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Epilepsy, Ketogenic Diet, and Gut Microbiota: Implications for Clinical Nutrition - A Systematic Review

Vicente M. Martínez Cárdenas, MD[1], Vivian R. Mena Miranda, MD, PhD[2]

[1]Children's Medical Center, Live Oak, Florida, Estados Unidos
Corresponding Author Email: *vicente7757[at]yahoo.com*
ORCID: https://orcid.org/0000-0002-6273-2501

[2]Hospital Pediátrico Universitario Centro Habana, La Habana, Cuba
ORCID: https://orcid.org/0000-0002-6853-5429

Abstract: ***Background: Ketogenic diet therapy is an established nonpharmacological treatment for drug-resistant epilepsy. Experimental and clinical evidence suggests that diet-induced changes in the intestinal microbiome may influence seizure susceptibility and treatment response, but the direction and clinical significance of these changes remain incompletely defined. Objective: To synthesize primary evidence published from 2018 through March 31, 2026 concerning the relationships among ketogenic diet therapy, the gut microbiota, seizure outcomes, and clinically relevant nutritional effects. Methods: A systematic review was conducted in accordance with PRISMA 2020. PubMed/MEDLINE, Scopus, and Web of Science were searched for original human and animal studies. Eligible studies evaluated at least two components of the epilepsy-ketogenic diet-gut microbiota relationship and reported microbiological, metabolomic, seizure, or nutrition-related outcomes. Two reviewers independently performed study selection and data extraction. Human observational studies were appraised with design-appropriate Joanna Briggs Institute tools, and animal studies with the SYRCLE risk-of-bias tool. Owing to marked clinical and methodological heterogeneity, findings were synthesized narratively. Results: Fourteen primary studies were included: seven human or translational studies and seven animal studies. Pediatric studies consistently showed that ketogenic diet therapy altered microbial composition or function, but taxonomic findings were heterogeneous. Several studies reported reduced microbial diversity, lower bifidobacterial abundance, or reduced fecal short-chain fatty acids, whereas others identified baseline microbial or inflammatory signatures associated with treatment response. In mechanistic models, microbiota depletion, microbial transfer, probiotic manipulation, diet withdrawal, and variation in dietary fiber modified seizure susceptibility or ketogenic diet effects. Transfer of post-ketogenic-diet microbiota from children with refractory epilepsy increased seizure resistance in recipient mice. However, most human studies were small, nonrandomized, and unable to establish microbiota-mediated causality. Conclusions: Ketogenic diet therapy reproducibly modifies the intestinal microbiome, and experimental evidence supports a contributory role of microbial and metabolic pathways in seizure protection. Human evidence remains preliminary and does not justify microbiome-based treatment selection or routine probiotic supplementation. Clinical nutrition protocols should preserve seizure control while monitoring gastrointestinal symptoms, growth, micronutrient adequacy, fiber intake, and metabolic complications. Larger prospective studies with standardized diet formulations, microbiome methods, and seizure outcomes are required.***

Keywords: drug-resistant epilepsy, ketogenic diet, gut microbiota, gut-brain axis, short-chain fatty acids, clinical nutrition, seizures

1. Introduction

Epilepsy is a chronic neurological disorder characterized by an enduring predisposition to generate epileptic seizures. Drug-resistant epilepsy is commonly defined as failure of adequate trials of two tolerated, appropriately selected, and correctly used antiseizure medication schedules to achieve sustained seizure freedom [1,2]. Ketogenic diet therapy is an established treatment for selected patients with drug-resistant epilepsy, particularly children, and encompasses the classical ketogenic diet and less restrictive variants such as the medium-chain triglyceride diet and modified Atkins diet [3,4].

The antiseizure effects of ketogenic diet therapy are unlikely to depend on a single pathway. Proposed mechanisms include altered cerebral energy metabolism, ketone-body signaling, mitochondrial function, neurotransmitter balance, ion-channel activity, inflammation, and adenosine signaling [4,5]. The intestinal microbiota has emerged as an additional candidate mediator because dietary macronutrient and fiber composition can rapidly alter microbial ecology and microbial metabolite production.

The gut-brain axis integrates microbial, metabolic, immune, endocrine, vagal, and central nervous system signaling [6]. In epilepsy, this framework is clinically relevant for two related reasons. First, gut microbial profiles may differ according to epilepsy phenotype or treatment responsiveness. Second, ketogenic diets profoundly alter the substrates available for microbial fermentation, particularly by restricting carbohydrate and often dietary fiber intake.

In a seminal mouse study, Olson et al. demonstrated that microbiota depletion abolished ketogenic-diet-associated seizure protection and that selected bacteria and associated metabolic changes could restore protection [7]. Subsequent pediatric studies demonstrated diet-associated changes in microbiome composition, metagenomic function, inflammatory markers, and short-chain fatty acids, although the affected taxa and direction of change varied [8-13]. More recent translational work showed that microbiota obtained from children after ketogenic diet treatment conferred increased seizure resistance when transferred to mice [14]. Animal studies have also examined genetic epilepsy, infantile spasms, diet discontinuation, probiotic supplementation, ketone esters, and dietary fiber [15-20].

The clinical interpretation of this literature requires caution. A microorganism associated with seizure protection in one experimental model may decline during successful treatment in a human cohort. Reduced abundance of fiber-fermenting organisms or short-chain fatty acids may coexist with seizure improvement and could represent a nutritional trade-off rather than a therapeutic mechanism. The objective of this systematic review was therefore to synthesize primary human and animal evidence on ketogenic diet-microbiota interactions in epilepsy, distinguish association from causality, and identify implications for clinical nutrition.

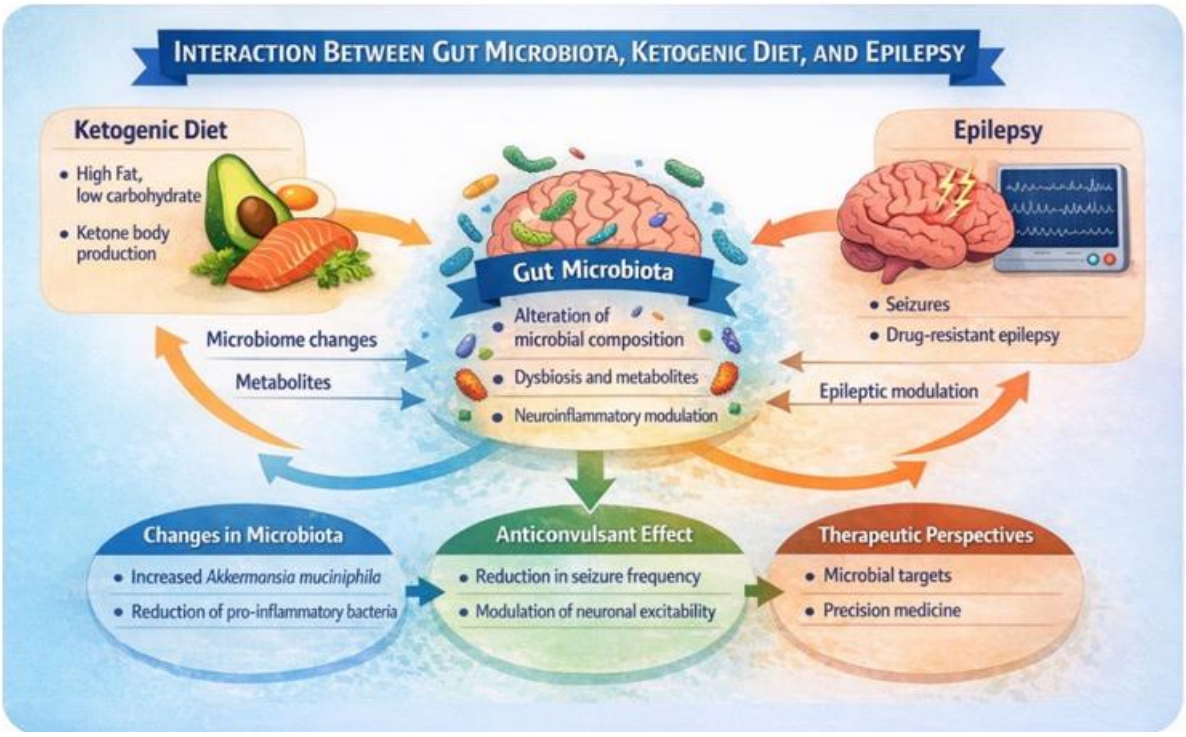

Figure 1: Conceptual interaction between the ketogenic diet, gut microbiota, and epilepsy

Figure legend: The ketogenic diet modifies the composition and metabolic activity of the gut microbiota through its high fat and low carbohydrate content and the production of ketone bodies. These changes may influence microbial metabolites, immune and neuroinflammatory signaling, neuronal excitability, and seizure susceptibility through the microbiota gut-brain axis. Experimental evidence supports a contributory role of the microbiota in ketogenic-diet-associated seizure protection; however, specific microbial changes are heterogeneous in humans, and microbiome-directed interventions remain investigational.

Source: Authors' own elaboration based on the primary studies included in the systematic review [7-20].

2. Methods

Design and reporting

This systematic review was conducted and reported according to the PRISMA 2020 statement. A prospective protocol was not registered; this is acknowledged as a limitation.

Information sources and search strategy

PubMed/MEDLINE, Scopus, and Web of Science were searched from January 1, 2018, through March 31, 2026. Controlled vocabulary and free-text terms were combined for epilepsy, seizures, ketogenic diet therapy, gut microbiota, microbiome, microbial metabolites, short-chain fatty acids, probiotics, and fecal microbiota transplantation. Reference lists of eligible articles and relevant reviews were examined to identify additional primary studies. Complete source-specific strategies and search dates are provided in Supplementary Table S1.

Eligibility criteria

Original studies in humans or animal models were eligible when they evaluated at least two components of the epilepsy-ketogenic diet-gut microbiota relationship and reported one or more of the following: microbiota composition or function; microbial metabolites; seizure frequency, severity, or susceptibility; response to ketogenic diet therapy; gastrointestinal or nutritional outcomes; or effects of microbiota-directed manipulation. Eligible designs included prospective and retrospective cohorts, cross-sectional studies, controlled experiments, and translational studies combining human samples with animal experiments.

Reviews, editorials, commentaries, protocols, conference abstracts without sufficient data, studies outside epilepsy or seizure models, and reports without microbiota- or microbiome-related outcomes were excluded. Studies of ketogenic diets in unrelated conditions were excluded. The search was restricted to English- or Spanish-language reports.

Study selection and data extraction

Two reviewers independently screened titles and abstracts and assessed potentially eligible full texts. Disagreements were resolved by discussion and consensus. Extracted variables included author, year, country, design, population or animal model, sample size when reported, dietary or

microbiota-directed intervention, microbiome method, seizure outcome, nutritional outcome, principal findings, and limitations.

The study-level data-extraction summary is provided in Supplementary Table S3.

Risk-of-bias assessment

Human cohort and cross-sectional studies were evaluated with the corresponding Joanna Briggs Institute critical appraisal checklists. Animal experiments were assessed using the SYRCLE risk-of-bias tool. Domains included selection, allocation and baseline comparability, blinding where applicable, exposure and outcome measurement, completeness of follow-up, selective reporting, and control of confounding. Because these instruments evaluate different designs, results were not converted into a common numerical score.

The study-level risk-of-bias assessments are provided in Supplementary Table S2.

3. Synthesis

Meta-analysis was not undertaken because of substantial heterogeneity in epilepsy syndromes, age, diet formulation, duration, microbial sequencing and bioinformatic methods, animal models, and outcome definitions. Findings were synthesized narratively in three domains: human clinical evidence, mechanistic animal evidence, and implications for clinical nutrition.

Study Selection

The search identified 85 records: 31 from PubMed/MEDLINE, 26 from Scopus, and 28 from Web of Science. After removal of 10 duplicate records, 75 records underwent title and abstract screening, of which 40 were excluded. Thirty-five reports were sought for retrieval, and all were successfully retrieved and assessed in full text. Twenty-one full-text reports were excluded for the following mutually exclusive reasons: no microbiota or microbiome assessment (n = 7), no ketogenic diet assessment (n = 5), population or model unrelated to epilepsy or seizures (n = 3), review, editorial, or absence of primary data (n = 4), and insufficient information or another eligibility-related reason (n = 2). Fourteen primary studies were included in the qualitative synthesis. The complete study-selection process is presented in Figure 2.

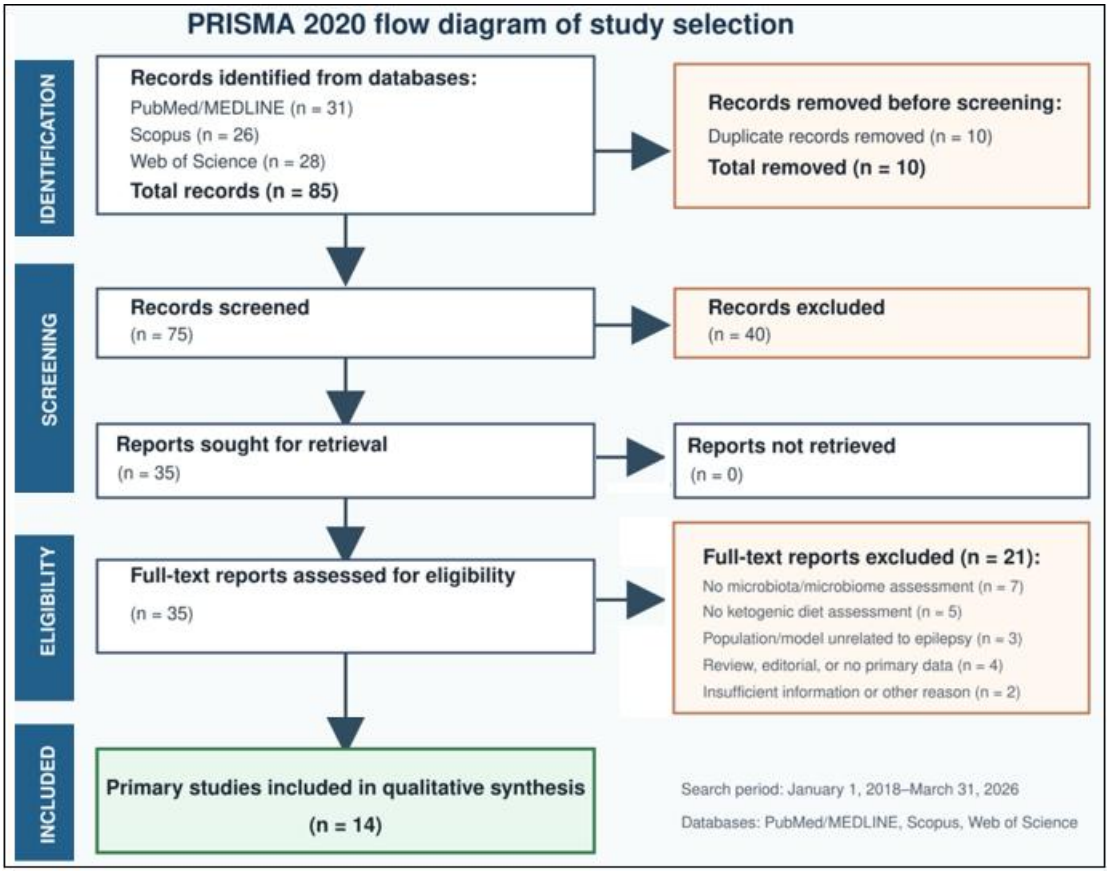

Figure 2: PRISMA 2020 flow diagram of study selection

The database searches identified 85 records: 31 from PubMed/MEDLINE, 26 from Scopus, and 28 from Web of Science. After removal of 10 duplicates, 75 records underwent title and abstract screening, and 40 were excluded. All 35 reports sought for retrieval were obtained and assessed for eligibility. Twenty-one full-text reports were excluded because they did not assess the microbiota or microbiome (n = 7), did not assess ketogenic diet therapy (n = 5), involved a population or model unrelated to epilepsy or seizures (n = 3), were reviews, editorials, or reports without primary data (n = 4), or contained insufficient information or failed another eligibility requirement (n = 2). Fourteen primary studies were included in the qualitative synthesis. The final search was conducted on March 31, 2026.

Fuente
Source: Authors' own elaboration according to the PRISMA 2020 statement.

4. Results

1) Characteristics of included studies

Fourteen primary studies published between 2018 and 2025 were included [7-20]. Seven incorporated human participants or human microbiota, and seven were animal experiments. Most clinical studies involved small cohorts of children with drug-resistant epilepsy. Follow-up ranged from approximately one to six months in prospective pediatric studies, whereas one cross-sectional study evaluated children receiving longer-term ketogenic diet therapy. Microbiome methods included 16S rRNA sequencing, shotgun metagenomics, metabolomics, measurement of fecal short-chain fatty acids, and microbiota transfer to germ-free or antibiotic-treated animals.

2) Human clinical and translational evidence

Zhang et al. prospectively followed 20 children receiving ketogenic diet therapy. Half achieved at least a 50% seizure reduction at six months. Microbial richness declined, Firmicutes decreased, and Bacteroidetes increased; several taxa were enriched among nonresponders [8]. Lindefeldt et al. studied 12 children before and after three months of treatment and used their parents as diet controls. Shotgun metagenomics demonstrated compositional and functional changes, including lower bifidobacteria and reduced carbohydrate-metabolism pathways, without a significant change in alpha diversity [9].

Ferraris et al. evaluated seven patients before and after one month of a classical 4:1 ketogenic diet. All reported more than 50% reduction in seizures or involuntary movements, but total fecal short-chain fatty acids fell by 55%, including reductions in acetate, propionate, and butyrate [10]. Gong et al. followed 12 children with drug-resistant epilepsy and 12 matched controls. After six months of ketogenic diet treatment, several epilepsy-associated microbial differences were partially reversed and fecal short-chain fatty acids increased, illustrating that metabolite findings are not consistent across cohorts [11].

Dahlin et al. followed 28 children for three months and found that higher baseline levels of selected bifidobacteria and tumor necrosis factor were associated with antiseizure response. The findings support biomarker development but require external validation [12]. Menzies et al. compared 14 children receiving ketogenic diet therapy with 13 controls. Children on the diet had lower microbial diversity and lower quality-of-life scores, although the measured intestinal inflammatory marker was not elevated [13].

Lum et al. analyzed samples from 10 children before and approximately one month after ketogenic diet initiation and transferred paired microbiota to mice. Recipient mice colonized with post-treatment microbiota exhibited greater seizure resistance than mice receiving pretreatment microbiota, providing translational evidence that human diet-associated microbial communities can influence seizure susceptibility in an experimental host [14].

3) Mechanistic animal evidence

Olson et al. demonstrated in mouse seizure models that ketogenic diet protection depended on the microbiota. Antibiotic treatment or germ-free status reduced protection, whereas colonization with Akkermansia muciniphila and Parabacteroides species restored it through metabolic pathways associated with reduced peripheral gamma-glutamylated amino acids and an increased hippocampal GABA/glutamate ratio [7].

Eor et al. found that ketogenic diet reduced pentylenetetrazole-induced seizure frequency in mice and that supplementation with Lactobacillus fermentum MSK 408 modified microbiota-derived short-chain fatty acids and predicted GABA metabolism without abolishing the diet's antiseizure effect [15]. Miljanovic and Potschka reported distinct dysbiosis in Scn1a-deficient Dravet mice and observed that ketogenic-diet-induced microbial changes correlated with reductions in motor-seizure frequency and duration [16].

In a neonatal rat model of infantile spasms, Mu et al. found that ketogenic diet reduced seizures but produced hepatic steatosis; a defined probiotic blend prevented much of the hepatic metabolic phenotype without negating seizure control [17]. Shearer et al. subsequently demonstrated that switching from ketogenic to normal diet rapidly increased spasms and was accompanied by changes in gut microbiota and hippocampal mitochondrial bioenergetics [18].

Li et al. reported that ketogenic diet reduced seizure susceptibility and cognitive impairment in a pilocarpine model and that antibiotic disruption attenuated selected benefits, supporting microbiome involvement while not establishing a single protective taxonomic signature [19]. Özcan et al. showed that fiber content in clinical ketogenic formulas drove metagenomic differences in a model infant community and altered seizure resistance in mice. Adding selected fibers to a fiber-deficient formula restored or enhanced seizure protection [20].

5. Methodological Appraisal

The human evidence was limited principally by small samples, nonrandomized designs, heterogeneity of epilepsy etiologies and ketogenic formulations, short follow-up, multiple microbiome comparisons, and incomplete control of medications, age, baseline diet, antibiotic exposure, and other confounders. Animal experiments permitted stronger mechanistic inference but showed variable reporting of randomization, allocation concealment, and blinded outcome assessment. External validity was limited by model-specific effects and differences between experimental diets and individualized clinical ketogenic protocols.

Table 1: Characteristics and methodological limitations of the 14 included primary studies

Study	Design/population	Intervention and microbiome assessment	Principal findings	Principal limitations
Olson et al., 2018 [7]	Controlled mouse experiments; 6-Hz and Kcna1-null seizure models	Ketogenic diet, antibiotics, germ-free mice, bacterial colonization, and metabolomics	The microbiota was required for full seizure protection; selected bacterial consortia and metabolic changes restored protection	Preclinical models; translation of specific taxa to humans remains uncertain
Zhang et al., 2018 [8]	Prospective cohort of 20 children with refractory epilepsy	Ketogenic diet for 6 months; 16S rDNA sequencing	Ten children achieved ≥50% seizure reduction; microbial diversity declined, and taxa differed between responders and nonresponders	Small uncontrolled cohort; heterogeneous epilepsy etiologies and medications
Lindefeldt et al., 2019 [9]	Prospective study of 12 children and parental diet controls	Ketogenic diet for 3 months; shotgun metagenomics	Functional and compositional changes occurred, including lower bifidobacterial abundance and reduced carbohydrate-metabolism pathways	Small cohort; parents were imperfect controls; short follow-up
Eor et al., 2021 [15]	Controlled mouse pentylenetetrazole seizure model	Ketogenic diet with or without *Lactobacillus fermentum* MSK 408; 16S-based functional prediction and SCFA assessment	The diet reduced seizures; probiotic supplementation modified the microbiota and SCFAs without interfering with the antiseizure effect	Acute experimental model; microbial functions were predicted rather than directly measured
Ferraris et al., 2021 [10]	Prospective before-after study of 7 patients	Classical 4:1 ketogenic diet for 1 month; fecal SCFA and fecal-water analyses	Seizure improvement was accompanied by a marked reduction in fecal SCFAs	Very small sample; no control group; no sequencing; short follow-up
Gong et al., 2021 [11]	Cross-sectional and prospective cohort of 12 children and 12 matched controls	Ketogenic diet for 6 months; 16S sequencing and fecal SCFA measurement	Partial reversal of dysbiosis and increased SCFAs; microbial taxa differed according to treatment response	Small sample; multiple comparisons; residual confounding
Miljanovic and Potschka, 2021 [16]	Genetic Dravet mouse model	Ketogenic versus control diet; 16S rRNA sequencing	Genotype- and diet-associated dysbiosis was observed; selected microbial changes correlated with seizure phenotype	Correlational microbiome-seizure analyses; model-specific findings
Mu et al., 2022 [17]	Neonatal rat model of infantile spasms	Ketogenic diet with or without a defined probiotic blend	The diet reduced seizures but caused hepatic steatosis; probiotics improved the hepatic metabolic phenotype	Preclinical safety model; limited direct assessment of microbiome function
Dahlin et al., 2022 [12]	Prospective cohort of 28 children with drug-resistant epilepsy	Ketogenic diet for 3 months; microbiome analysis, cytokine measurement, and machine learning	Baseline bifidobacteria and TNF profiles were associated with antiseizure response	Small cohort; exploratory machine-learning analysis; no external validation
Shearer et al., 2023 [18]	Neonatal rat model of infantile spasms	Continuous ketogenic diet versus transition to a normal diet; 16S sequencing and mitochondrial assays	Diet withdrawal rapidly increased spasms and altered the microbiota and mitochondrial bioenergetics	Small animal groups; observed correlations do not prove microbial mediation
Lum et al., 2023 [14]	Translational study involving 10 children and recipient mice	Pre- and post-ketogenic-diet microbiota transfer; metagenomics and metabolomics	Post-treatment microbiota increased seizure resistance in recipient mice	Small, heterogeneous donor cohort; mouse outcomes are not equivalent to clinical efficacy
Menzies et al., 2023 [13]	Cross-sectional study of 14 children receiving ketogenic diet therapy and 13 controls	Long-term ketogenic diets; microbiome, growth, gastrointestinal symptoms, S100A12, and quality-of-life assessment	Children receiving the diet had lower microbial diversity and quality-of-life scores, without a detectable increase in the measured marker of intestinal inflammation	Cross-sectional design; small sample; treatment duration varied considerably
Li et al., 2024 [19]	Rat lithium-pilocarpine model	Ketogenic diet with or without microbiota disruption; 16S sequencing	The diet improved seizure susceptibility and cognition; antibiotic disruption compromised selected benefits	Preclinical model; antibiotic effects may not be microbiota-specific
Özcan et al., 2025 [20]	Mouse 6-Hz seizure model and model human infant microbial community	Clinical ketogenic formulas with different fiber contents; metagenomic analysis	Fiber composition modified microbial function and seizure resistance	Preclinical formulation study; findings require clinical confirmation

Abbreviations: GABA, gamma-aminobutyric acid; PTZ, pentylenetetrazole; SCFAs, short-chain fatty acids; TNF, tumor necrosis factor.

Source: Authors' own elaboration based on the primary studies included in the systematic review.

6. Discussion

This review identifies a coherent biological signal but a heterogeneous clinical literature. Ketogenic diet therapy changes the intestinal microbiome, and several animal experiments support a functional contribution of microorganisms or microbial metabolites to seizure protection. The strongest mechanistic evidence derives from microbiota depletion, gnotobiotic colonization, bacterial supplementation, diet switching, and transfer of post-treatment human microbiota into experimental animals [7,14-20]. These studies justify continued investigation of the microbiome as one component of ketogenic diet action.

The human evidence does not support a universal "antiseizure microbiome." Pediatric cohorts differed in alpha diversity, bifidobacterial abundance, phylum-level changes, and short-chain fatty acid responses [8-14]. Differences in age, epilepsy etiology, medications, diet ratio, fat source, fiber intake, treatment duration, sequencing platform, and statistical workflow probably contribute to this inconsistency. Taxonomic associations should therefore not be interpreted as diagnostic biomarkers or treatment targets without external validation.

The findings have direct implications for clinical nutrition. Ketogenic diet therapy can reduce fermentable carbohydrate and fiber intake, lower bifidobacteria, and decrease short-chain fatty acids in some patients [9,10,13]. These effects may contribute to constipation, gastrointestinal symptoms, or other long-term concerns even when seizures improve. Conversely, experimental evidence suggests that selected fibers can preserve or enhance seizure resistance by modifying microbial function [20]. This supports careful attention to diet formulation rather than indiscriminate carbohydrate restriction, but it does not yet justify a specific fiber prescription for seizure control.

Probiotics should also be interpreted cautiously. Experimental studies suggest that defined strains may modify microbial metabolites or reduce ketogenic-diet-associated hepatic effects [15,17]. These results cannot be generalized to commercial probiotic products or routine pediatric practice. Probiotic selection, dose, viability, safety, and interaction with the patient's diet and immune status require clinical evaluation.

Clinical care should remain based on established ketogenic diet protocols administered by a multidisciplinary team. Baseline and longitudinal assessment should include growth, gastrointestinal tolerance, dietary adequacy, hydration, lipid profile, liver and renal parameters when indicated, micronutrient supplementation, and adherence. Microbiome testing is not currently a validated method for selecting candidates, choosing a ketogenic formulation, or predicting seizure response.

The review has important limitations. Only 14 studies met the defined scope, and several included very small cohorts. Human studies were predominantly observational and susceptible to confounding and multiple-testing error. Animal models allowed mechanistic experiments but differed substantially in developmental stage, epilepsy phenotype, diet composition, and microbiome context. The absence of a prospectively registered protocol and the restriction to English and Spanish may have introduced reporting and language bias. Quantitative pooling was not appropriate.

Future research should use multicenter prospective designs, standardized reporting of ketogenic ratios and nutrient composition, detailed medication and antibiotic exposure, repeated microbiome and metabolome sampling, validated seizure outcomes, and prespecified statistical analysis. Trials of fiber, prebiotic, probiotic, postbiotic, or microbiota-derived interventions should evaluate both seizure outcomes and nutritional safety.

7. Conclusions

Ketogenic diet therapy alters the intestinal microbiome in children and experimental animals, but the direction and clinical meaning of individual taxonomic changes are inconsistent. Experimental studies provide credible evidence that gut microorganisms and microbial metabolic pathways can modify seizure susceptibility and contribute to ketogenic-diet-associated seizure protection. Human studies remain too small and heterogeneous to establish a causal microbial signature or support routine microbiome-guided treatment.

For clinical nutrition, the principal implication is to optimize the entire ketogenic formulation—including fiber and fat sources- while monitoring seizure control, gastrointestinal function, growth, nutrient adequacy, and metabolic complications. Microbiome-directed therapies remain investigational.

Declarations

Author contributions: Vicente M. Martínez Cárdenas and Vivian R. Mena Miranda contributed to conceptualization, literature searching, study selection, data extraction, evidence synthesis, manuscript drafting, critical revision, and final approval. Both authors agree to be accountable for the work.

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Conflicts of interest: The authors declare no conflicts of interest related to this study.

Ethics statement: Ethical approval was not required because this systematic review analyzed previously published data and involved no identifiable participant information.

Data availability: All data synthesized in this review are contained in the cited publications. The completed extraction form and study-level appraisal should be supplied as supplementary material upon submission.

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Supplementary Table S1. Search strategies used in the systematic review.

Source	Search strategy	Coverage
PubMed/MEDLINE	(("Epilepsy"[Mesh] OR epilep*[Title/Abstract] OR seizure*[Title/Abstract]) AND ("Diet, Ketogenic"[Mesh] OR "ketogenic diet*"[Title/Abstract] OR "ketogenic therap*"[Title/Abstract] OR "ketone ester*"[Title/Abstract]) AND ("Gastrointestinal Microbiome"[Mesh] OR microbiota[Title/Abstract] OR microbiome[Title/Abstract] OR "gut-brain axis"[Title/Abstract] OR "short-chain fatty acid*"[Title/Abstract] OR	January 1, 2018-March 31, 2026; 31 records retrieved

	SCFA[Title/Abstract] OR probiotic*[Title/Abstract] OR prebiotic*[Title/Abstract] OR "fecal microbiota transplant*"[Title/Abstract]))	
Scopus	TITLE-ABS-KEY (epilep* OR seizure*) AND TITLE-ABS-KEY ("ketogenic diet" OR "ketogenic therapy" OR "ketone ester") AND TITLE-ABS-KEY (microbiota OR microbiome OR "gut-brain axis" OR "short-chain fatty acid*" OR SCFA OR probiotic* OR prebiotic* OR "fecal microbiota transplant*")	January 1, 2018-March 31, 2026; 26 records retrieved
Web of Science Core Collection	TS=(epilep* OR seizure*) AND TS=("ketogenic diet" OR "ketogenic therapy" OR "ketone ester") AND TS=(microbiota OR microbiome OR "gut-brain axis" OR "short-chain fatty acid*" OR SCFA OR probiotic* OR prebiotic* OR "fecal microbiota transplant*")	January 1, 2018-March 31, 2026; 28 records retrieved

Total records retrieved: 85.

Abbreviations: MeSH, Medical Subject Headings; SCFA, short-chain fatty acid; TITLE-ABS-KEY, title, abstract, and keywords; TS, topic search.

Source: Authors' own elaboration.

Supplementary Table S2. Study-level risk-of-bias assessment of the 14 primary studies included in the systematic review
A. Human and translational studies

Study	Design and appraisal tool	Participant selection and eligibility	Exposure/intervention measurement	Confounding/comparability	Outcome measurement	Follow-up/completeness	Main study-level concerns
Zhang et al., 2018 [8]	Prospective before-after cohort; JBI Checklist for Cohort Studies	Eligibility and pediatric refractory-epilepsy population adequately described	Ketogenic diet exposure and microbiome sampling were defined	No concurrent untreated epilepsy group; limited adjustment for epilepsy etiology, medications, baseline diet, and antibiotic exposure	Seizure response, EEG, laboratory findings, and 16S rDNA sequencing were reported	Six-month assessment reported for 20 participants; small cohort	Uncontrolled design, small sample, multiple microbial comparisons, and residual confounding
Lindefeldt et al., 2019 [9]	Prospective before-after study; JBI Checklist for Quasi-Experimental Studies	Children with therapy-resistant epilepsy were defined; parental controls were recruited	Three-month ketogenic diet exposure and pre/post sampling were clearly reported	Parents provided diet controls but were not age- or disease-matched; limited control of medication and dietary confounding	Shotgun metagenomic taxonomic and functional outcomes were objectively measured	Pre/post data were available for the reported pediatric cohort	Small sample, imperfect comparator group, short follow-up, and multiple metagenomic comparisons
Ferraris et al., 2021 [10]	Prospective single-group before-after study; JBI Checklist for Quasi-Experimental Studies	Inclusion of seven patients receiving a classical ketogenic diet was described	Classical 4:1 ketogenic diet and adherence through ketonemia were reported	No control group and limited control of concurrent clinical or dietary factors	Fecal short-chain fatty acids were objectively measured; seizure improvement was clinically reported	All seven participants completed one month	Very small sample, no comparator, short follow-up, and seizure outcomes not the primary standardized endpoint
Gong et al. 2021 [11]	Cross-sectional comparison with prospective follow-up; JBI cohort and analytical cross-sectional checklists	Children with drug-resistant epilepsy and matched healthy controls were described	Six-month ketogenic diet exposure, fecal sampling, and laboratory methods were specified	Matching reduced some confounding, but medication, diet, and other microbiome-related factors remained incompletely controlled	16S sequencing, fecal short-chain fatty acids, and seizure response were assessed	Twelve children underwent baseline and six-month assessment	Small sample, combined cross-sectional/longitudinal design, multiple comparisons, and residual confounding

Dahlin et al., 2022 [12]	Prospective observational cohort; JBI Checklist for Cohort Studies	Twenty-eight children with drug-resistant epilepsy were enrolled and followed	Three-month ketogenic diet exposure and paired fecal/serum sampling were defined	Potential confounding from epilepsy type, medications, baseline diet, age, and antibiotics could not be fully excluded	Microbiome, inflammatory biomarkers, and antiseizure response were assessed using prespecified clinical and laboratory procedures	Three-month follow-up was reported; sample remained small for predictive modeling	Exploratory machine-learning analysis, risk of overfitting, small cohort, and absence of external validation
Menzies et al., 2023 [13]	Analytical cross-sectional study; JBI Checklist for Analytical Cross-Sectional Studies	Ketogenic-diet and control groups were defined	Current ketogenic diet exposure was documented, but treatment duration varied substantially	Group differences and treatment indication could generate selection and confounding effects	Gastrointestinal symptoms, growth, quality of life, S100A12, and microbiome measures were reported	Cross-sectional design; longitudinal completeness not applicable	Small sample, variable exposure duration, nonrandom group assignment, and inability to establish temporality
Lum et al., 2023 [14]	Translational paired human study with microbiota-transfer experiments; JBI quasi-experimental checklist for human component and SYRCLE for animal component	Ten children with refractory epilepsy provided paired samples	Pre/post ketogenic diet sampling and microbiota-transfer procedures were clearly described	Human donors were heterogeneous in epilepsy type and diet formulation; paired design reduced between-person variability	Metagenomics, metabolomics, brain transcriptomics, and experimental seizure thresholds were objectively measured	Paired human samples and recipient experiments were reported	Small donor cohort, heterogeneous clinical treatments, multiple omics comparisons, and indirect translation of mouse seizure resistance to human efficacy

B. Animal studies

SYRCLE domain abbreviations: SG, sequence generation; BC, baseline comparability; AC, allocation concealment; RH, random housing; BP, blinding of caregivers/investigators; ROA, random outcome assessment; BOA, blinded outcome assessment; ID, incomplete outcome data; SR, selective reporting; OB, other bias. Y, low concern/adequately reported; N, high concern; U, unclear because reporting was insufficient.

Study	Model	SG	BC	AC	RH	BP	ROA	BOA	ID	SR	OB	Main study-level concerns
Olson et al., 2018 [7]	Mouse 6-Hz and Kcna1-null seizure models	U	Y	U	U	U	U	U	Y	Y	Y	Randomization and blinding procedures were incompletely reported; multiple experimental cohorts and mechanistic comparisons limit a single global judgment
Eor et al., 2021 [15]	Pentylenetetrazole-induced acute seizure model in mice	U	Y	U	U	U	U	U	Y	Y	U	Allocation, housing, and blinding were insufficiently reported; acute seizure model limits external validity
Miljanovic and Potschka, 2021 [16]	Scn1a-deficient Dravet mouse model	U	Y	U	U	U	U	U	Y	Y	Y	Microbiome-seizure associations were principally correlational; randomization and blinding details were unclear
Mu et al., 2022 [17]	Neonatal rat model of infantile spasms	U	Y	U	U	U	U	U	Y	Y	U	Experimental allocation and blinding were incompletely reported; industry-linked probiotic involvement should be considered when interpreting findings
Shearer et al., 2023 [18]	Neonatal rat model of infantile spasms	Y	Y	U	U	U	U	Y	Y	Y	Y	Random group assignment and blinded seizure quantification strengthened the study; small experimental groups and short observation remained limitations
Li et al., 2024 [19]	Lithium-pilocarpine rat model	U	Y	U	U	U	U	U	Y	Y	U	Antibiotics may exert effects unrelated to microbiota depletion; allocation and blinding procedures were not sufficiently clear
Özcan et al., 2025 [20]	Mouse 6-Hz model and model human infant microbial community	U	Y	U	U	U	U	U	Y	Y	Y	Multiple formula and fiber experiments complicate a single bias judgment; randomization and blinding were incompletely reported

Table note: Appraisal was performed at study level using design-appropriate JBI instruments for human studies and the SYRCLE risk-of-bias tool for animal studies. JBI and SYRCLE findings were not combined into a common numerical score or converted into unified "high," "moderate," or "low" quality categories because the instruments assess different methodological domains. "Unclear" indicates insufficient reporting and does not necessarily establish that the procedure was not performed.

Abbreviations: JBI, Joanna Briggs Institute; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation.

Source: Authors' own elaboration based on the included primary studies [7-20].

Supplementary Table S3: Study-level data-extraction summary of the 14 primary studies included in the systematic review

Study; country	Design and population/ model	Sample size	Intervention/ comparison and duration	Microbiota/ metabolite methods	Seizure and clinical outcomes	Principal extracted findings
Olson et al., 2018 [7]; USA	Controlled mechanistic experiments in mouse 6-Hz and Kcna1-null seizure models	Multiple experimental cohorts; numbers varied by experiment	Ketogenic versus control diet; germ-free status; antibiotics; bacterial colonization; fecal transfer	16S rRNA profiling and targeted/untargeted metabolomic analyses	Seizure threshold and spontaneous seizure outcomes	Microbiota depletion reduced ketogenic-diet-associated seizure protection. Colonization with *Akkermansia muciniphila* and *Parabacteroides* species restored protection and was associated with altered gamma-glutamylated amino acids and hippocampal GABA/glutamate balance
Zhang et al., 2018 [8]; China	Prospective cohort of children with refractory epilepsy	20 children	Ketogenic diet; baseline to 6 months	Fecal 16S rDNA sequencing	Seizure frequency, EEG, and laboratory outcomes	Two children became seizure-free, three achieved ≥90% reduction, five achieved 50%-89% reduction, and ten achieved <50% reduction. Richness declined; Firmicutes decreased and Bacteroidetes increased; selected taxa were enriched in nonresponders
Lindefeldt et al., 2019 [9]; Sweden	Prospective pediatric before-after study with parental diet controls	12 children; parents served as controls	Ketogenic diet; baseline to 3 months	Shotgun metagenomic sequencing with taxonomic and functional profiling	Microbiome composition/function; clinical treatment context	Alpha diversity did not change significantly, but taxonomic and functional composition changed. Bifidobacteria, *Eubacterium rectale*, and *Dialister* decreased; several carbohydrate-metabolism pathways were reduced
Eor et al., 2021 [15]; Republic of Korea	Controlled pentylenetetrazole-induced acute seizure model in mice	30 mice across six groups	Normal or ketogenic diet with/without Lactobacillus fermentum MSK 408; 4 weeks	Fecal microbiota profiling, fecal short-chain fatty acids, and PICRUSt functional prediction	Seizure frequency and barrier/metabolic outcomes	Ketogenic diet reduced seizure frequency. Probiotic supplementation altered microbiota and short-chain fatty acids, improved selected barrier and lipid measures, and did not interfere with the antiseizure effect
Ferraris et al., 2021 [10]; Italy	Prospective single-group before-after study in patients with epilepsy	7 patients	Classical 4:1 ketogenic diet; 1 month	Gas chromatography measurement of fecal short-chain fatty acids; fecal-water toxicity assays	Seizures/involuntary movements and diet tolerance	All participants reported >50% improvement. Total fecal short-chain fatty acids decreased by 55%; acetate decreased by 64%, propionate by 33%, and butyrate by 20%
Gong et al., 2021 [11]; China	Cross-sectional comparison plus prospective follow-up in children with drug-resistant epilepsy and matched healthy controls	12 children with epilepsy and 12 controls	Ketogenic diet in epilepsy group; baseline to 6 months	Fecal 16S sequencing and short-chain-fatty-acid measurement	Seizure response and cognitive observations	Several epilepsy-associated microbial differences were partially reversed after treatment. Fecal short-chain fatty acids increased and correlated with bacterial changes; taxa differed between adequate and inadequate responders
Miljanovic and Potschka,	Genetic Dravet mouse model	Experimental group sizes	Ketogenic versus control diet	Fecal 16S rRNA sequencing	Motor-seizure frequency and duration	*Scn1a* deficiency was associated with dysbiosis. Ketogenic diet altered Firmicutes, Bacteroidetes, and selected genera; magnitude of

2021 [16]; Germany		reported by genotype/ diet comparison				selected microbial changes correlated with reduced seizure frequency and duration
Mu et al., 2022 [17]; Canada/ USA	Neonatal Sprague-Dawley rat model of infantile spasms	Multiple experimental groups	Ketogenic diet with/without Streptococcus thermophilus HA-110 and Lactococcus lactissubsp. lactis HA-136	Microbiota-directed probiotic intervention with hepatic biochemical, lipid, inflammatory, and mitochondrial analyses	Spasms and hepatic safety outcomes	Ketogenic diet reduced seizures but caused hepatic steatosis. The defined probiotic blend improved hepatic triglyceride accumulation, oxidative stress, and lipid-oxidation signaling without negating seizure control
Dahlin et al., 2022 [12]; Sweden/ USA	Prospective observational cohort of children with drug-resistant epilepsy	28 children	Ketogenic diet; baseline to 3 months	Fecal microbiome profiling, serum inflammatory biomarkers, bioinformatics, and machine learning	Antiseizure response	The diet had a general anti-inflammatory effect. Higher baseline levels of selected bifidobacteria and TNF were associated with treatment response; the exploratory signature was not externally validated
Shearer et al., 2023 [18]; Canada/ USA	Neonatal rat model of infantile spasms	KD→KD: n=5 at final assessment; KD→normal diet: n=6 at final assessment	Continuous ketogenic diet versus switch to normal diet after 3 days; final comparison after an additional 3 days	Fecal 16S rRNA sequencing and hippocampal mitochondrial respirometry	Spasm frequency, behavior, ketones, and mitochondrial function	Switching to normal diet approximately doubled spasms relative to continuous treatment and rapidly altered microbiota and mitochondrial bioenergetics. Selected *Streptococcus* taxa correlated inversely with spasms
Lum et al., 2023 [14]; USA	Translational paired pediatric study with microbiota transfer to germ-free or antibiotic-treated mice	10 children; recipient mice in multiple experimental cohorts	Human samples immediately before and approximately 1 month after ketogenic diet initiation; paired microbiota transfer	Shotgun metagenomics, metabolomics, and recipient-mouse brain transcriptomics	Experimental seizure threshold in recipient mice; clinical donor context	Post-treatment human microbiota increased seizure resistance in recipient mice by approximately 22% relative to paired pretreatment microbiota and transferred selected metagenomic/metabolomic features
Menzies et al., 2023 [13]; Australia	Analytical cross-sectional pediatric study	14 children receiving ketogenic diet and 13 controls	Established ketogenic diet; median duration 15 months versus normal-diet controls	Gut microbiome analysis and fecal S100A12	Gastrointestinal symptoms, growth, quality of life, and intestinal inflammation marker	Ketogenic-diet participants had lower microbial diversity and lower quality-of-life scores; gastrointestinal symptoms tended to be greater, while S100A12 did not indicate detectable intestinal inflammation
Li et al., 2024 [19]; China	Lithium-pilocarpine rat model of temporal-lobe epilepsy/status epilepticus	Multiple experimental groups	Ketogenic versus normal diet with/without antibiotic-mediated microbiota disruption	Fecal 16S rRNA sequencing	Seizure susceptibility, epileptic activity, neuronal injury, learning, and memory	Ketogenic diet reduced seizure susceptibility and cognitive impairment and altered microbial composition. Antibiotic disruption compromised selected antiseizure and neuroprotective effects
Özcan et al., 2025 [20]; USA	Mouse 6-Hz seizure model and model human infant microbial community	Multiple experiments using clinical formulas and fiber conditions	Clinical ketogenic infant formulas with differing fat ratios, fat sources, and fiber contents; screening of 13 fiber sources/types	Metagenomic profiling of model microbial community and experimental seizure testing	Resistance to 6-Hz seizures and microbial functional pathways	Fiber content was a major determinant of microbial function and seizure protection. Addition of selected fibers restored or potentiated protection in fiber-deficient or protective ketogenic formulas

Abbreviations: EEG, electroencephalography; GABA, gamma-aminobutyric acid; JBI, Joanna Briggs Institute; KD, ketogenic diet; PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; SCFA, short-chain fatty acid; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation; TNF, tumor necrosis factor.

Table note: For studies containing several independent animal experiments, a single sample size was not assigned when the number varied by experiment. The corresponding publication should be consulted for experiment-specific denominators. Human and animal findings were not pooled quantitatively.

Source: Authors' own elaboration based on the included primary studies [7-20].