogenesis and stressed the need for intensive research efforts to unravel the link between gut microbiome and epilepsy.⁶⁶

4.5 | Antibiotic studies

Indirect evidence for the potential benefit of regulating gut microbiota in seizure suppression though antibiotic treatment can be derived from the following retrospective study, by Braakman and Van Ingen. In this study, researchers reported that five out of six patients with DRE became seizure-free during antibiotic treatment, the sixth had a significant reduction in seizure frequency and the improvement was sustained for two weeks after the antibiotic treatment cessation. The authors speculated that this beneficial effect of antibiotics on seizure frequency might be mediated by alterations in the gut microbiome, although this could not be objectively proven, since no microbiota profiling was performed.⁶⁷

4.6 | Probiotic studies

There is only one published clinical trial reporting on the administration of probiotics to patients with refractory epilepsy. Gómez-Eguílaz et al reported on the effects of four-month probiotic supplementation, with a combination of *Lactobacillus*, *Bacteroides* and *Streptococcus* subspecies, in 45 patients with drug-resistant epilepsy. Following intervention, patients displayed a reduction in seizure frequency ($\geq 50\%$ in 28.9% of the patients) and improvement in quality of life, assessed by Quality of Life score (mean \pm SD: 19.23 ± 6.04 vs 26.45 ± 9.7 ; $P = .013$). Overall, this study reveals that probiotics might constitute a safe, low-cost and effective supplementary therapy in patients with DRE. However, whether therapeutic effects of probiotics are mediated by modifications in gut microbiome could not be established, since, again, microbiota analysis was not performed.⁶⁸ No studies about prebiotic supplementation in drug-resistant epilepsy have been published yet.

5 | FUTURE DIRECTIONS

The study of the enteric microbiome in DRE represents a promising area for the enhancement of epilepsy care. Gut microbiome “profiling” could potentially serve as a biomarker in epilepsy management. Once the alterations in the composition or function of gut microbiota in refractory epilepsy are clarified, this could be used in clinical practice in order to identify drug resistance from an early stage and treat patients with more targeted interventions (eg, KD, probiotics, and prebiotics). This would require the conduction of large studies, recruiting cohorts of patients who have experienced the first seizure (or early in the course of the disease) and prospective analysis and examination of their gut microbiome, in association with the progress of the disease (number of seizures, response to treatment, etc). Furthermore, gut microbiome analysis might be used to monitor response to treatment in those patients treated with microbiota-targeted therapies. The impact of antiepileptic drug treatment on gut microbiota, and vice versa, should be investigated by future studies. Like many other, nonantibiotic drugs, it is assumed that some of the antiepileptic drugs might influence gut bacteria growth and survival. Such evidence is derived from animal studies involving AEDs and their effects on gut microbiota^{69,70}; however, no studies in humans have been conducted so far. On the other hand, the gut microbiome can affect drug metabolism altering absorption, bioavailability and efficacy of the medication, through various direct and indirect mechanisms.^{71,72} Furthermore, future research may establish whether altering bacterial composition may serve in the management of refractory epilepsy through antibiotics, pre- or probiotic supplementation and FMT. The overall side effects of the KD for the general health of the patients in terms of microbiota modifications will also need to be established. Finally, there is a need to expand the research of gut dysbiosis as a contributor to epileptogenesis, beyond the DRE patients. Alterations of the gut microbiome might also be present in non-drug-resistant epileptic cases, and this remains to be studied.

6 | CONCLUSION

There is some evidence for a difference in the enteric microbial composition between those with refractory epilepsy and both healthy control and nonrefractory epileptic groups. We note a consensus among studies concerning increased prevalence of Firmicutes relative to Bacteroides in individuals with refractory epilepsy. However, detailed taxonomic analysis revealed considerable variations in the proportions of reported taxa among the studies. Furthermore, the conclusions were conflicting as to whether α -diversity is altered in the drug-resistant epilepsy microbiota populations.

Comparison of the structure and composition of the fecal microbiota among epileptic patients with different clinical phenotypes (DRE and DSE) has so far been conducted by two studies.^{42–43} In the study by Peng et al,⁴² the DSE group showed no statistically important differences compared to controls, while, in the study by Gong et al, it was reported that both DRE and DSE patients exhibited alterations in gut microbiota composition compared to controls. After further analysis, DRE patients' intestines were found to be more dysbiotic, compared to DSE, with an increased abundance in rare flora.⁴³ It is possible that, once the alterations of fecal microbiota properties among different epileptic groups are adequately studied, gut profiling could serve as a potential biomarker for disease prognosis, in clinical practice.

Importantly, there are certain inherent limitations to the study design and instruments that were used. Firstly, many of the studies included relatively small sample sizes. Also, there is a lack of homogeneity among the studies with regard to the age of subjects and the sequencing methodology used to create gut microbiota profiles. The evidence should be judged with caution since the impact of genetic and environmental factors on the composition of the gut microbiota was not evaluated in any of the studies. This may be an important confounding factor of the reported results.

There has been limited research conducted so far, concerning the interventions aiming to alter the enteric microbiota as a potential treatment in DRE. Proposed modifications include probiotics, prebiotics, antibiotics, or dietary modifications. The KD diet is already used for medically refractory epilepsy although the exact mechanism for its therapeutic effects is still not completely understood.

In spite of the limitations, research and clinical study findings suggest that gut microbiome sequencing merits further investigation as a potential biomarker in DRE. There is a need for replicating these findings in large cohorts with adequate control populations, considering variables like age, environmental exposure including medication and diet, genetic factors, and comorbidities. The use of unified microbiome analysis protocols, including both taxa and metabolomic analysis, would provide more extensive and comparable results, between studies. Preliminary study evidence shows promising results; however, more research is warranted so that these modifications might be considered as “evidence-based.”

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CONFLICT OF INTERESTS

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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