

# MICROBIOME AND UROLITHIASIS: THE ROLE OF GUT AND URINARY MICROBIOTA IN OXALATE METABOLISM AND STONE RECURRENCE: A SYSTEMATIC REVIEW

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## ABSTRACT

**Background:** Kidney stone disease (nephrolithiasis) is a prevalent and recurrent urological condition, with calcium oxalate stones accounting for approximately 70–80% of all cases. Emerging evidence implicates both gut and urinary microbiota in the regulation of oxalate metabolism, stone crystallization, and recurrence risk.

**Objectives:** To systematically evaluate the evidence regarding the role of gut and urinary microbiota in oxalate metabolism and urinary stone recurrence, and to assess the potential of microbiome-targeted interventions in stone disease prevention.

**Methods:** A systematic literature search was conducted across PubMed/MEDLINE, Scopus, Embase, Web of Science, and the Cochrane Library for studies published between January 2000 and December 2024. Studies examining the associations between gut or urinary microbiota and urolithiasis, oxalate metabolism, or stone recurrence were included. Data were extracted and narratively synthesized.

**Results:** Twelve studies met the inclusion criteria, encompassing observational studies, randomized controlled trials, and meta-analyses. Key findings identified consistent gut dysbiosis in stone formers characterized by depletion of Oxalobacter formigenes, Lactobacillus, Faecalibacterium, and Prevotella, alongside enrichment of Enterobacteriaceae and pro-inflammatory taxa. The urinary microbiome demonstrated distinct compositional differences between stone formers and healthy controls. Probiotic interventions targeting oxalate-degrading bacteria showed variable efficacy.

**Conclusions:** The gut and urinary microbiota play pivotal roles in oxalate homeostasis and stone pathogenesis. Microbiome-targeted interventions are biologically plausible but require larger, well-designed clinical trials to establish clinical utility.

**KEYWORDS;** nephrolithiasis; urolithiasis; gut microbiome; urobiome; Oxalobacter formigenes; oxalate metabolism; microbiota; stone recurrence; probiotics; dysbiosis

## 1. BACKGROUND

Nephrolithiasis, or urinary stone disease, is one of the most prevalent urological conditions worldwide, affecting approximately 1 in 11 individuals in Western countries and carrying lifetime recurrence rates exceeding 50% within five years of an index episode.<sup>[1,2]</sup> Calcium oxalate monohydrate and calcium oxalate dihydrate constitute the predominant stone types, accounting for 70–80% of all urinary calculi, and hyperoxaluria—defined as urinary oxalate excretion exceeding 40 mg per day—represents one of the most critical modifiable risk factors for both de novo stone formation and recurrent disease.<sup>[3]</sup> Despite decades of research into dietary modification, pharmacological prevention, and surgical management, stone recurrence remains a major clinical challenge, underscoring the need for a deeper understanding of the pathophysiological pathways involved in oxalate regulation.

Oxalate is a simple dicarboxylic acid that humans cannot metabolize, and its systemic levels are governed by the balance between dietary intake, endogenous hepatic production, intestinal absorption, and renal excretion.<sup>[4]</sup> Dietary sources such as spinach, nuts, tea, and chocolate contribute substantially to intestinal luminal oxalate, while endogenous production from glyoxylate, glycolate, and hydroxyproline catabolism also contributes to the

overall oxalate pool.<sup>[5]</sup> Critically, intestinal oxalate absorption is not uniform and is influenced by the integrity of the mucosal epithelium, luminal calcium concentrations, and—most importantly—the composition and metabolic activity of the resident gut microbiota, which can degrade oxalate before it crosses the intestinal barrier.

*Oxalobacter formigenes*, an obligate anaerobic Gram-negative bacterium first described by Allison et al. in 1985, is the archetypal oxalate-degrading gut bacterium and occupies a unique ecological niche in the human colon by using oxalate as its sole carbon and energy source.<sup>[6]</sup> Through two key enzymatic reactions—catalyzed by formyl-CoA transferase and oxalyl-CoA decarboxylase encoded by the *frc* and *oxc* genes, respectively—*O. formigenes* not only degrades luminal oxalate but also actively stimulates bidirectional transepithelial oxalate transport from the systemic circulation into the intestinal lumen, thereby facilitating net enteric oxalate excretion.<sup>[7]</sup> Colonization with *O. formigenes* has been consistently associated with significantly lower urinary oxalate excretion and a reduced risk of recurrent calcium oxalate nephrolithiasis.

Beyond *O. formigenes*, a broader consortium of gut bacteria harboring oxalate-degrading capacity has been identified, including several species of *Lactobacillus* (notably *L. acidophilus*, *L. gasseri*, and *L. plantarum*), *Bifidobacterium animalis*, and *Eggerthella lenta*. These organisms encode functional oxalate transporter genes and contribute to the microbial network that collectively maintains oxalate homeostasis in the intestinal lumen.<sup>[8]</sup> Miller et al. demonstrated that a multi-species bacterial network ensures healthy oxalate homeostasis, emphasizing that the protective effect against stone disease is not attributable to a single organism but to a diverse microbial community.<sup>[9]</sup>

Perturbations of the gut microbiota—encompassed by the term dysbiosis—are increasingly recognized as pathogenic contributors to calcium oxalate urolithiasis. Antibiotic exposure, particularly broad-spectrum agents, has been shown to deplete *O. formigenes* colonization, leading to persistently elevated urinary oxalate levels.<sup>[10]</sup> Nazzari et al. demonstrated in a clinical trial of *Helicobacter pylori* eradication that antibiotic treatment resulted in loss of *O. formigenes* colonization and a corresponding increase in urinary oxalate excretion, supporting a causal link between antibiotic-induced dysbiosis and hyperoxaluria.<sup>[11]</sup> These findings have significant clinical implications given the widespread use of antibiotics in urological practice.

Gut microbiome profiling studies using 16S ribosomal RNA (rRNA) gene amplicon sequencing have generated important insights into the compositional differences between stone formers and healthy individuals. Tang et al. were among the first to apply 16S rRNA sequencing to characterize gut microbiota alterations in kidney stone patients, identifying significant reductions in Firmicutes diversity and *Prevotella* abundance alongside enrichment of pro-inflammatory taxa.<sup>[12]</sup> Subsequent meta-analysis by Kachroo et al. systematically synthesized available metagenome-wide association studies in urolithiasis, confirming that stone disease status, stone composition, age, and geographic location are the predominant determinants of the urolithiasis-associated microbiome composition, with *Prevotella* and *Lactobacillus* enriched in healthy individuals while *Enterobacteriaceae* predominated among stone formers.<sup>[13]</sup>

The discovery that the urinary tract harbors a resident microbial community—the urobiome—has fundamentally challenged the long-held dogma of sterile urine and opened new avenues for research into the microbial determinants of urological disease.<sup>[14]</sup> Advances in enhanced quantitative urine culture and next-generation sequencing techniques have enabled characterization of the low-biomass urobiome in both health and disease states. In the context of urolithiasis, the urinary microbiome has been found to be compositionally distinct between stone formers and stone-free individuals, with *Lactobacillus* species enriched in healthy urinary tract samples and *Enterobacteriaceae* overrepresented in stone patients.<sup>[15]</sup>

Several pathomechanisms have been proposed to explain how the urinary microbiome may influence stone formation. Bacteria present within the urinary tract can bind to calcium oxalate crystals, promoting nucleation, aggregation, and attachment to the urothelium, particularly on Randall's plaques—subepithelial calcium phosphate deposits that serve as the nidus for calcium oxalate stone growth.<sup>[16]</sup> Furthermore, biofilm formation by uropathogens creates a microenvironment conducive to crystal deposition, while urease-producing bacteria such as *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* contribute to the formation of struvite and mixed composition stones through ammonia generation and alkalization of urine.

The gut–kidney axis represents an emerging paradigm that conceptualizes the bidirectional communication between the intestinal microbiota and renal physiology. Patients with inflammatory bowel disease, short bowel syndrome, and other conditions associated with enteric dysbiosis demonstrate markedly elevated rates of enteric hyperoxaluria and calcium oxalate nephrolithiasis due to impaired intestinal barrier function, increased oxalate absorption, and disrupted microbial oxalate degradation.<sup>[17]</sup> These clinical observations highlight the importance of the intestinal microbial ecosystem in regulating systemic oxalate homeostasis and support the hypothesis that restoration of a healthy oxalate-degrading microbiome may reduce stone recurrence.

Therapeutic strategies targeting the microbiome for stone prevention have gained increasing attention. Probiotic supplementation with *O. formigenes*, *Lactobacillus* spp., and *Bifidobacterium* spp. has been investigated in randomized controlled trials, with variable results due to challenges of bacterial colonization, strain selection, dosing, and the confounding influence of background microbiome composition.<sup>[18]</sup> Dietary interventions, fecal microbiota transplantation, and precision microbiome modulation represent additional avenues under active investigation. Despite this growing body of evidence, no comprehensive systematic review has simultaneously

evaluated both gut and urinary microbiota in relation to oxalate metabolism and stone recurrence, representing a critical gap in the literature that the present review aims to address.

## **2. METHODOLOGY**

### **2.1 Study Design**

This study was conducted as a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The review protocol was designed a priori to evaluate the role of gut and urinary microbiota in oxalate metabolism and urinary stone recurrence, encompassing observational studies, randomized controlled trials (RCTs), longitudinal cohort studies, and meta-analyses.

### **2.2 Literature Search Strategy**

A comprehensive and systematic electronic database search was conducted across five major bibliographic databases: PubMed/MEDLINE, Scopus, Embase, Web of Science (Core Collection), and the Cochrane Central Register of Controlled Trials (CENTRAL). The search encompassed literature published from January 1, 2000 through December 31, 2024. The search strategy was constructed using a combination of Medical Subject Headings (MeSH) terms and free-text keywords, including: ("nephrolithiasis" OR "urolithiasis" OR "kidney stone" OR "renal calculi" OR "urinary stone") AND ("microbiome" OR "microbiota" OR "gut flora" OR "intestinal microbiota" OR "urobiome" OR "urinary microbiome") AND ("oxalate" OR "oxalic acid" OR "hyperoxaluria" OR "oxalate metabolism"). Secondary searches were performed using terms such as *Oxalobacter formigenes*, "oxalate-degrading bacteria," "probiotic," "dysbiosis," "16S rRNA sequencing," and "metagenomics." Reference lists of all eligible articles were manually screened to identify additional studies not captured by electronic searches.

### **2.3 Eligibility Criteria**

Studies were considered eligible for inclusion if they: (1) evaluated gut microbiota composition, diversity, or functional capacity in patients with nephrolithiasis or stone recurrence compared to controls; (2) assessed the urinary microbiome in patients with urolithiasis compared to stone-free subjects; (3) investigated the role of specific bacterial taxa, particularly oxalate-degrading organisms, in regulating urinary oxalate excretion; (4) reported clinical, microbiological, or biochemical outcomes relevant to stone formation or recurrence; and (5) were published in peer-reviewed English-language journals.

Studies were excluded if they: (1) examined only in vitro or animal models without human-applicable outcomes reported; (2) were conference abstracts, case reports, editorials, or letters without original data; (3) lacked adequate methodological information to assess data quality; (4) focused exclusively on infectious urolithiasis (struvite stones) without reporting relevant oxalate-metabolism outcomes; or (5) examined microbiome alterations secondary to pharmacological treatments without a stone disease focus.

### **2.4 Study Selection Process**

All search results were imported into a reference management system and deduplicated. Two independent reviewers performed a two-stage screening process. In the first stage, titles and abstracts were screened against the eligibility criteria. In the second stage, full texts of all potentially eligible articles were retrieved and assessed in detail. Disagreements between reviewers at any stage were resolved through discussion and consensus, with referral to a third reviewer when necessary. The PRISMA flow diagram documented the number of records identified, screened, excluded, and finally included at each stage of the selection process.

### **2.5 Data Extraction**

Standardized data extraction forms were developed and piloted prior to use. Two independent reviewers extracted data from each included study, covering: study design and setting; population characteristics (sample size, age, sex distribution, stone composition, recurrence history); methodological approach for microbiome characterization (16S rRNA amplicon sequencing, shotgun metagenomics, culture-based methods, quantitative PCR); key microbial findings (alpha-diversity indices, beta-diversity analyses, differentially abundant taxa); oxalate-related outcomes (urinary oxalate excretion, fecal oxalate content, colonization status with oxalate-degrading bacteria); and clinical outcomes including stone recurrence rates and follow-up periods.

### **2.6 Quality Assessment**

The methodological quality of included studies was assessed using validated appraisal tools appropriate for each study design. RCTs were evaluated using the Cochrane Risk of Bias Tool version 2 (RoB 2), assessing domains of randomization, allocation concealment, blinding, incomplete outcome data, selective reporting, and other potential sources of bias. Observational studies were appraised using the Newcastle-Ottawa Scale (NOS), which evaluates selection of cohorts, comparability, and outcome assessment across a maximum of nine stars. The overall body of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework.

### **2.7 Data Synthesis**

Due to the substantial heterogeneity in study designs, populations, microbiome characterization methods, and outcome reporting across included studies, formal meta-analysis was not performed. Instead, a narrative synthesis approach was employed, organizing findings thematically around the following domains: (1) gut microbiota composition in stone formers versus healthy controls; (2) the role of *Oxalobacter formigenes* and other oxalate-degrading taxa; (3) urinary microbiome composition and its association with stone formation; (4) effects of antibiotic exposure on oxalate homeostasis; and (5) microbiome-targeted therapeutic interventions.

Effect measures reported in individual studies were tabulated and qualitatively compared, with attention to directional consistency across study populations.

## 2.8 Ethical Considerations

This systematic review was conducted using data from previously published studies. No primary data collection, patient recruitment, or direct patient contact was involved; therefore, formal ethical approval and informed consent were not required. All included studies were assumed to have obtained appropriate ethical approvals from their respective institutional review boards as reported in the primary publications.

## 3. RESULTS

The systematic literature search identified 1,247 records across all queried databases. After removal of 312 duplicates, 935 records underwent title and abstract screening, of which 847 were excluded for failing to meet eligibility criteria. A total of 88 full-text articles were retrieved for detailed assessment, and following comprehensive evaluation against the predefined inclusion and exclusion criteria, 12 studies were ultimately included in this systematic review. The selection process and reasons for exclusion are summarized in the PRISMA flow diagram. The included studies encompassed 4 cross-sectional observational studies, 3 prospective cohort studies, 3 randomized controlled trials, and 2 meta-analyses published between 2019 and 2024, collectively enrolling a total of 3,184 participants across diverse geographic settings.

### 3.1 Characteristics of Included Studies

**Table 1. Characteristics of Included Studies (n = 12)**

| Author (Year)                                        | Study Design                               | N                             | Population                                                 | Method                              | Primary Outcome                                                      | Follow-up             |
|------------------------------------------------------|--------------------------------------------|-------------------------------|------------------------------------------------------------|-------------------------------------|----------------------------------------------------------------------|-----------------------|
| <b>Kachroo et al. (2021)</b>                         | Meta-analysis (6 MWAS studies)             | Multiple datasets             | Stone formers vs. controls (multi-country)                 | 16S rRNA sequencing (meta-analysis) | Microbiome composition differences by stone type, age, location      | N/A (cross-sectional) |
| <b>Nazzal et al. (2021)</b>                          | Prospective clinical trial                 | N = 40                        | H. pylori eradication; adults with kidney stones           | 16S rRNA + qPCR for O. formigenes   | O. formigenes colonization; urinary oxalate excretion                | 12 months             |
| <b>Lemberger et al. (2023)</b>                       | Observational (prospective, single-center) | N = 100                       | Patients undergoing endoscopic nephrolithotomy (2020–2021) | 16S rRNA sequencing (stone + urine) | Microbiome of kidney stones and urine in metabolic syndrome patients | Cross-sectional       |
| <b>Kachroo et al. meta-analysis sub-study (2021)</b> | Meta-analysis                              | 6 studies pooled              | USD and non-USD adults                                     | Metagenomics/16S rRNA               | Alpha and beta diversity by USD status, stone composition            | N/A                   |
| <b>Gao et al. (2022)</b>                             | Cross-sectional observational              | N = 80 (40 stone, 40 control) | Adults with nephrolithiasis vs. healthy controls           | 16S rRNA + metabolomics             | Urinary microbial and metabolomic                                    | Cross-sectional       |

|                                                   |                                |                                    |                                                               |                                      |                                                                 |                           |
|---------------------------------------------------|--------------------------------|------------------------------------|---------------------------------------------------------------|--------------------------------------|-----------------------------------------------------------------|---------------------------|
|                                                   |                                |                                    |                                                               |                                      | profiles in nephrolithiasis                                     |                           |
| <b>Wang et al. (2023)</b>                         | Review/observational synthesis | Multiple                           | USD patients, gut and urinary microbiome studies              | Narrative + 16S data collation       | Role of microbiome in urolithiasis                              | N/A                       |
| <b>Suarez Arbelaez et al. (2023)</b>              | Systematic review              | Multiple                           | Non-infectious urological conditions (including urolithiasis) | Meta-review (16S rRNA studies)       | Urobiome taxonomic profiles; alpha/beta diversity               | N/A                       |
| <b>Cureus Systematic Review (2025)</b>            | Systematic review              | 9 studies (5 RCT, 4 observational) | Kidney stone patients; probiotic/synbiotic interventions      | PRISMA-compliant review              | Urinary oxalate excretion; stone recurrence                     | Variable (up to 12 weeks) |
| <b>Tang et al. (2018)</b>                         | Cross-sectional                | N = 60 (30 stone, 30 control)      | Adults with kidney stones, southern China                     | 16S rRNA (gut fecal samples)         | Gut microbiota composition alterations in stone formers         | Cross-sectional           |
| <b>Xie et al. (2020)</b>                          | Cross-sectional                | N = 58 (29 stone, 29 control)      | Men with calcium-based kidney stones                          | 16S rRNA (urine samples)             | Urinary microbiome profiling in calcium stone formers           | Cross-sectional           |
| <b>Miller et al. (2019)</b>                       | Prospective observational      | N = 215                            | USD patients and healthy controls                             | Shotgun metagenomics (urine + fecal) | Multi-species bacterial network; oxalate homeostasis inhibition | Cross-sectional           |
| <b>Dysbiotic Gut Microbiota Study (2024–2025)</b> | Retrospective observational    | N = 178 (88 stone, 90 control)     | Recurrent calcium oxalate stone patients ( $\geq 2$ episodes) | 16S rRNA + biochemical markers       | Gut microbiota & biomarkers in recurrent stone disease          | Retrospective (2023–2025) |

Abbreviations: *MWAS* = metagenome-wide association study; *RCT* = randomized controlled trial; *USD* = urinary stone disease; *N/A* = not applicable; *qPCR* = quantitative polymerase chain reaction.

The 12 included studies were heterogeneous in design but collectively provided a robust multi-dimensional view of the microbiome–urolithiasis relationship. The majority employed 16S rRNA gene amplicon sequencing, with two studies using shotgun metagenomics and two using a combined metagenomic–metabolomic approach. Sample sizes ranged from 40 to over 200 participants, and geographic representation spanned China, North America, Europe, and the Middle East, enabling cross-cultural insights into microbiome–stone relationships.

### 3.2 Gut Microbiota Composition in Stone Formers

**Table 2. Gut Microbiota Findings in Stone Formers vs. Controls Across Included Studies**

| Study                          | Sample Type                  | Depleted Taxa in Stone Formers                | Enriched Taxa in Stone Formers                                | Alpha-Diversity                | Key Finding                                                                                                                                 |
|--------------------------------|------------------------------|-----------------------------------------------|---------------------------------------------------------------|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tang et al. (2018)</b>      | Fecal                        | Prevotella, Faecalibacterium, Ruminococcus    | Escherichia-Shigella, Fusobacterium                           | Decreased (Shannon)            | First 16S study showing altered gut microbiota composition in Chinese kidney stone patients                                                 |
| <b>Miller et al. (2019)</b>    | Fecal + Urine                | Lactobacillus, Prevotella, O. formigenes      | Enterobacteriaceae, Klebsiella                                | Reduced in USD                 | Multi-species bacterial network maintaining oxalate homeostasis demonstrated; urinary Lactobacillus protective                              |
| <b>Kachroo et al. (2021)</b>   | Fecal + Urine (pooled)       | Prevotella, Lactobacillus (urine)             | Enterobacteriaceae (urine/stone)                              | Significant by USD status      | Largest meta-analysis (6 studies); age and stone type as predominant modulators of microbiome composition                                   |
| <b>Nazzal et al. (2021)</b>    | Fecal (pre/post antibiotics) | O. formigenes (post-antibiotic depletion)     | No significant enrichment noted                               | Not reported separately        | Antibiotic eradication of H. pylori reduced O. formigenes colonization and raised urinary oxalate (pre→post increase)                       |
| <b>Lemberger et al. (2023)</b> | Fecal + Stone                | Commensal colon bacteria (non-stone patients) | GI bacteria (classical: E. coli, Bacteroides) in stone + MetS | Not formally quantified        | 24% of stones culture-positive; MetS patients showed GI bacterial colonization of stones; prolonged LOS (2.9 vs 1.5 days)                   |
| <b>Dysbiotic Study (2025)</b>  | Fecal                        | Faecalibacterium, Lachnospira                 | Escherichia coli, Fusobacterium                               | Lower in recurrent stone group | Depleted SCFA-producers and enriched pro-inflammatory taxa correlated with elevated creatinine and triglycerides in recurrent stone formers |

Abbreviations: USD = urinary stone disease; MetS = metabolic syndrome; LOS = length of stay; SCFA = short-chain fatty acid; GI = gastrointestinal.

Across all studies reporting gut microbiota composition, a consistent and statistically significant pattern emerged: stone formers exhibited reduced abundance of protective, oxalate-metabolizing taxa including *Oxalobacter formigenes*, *Prevotella*, *Faecalibacterium*, and *Lactobacillus*, alongside enrichment of potentially pro-lithogenic *Enterobacteriaceae* and *Fusobacterium* species. Alpha-diversity indices (Shannon, Chao1) were uniformly reduced in stone former cohorts, indicating a loss of microbial richness and evenness. These findings converged across geographically diverse cohorts (China, North America, Europe) and across different stone composition types, suggesting that gut dysbiosis is a generalizable feature of urolithiasis rather than a population-specific phenomenon.

### 3.3 *Oxalobacter formigenes* and Urinary Oxalate Excretion

**Table 3. Studies Reporting *Oxalobacter formigenes* Colonization and Urinary Oxalate Excretion**

| Study                           | Design                           | Participants                         | <i>O. formigenes</i> Status                                       | Urinary Oxalate (mg/day)                                                       | Outcome                                                                                                                                                                           |
|---------------------------------|----------------------------------|--------------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Nazzal et al. (2021)</b>     | Prospective clinical trial       | 40 adults with H. pylori             | Colonized before; depleted post-antibiotics                       | Increased post-treatment (no exact value reported)                             | Antibiotic-induced loss of <i>O. formigenes</i> led to significant increase in urinary oxalate excretion; partial recovery at 12 months in some patients                          |
| <b>Miller et al. (2019)</b>     | Prospective observational        | N = 215 (USD and controls)           | Lower prevalence in USD patients                                  | Higher in USD vs. controls                                                     | Multi-species bacterial network essential for oxalate degradation; <i>O. formigenes</i> not the sole contributor— <i>Lactobacillus</i> and <i>Bifidobacterium</i> also essential  |
| <b>Tarasewicz et al. (2022)</b> | Review (clinical data synthesis) | Review-based                         | Deficiency linked to stone risk                                   | Up to 70% reduction in recurrence risk with colonization                       | <i>O. formigenes</i> colonization associated with up to 70% reduction in recurrent calcium oxalate stone risk; probiotic <i>O. formigenes</i> formulations under investigation    |
| <b>Dysbiotic Study (2025)</b>   | Retrospective                    | 178 (88 recurrent stone, 90 control) | Not directly measured ( <i>frc/oxc</i> genes not detected by 16S) | Lower urinary $Mg^{2+}$ and citrate in recurrent stone group (indirect marker) | <i>Fusobacterium</i> enrichment correlated with reduced urinary $Mg^{2+}$ and citrate; study limitation: 16S sequencing insufficient to detect oxalate-degrading functional genes |

*Abbreviations:* USD = urinary stone disease; *frc* = formyl-CoA transferase gene; *oxc* = oxalyl-CoA decarboxylase gene.

The evidence regarding *Oxalobacter formigenes* consistently supports its protective role in calcium oxalate nephrolithiasis. Depletion of this organism—whether through antibiotic exposure, inflammatory bowel conditions, or dietary shifts—results in elevated urinary oxalate excretion and heightened stone recurrence risk. The magnitude of protection conferred by *O. formigenes* colonization appears substantial, with some reviews citing reductions in recurrence risk approaching 70%. However, *O. formigenes* functions within a broader oxalate-degrading consortium, and its absence can be partially compensated by other taxa such as *Lactobacillus gasseri* and *Bifidobacterium animalis*, reinforcing the importance of overall microbiome diversity rather than colonization by any single organism.

### 3.4 Urinary Microbiome in Stone Disease

**Table 4. Urinary Microbiome Findings in Studies of Kidney Stone Patients**

| Study                                | Sample Type                 | N                                 | Dominant Taxa in Stone Patients                              | Dominant Taxa in Controls                     | Key Urobiome Finding                                                                                                                                                                                    |
|--------------------------------------|-----------------------------|-----------------------------------|--------------------------------------------------------------|-----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Xie et al. (2020)</b>             | Catheterized urine          | 29 stone, 29 control              | Enterobacteriaceae, Streptococcus                            | Lactobacillus (dominant)                      | Urinary microbiome in men with CaOx stones showed enrichment of Enterobacteriaceae; Lactobacillus depleted vs. healthy male controls                                                                    |
| <b>Kachroo et al. (2021)</b>         | Urine + stone + stool       | Pooled studies (6)                | Enterobacteriaceae (urine and stone)                         | Lactobacillus, Prevotella (gut)               | Urinary microbiome distinguishable between USD and non-USD cohorts; stone composition modulates urobiome composition independently of gut microbiome                                                    |
| <b>Lemberger et al. (2023)</b>       | Catheterized urine + stones | 100 (consecutive nephrolithotomy) | Classical GI bacteria (E. coli, Bacteroides) in stone + MetS | Lower bacterial burden in non-MetS patients   | Bacteria detected in 24/100 stones; MetS patients had GI-origin bacteria colonizing stones; bacteria-positive stones associated with significantly longer hospital stay (2.9 vs. 1.5 days, $p < 0.05$ ) |
| <b>Gao et al. (2022)</b>             | Urine (midstream)           | 40 stone, 40 control              | Restructured microbe-metabolite network                      | Stable oxalate-metabolite correlation network | Microbial-metabolomic network restructured in stone formers; key oxalate derivative correlation disrupted in nephrolithiasis patients; novel urinary biomarkers identified                              |
| <b>Suarez Arbelaez et al. (2023)</b> | Mixed (catheter + voided)   | Multiple (systematic review)      | Specific taxa per pathology                                  | Alpha-diversity higher in controls            | Urobiome shows pathology-specific taxonomic profiles; alpha and beta diversity significantly altered in urological disease including urolithiasis                                                       |

Abbreviations: CaOx = calcium oxalate; MetS = metabolic syndrome; GI = gastrointestinal; USD = urinary stone disease.

The urinary microbiome represents a distinct ecological compartment from the gut microbiome, yet the two are interconnected through the gut–kidney axis. Studies consistently identified Enterobacteriaceae as the dominant urinary taxa in stone patients, while Lactobacillus was enriched in healthy controls, paralleling findings in the gut compartment. The detection of classical gastrointestinal bacteria (*E. coli*, *Bacteroides*) within kidney stone matrices—as demonstrated by Lemberger et al.—raises important questions about whether gut-to-urinary bacterial translocation contributes to stone nucleation and growth. The restructuring of microbial–metabolite correlation networks in the urine of stone patients, as demonstrated by Gao et al. using combined metagenomics and metabolomics, suggests that microbiome-driven metabolic reprogramming may serve as a novel biomarker pathway for nephrolithiasis.

### 3.5 Probiotic and Microbiome-Targeted Interventions

**Table 5. Randomized Controlled Trials and Intervention Studies on Microbiome-Targeted Therapies in Urolithiasis**

| Study                                          | Design                                           | N                              | Intervention                                       | Probiotic Organism                                                                    | Duration   | Primary Outcome                                                                                                     |
|------------------------------------------------|--------------------------------------------------|--------------------------------|----------------------------------------------------|---------------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------------------------------------|
| <b>Cureus SR (2025) – RCT subset</b>           | Systematic review of 5 RCTs                      | 5 RCTs (pooled)                | Oral probiotic capsules/sachets                    | <i>O. formigenes</i> , <i>Lactobacillus</i> spp., <i>Bifidobacterium</i> spp.         | 2–12 weeks | Urinary oxalate excretion (primary); stone recurrence (secondary) — no significant reduction demonstrated           |
| <b>Cureus SR (2025) – Observational subset</b> | 4 observational studies (within SR)              | Variable                       | Dietary modulation; probiotic foods                | Mixed strains ( <i>Lactobacillus</i> , <i>O. formigenes</i> )                         | Variable   | Associations between probiotic use and lower urinary oxalate reported; evidence quality low (no RCT confirmation)   |
| <b>Goldfarb et al. (CJASN 2009)</b>            | Randomized double-blind placebo-controlled trial | N = 68                         | Lactic acid bacteria capsule (Oxadrop) vs. placebo | <i>Lactobacillus acidophilus</i> + <i>L. brevis</i> + <i>Bifidobacterium infantis</i> | 4 weeks    | No significant reduction in urinary oxalate excretion vs. placebo ( $p = 0.18$ ); tolerated without adverse effects |
| <b>Milliner et al. (2018)</b>                  | Phase II/III RCT                                 | N = 39 (primary hyperoxaluria) | Oral <i>O. formigenes</i> (OXBE19) vs. placebo     | <i>Oxalobacter formigenes</i>                                                         | 24 weeks   | No significant reduction in 24h urinary oxalate in primary hyperoxaluria patients; colonization difficult to        |

|                                                  |                        |                     |                                         |                                                                         |                                |                                                                                                                                                              |
|--------------------------------------------------|------------------------|---------------------|-----------------------------------------|-------------------------------------------------------------------------|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                  |                        |                     |                                         |                                                                         |                                | establish in this population                                                                                                                                 |
| <b>Mini SR – Probiotics (Therapeutics, 2024)</b> | Mini systematic review | 38 studies analyzed | Probiotic (LB/BF/O.formigenes) + herbal | O. formigenes, Lactobacillus, Bifidobacterium (min 10 <sup>7</sup> CFU) | 24h–14 days (in vitro/in vivo) | Positive in vitro/animal oxalate degradation; human clinical evidence limited to small studies; evidence supports biological plausibility not clinical proof |

*Abbreviations: SR = systematic review; RCT = randomized controlled trial; CFU = colony-forming units; LB = Lactobacillus; BF = Bifidobacterium.*

Despite the strong biological plausibility of microbiome-targeted interventions for urolithiasis prevention, the clinical trial evidence to date has been disappointing. Both the targeted Cureus systematic review (2025) and the earlier Goldfarb RCT using multi-strain lactic acid bacteria failed to demonstrate statistically significant reductions in urinary oxalate excretion compared to placebo in idiopathic stone formers. The Milliner Phase II/III RCT of oral O. formigenes also did not achieve colonization or urinary oxalate reduction in the challenging population of primary hyperoxaluria. The principal challenges identified across intervention studies include difficulty establishing durable gastrointestinal colonization by exogenous bacterial strains, heterogeneity in probiotic formulations and doses, short intervention durations, small sample sizes, and lack of imaging-confirmed stone recurrence as an outcome. These limitations collectively constrain the strength of conclusions that can be drawn from existing evidence.

### 3.6 Quality Assessment of Included Studies

**Table 6. Methodological Quality Assessment of Included Studies**

| Study                          | Study Design               | Quality Tool      | Selection Bias | Measurement Bias | Confounding Control | Overall Quality           |
|--------------------------------|----------------------------|-------------------|----------------|------------------|---------------------|---------------------------|
| <b>Kachroo et al. (2021)</b>   | Meta-analysis              | PRISMA / AMSTAR-2 | Low            | Low              | Moderate            | High                      |
| <b>Nazzal et al. (2021)</b>    | Prospective clinical trial | RoB 2             | Low            | Low              | Low                 | High                      |
| <b>Lemberger et al. (2023)</b> | Prospective observational  | Newcastle-Ottawa  | Low            | Moderate         | Moderate            | Moderate-High (7/9 stars) |
| <b>Gao et al. (2022)</b>       | Cross-sectional            | Newcastle-Ottawa  | Moderate       | Low              | Moderate            | Moderate (6/9 stars)      |
| <b>Miller et al. (2019)</b>    | Prospective observational  | Newcastle-Ottawa  | Low            | Low              | Moderate            | High (8/9 stars)          |
| <b>Tang et al. (2018)</b>      | Cross-sectional            | Newcastle-Ottawa  | Moderate       | Moderate         | Moderate            | Moderate (6/9 stars)      |
| <b>Cureus SR (2025)</b>        | Systematic review          | AMSTAR-2          | Low            | Moderate         | Moderate            | Moderate                  |
| <b>Goldfarb et al. (2009)</b>  | RCT                        | RoB 2 (Cochrane)  | Low            | Low              | Low                 | High                      |

|                                      |                             |                        |                      |          |          |                      |
|--------------------------------------|-----------------------------|------------------------|----------------------|----------|----------|----------------------|
| <b>Dysbiotic Study (2025)</b>        | Retrospective observational | Newcastle-Ottawa       | High (retrospective) | Moderate | Moderate | Moderate (6/9 stars) |
| <b>Suarez Arbelaez et al. (2023)</b> | Systematic review           | AMSTAR-2               | Low                  | Moderate | Moderate | Moderate             |
| <b>Xie et al. (2020)</b>             | Cross-sectional             | Newcastle-Ottawa       | Moderate             | Low      | Moderate | Moderate (6/9 stars) |
| <b>Tarasewicz et al. (2022)</b>      | Narrative review            | Descriptive assessment | N/A                  | Moderate | N/A      | Moderate             |

Quality tools used: RoB 2 = Cochrane Risk of Bias Tool version 2 for RCTs; Newcastle-Ottawa Scale (NOS) for observational studies; AMSTAR-2 for systematic reviews.

The overall methodological quality of the included body of evidence was moderate to high. The two included meta-analyses and the prospective clinical trial by Nazzal et al. demonstrated the highest methodological rigor, with low risk of bias across key domains. Cross-sectional observational studies were assigned moderate quality ratings primarily due to potential selection bias in patient recruitment and the inherent inability to establish temporal causality between microbiome composition and stone formation. The retrospective study on dysbiotic gut microbiota was limited by selection bias inherent to its retrospective design and by the use of 16S rRNA sequencing, which lacks the resolution to detect functional oxalate-degrading genes. The overall body of evidence was rated as moderate certainty by GRADE criteria, with the primary limitation being the heterogeneity in microbiome characterization methods and outcome reporting across studies.

#### 4. DISCUSSION

This systematic review synthesized evidence from 12 studies representing diverse methodological approaches and geographic settings to elucidate the complex interactions between gut and urinary microbiota, oxalate metabolism, and urinary stone disease. The convergent findings across studies support a central role for microbial dysbiosis in the pathogenesis and recurrence of calcium oxalate nephrolithiasis, extending the traditional understanding of stone disease beyond dietary and metabolic determinants to encompass the dynamic influence of the human microbiome. These findings align with the emerging paradigm of the gut–kidney axis, in which intestinal microbial composition profoundly influences renal oxalate handling and, consequently, stone-forming risk.<sup>[1,4]</sup>

The depletion of *Oxalobacter formigenes* from the gut microbiota emerged as one of the most consistent and clinically significant findings across studies. As the only known obligate oxalotrophic bacterium in the human gut, *O. formigenes* uniquely couples luminal oxalate degradation with active transcellular secretion of oxalate from blood into the intestinal lumen through a proton-coupled transport mechanism.<sup>[6,7]</sup> The demonstration by Nazzal et al. that a single antibiotic course administered for *Helicobacter pylori* eradication substantially depleted *O. formigenes* colonization and increased urinary oxalate excretion has profound clinical implications, particularly in the context of the widespread use of antibiotics in urological and general medical practice.<sup>[11]</sup>

The meta-analysis by Kachroo et al., representing the most comprehensive synthesis of microbiome data in urolithiasis to date, provided critical insights into the determinants of microbiome composition in stone disease. The identification of age, stone composition, geographic location, and disease status as the principal factors modulating urolithiasis-associated microbiome composition suggests that stone disease is not associated with a single universal dysbiotic signature but rather with context-dependent microbiome alterations.<sup>[13]</sup> This heterogeneity may partly explain the inconsistent findings across individual studies and underscores the need for stratified analyses in future research, particularly with respect to stone composition—a major determinant of the pathophysiological pathway leading to crystallization and a likely driver of differential microbiome selection pressure.

Miller et al. introduced the concept of a multi-species bacterial network maintaining oxalate homeostasis, challenging the simplistic notion that *O. formigenes* alone determines oxalate balance.<sup>[9]</sup> The demonstration that urinary *Lactobacillus* was enriched in healthy individuals while *Enterobacteriaceae* predominated in stone patients across both gut and urinary compartments points to a coherent microbiome-wide shift associated with lithogenic risk. This network perspective has important therapeutic implications: if oxalate degradation is distributed across multiple taxa, then the loss of any single organism may be compensated by others, explaining why probiotic trials targeting single strains have generally failed to achieve significant oxalate reduction.

The characterization of the urinary microbiome—the urobiome—as a non-sterile microbial ecosystem has fundamentally shifted understanding of how bacteria interact with stone-forming processes within the collecting system.<sup>[14,15]</sup> The finding of Lemberger et al. that bacteria were detected in 24% of surgically removed kidney stones, with stone-colonized patients experiencing significantly prolonged hospital stays (2.9 vs. 1.5 days), highlights the clinical relevance of the stone microbiome beyond microbiome–host physiology interactions to

include direct clinical outcomes such as post-procedural morbidity.<sup>[10]</sup> The enrichment of gastrointestinal bacteria within stones of patients with metabolic syndrome further supports the hypothesis that gut-to-urinary bacterial translocation—facilitated by impaired intestinal barrier function in metabolic syndrome—contributes to stone nidation and complexity.

The urinary metabolomic-metagenomics integrated approach employed by Gao et al. provided a uniquely powerful lens through which to examine stone pathogenesis, demonstrating that the microbial-metabolite correlation network is fundamentally restructured in stone patients compared to controls.<sup>[15]</sup> Specifically, the disruption of oxalate derivative metabolic pathways in the urine of stone formers implicates urinary microbiota-driven metabolic reprogramming as a pathomechanistic contributor to stone formation rather than simply a bystander effect of altered urinary flow dynamics. These findings suggest that integrated multi-omics approaches combining microbiome profiling with metabolomics may yield superior diagnostic and prognostic biomarkers for nephrolithiasis compared to microbiome analysis alone.

The retrospective study of gut dysbiosis in recurrent calcium oxalate stone patients revealed additional mechanistic insights linking microbial imbalance to metabolic derangements relevant to stone formation. The enrichment of *Fusobacterium* in recurrent stone formers correlated with elevated serum triglycerides and creatinine, while depletion of short-chain fatty acid (SCFA)-producing organisms—*Faecalibacterium* and *Lachnospira*—was associated with dyslipidemia and reduced urinary magnesium and citrate excretion.<sup>[17]</sup> These correlations suggest that gut microbiota influences stone formation not only through direct oxalate degradation but also through broader metabolic pathways including lipid regulation, intestinal barrier maintenance, and mineral absorption—pathways mediated largely by SCFA production from dietary fiber fermentation.

The therapeutic landscape for microbiome-targeted stone prevention remains largely unproven despite its biological plausibility. The comprehensive systematic review by Cureus (2025) evaluating nine studies of probiotic and synbiotic interventions in urolithiasis concluded that current evidence does not support the clinical use of these agents for reducing urinary oxalate or preventing stone recurrence.<sup>[18]</sup> This conclusion aligns with earlier findings from the Goldfarb RCT and the Milliner Phase II/III trial of oral *O. formigenes*. The principal barriers to probiotic efficacy appear to be the difficulty in achieving durable gastrointestinal colonization by exogenous bacteria in individuals already harboring established microbial communities, the short duration of most intervention trials, and inadequate strain characterization with respect to colonization capacity and oxalate-degrading enzyme expression.

An important confounding variable across microbiome-urolithiasis studies is antibiotic exposure, which Kachroo et al. identified as a significant modulator of both alpha- and beta-diversity in stone patients.<sup>[13]</sup> Given that urinary tract infections are common among stone formers, antibiotic-induced perturbations of the gut and urinary microbiome may amplify the dysbiotic signature attributable to stone disease itself, creating a bidirectional relationship in which stones promote infections requiring antibiotic treatment, which in turn worsens dysbiosis and increases stone recurrence. Disentangling these reciprocal interactions requires carefully designed prospective studies with rigorous antibiotic exposure documentation and ideally pre-antibiotic baseline microbiome profiling.

The role of metabolic comorbidities—particularly metabolic syndrome and type 2 diabetes—in shaping the stone-associated microbiome deserves attention. The study of calcium oxalate stone patients with comorbid metabolic syndrome demonstrated significantly lower alpha-diversity and marked depletion of SCFA-producing probiotics in patients harboring both conditions compared to either condition alone, suggesting a synergistic dysbiotic effect.<sup>[18]</sup> Given the rising global prevalence of metabolic syndrome and its established association with uric acid and calcium oxalate nephrolithiasis, future microbiome research should systematically characterize the metabolic phenotype of stone patients alongside microbiome data to enable more precise stratification of lithogenic risk.

The methodological landscape of microbiome research in urolithiasis is rapidly evolving, with 16S rRNA sequencing transitioning toward shotgun metagenomics and, increasingly, metatranscriptomics and metabolomics.<sup>[14]</sup> The limitation of 16S rRNA-based approaches—namely, their inability to resolve functional oxalate-degrading genes such as *oxc* and *frc*—was explicitly acknowledged in the dysbiotic gut microbiota study, which could not detect these genes despite clinical evidence of altered oxalate handling. As metagenomics becomes more accessible and cost-effective, future studies should prioritize functional metagenomic profiling to directly quantify the oxalate-degrading gene repertoire of the stone-former gut microbiome rather than relying on taxonomic surrogates.

The geographic heterogeneity in microbiome composition across included studies—with studies from China, North America, and Europe reporting overlapping but not identical dysbiotic patterns—reflects the substantial influence of diet, host genetics, geographic environment, and lifestyle factors on baseline microbiome composition. Diet, in particular, is a powerful confounding variable in urolithiasis microbiome research: oxalate intake varies substantially across dietary patterns (Western vs. plant-based vs. Mediterranean diets), and dietary fiber content directly determines the substrate availability for SCFA-producing bacteria and indirectly influences *O. formigenes* colonization density.<sup>[4,5]</sup> Standardization of dietary assessment and control for dietary confounding is a critical unmet need in this field, as highlighted by the international consensus agreement on microbiome standardization in urolithiasis research published in Nature Reviews Urology.

Looking forward, several innovative therapeutic strategies merit investigation for their potential to restore a protective microbiome in stone formers. Fecal microbiota transplantation (FMT) from stone-free donors with demonstrated high *O. formigenes* colonization and oxalate-degrading capacity represents a potentially powerful but logistically complex approach to reconstituting the oxalate-protective microbiome in susceptible individuals. Dietary prebiotic supplementation to nourish endogenous oxalate-degrading taxa, precision synbiotics combining oxalate-degrading bacteria with specific prebiotic substrates, and engineered bacterial consortia expressing high-level oxalate-degrading enzyme activity are among the biotechnological approaches currently at early-stage development. The successful development of any such approach will require standardized, long-term randomized trials with imaging-confirmed stone recurrence as the primary endpoint and comprehensive microbiome profiling to establish colonization and persistence of the therapeutic strain.

The present systematic review carries several limitations that must be acknowledged. The substantial heterogeneity in study designs, populations, microbiome characterization methods, sequencing platforms, and outcome reporting precluded formal meta-analytic pooling of quantitative data, limiting the synthesis to a narrative approach. Many included studies were cross-sectional in design, precluding causal inference regarding the temporal relationship between dysbiosis and stone formation. Publication bias may have disproportionately represented studies with positive microbiome–stone associations. Additionally, the small sample sizes of several individual studies reduce statistical power and limit generalizability. Future research should prioritize large-scale, multicenter, prospective cohort studies with standardized microbiome methodologies, pre-specified outcomes, and long-term follow-up to generate the high-quality evidence base required to translate microbiome insights into clinical practice.

In summary, this systematic review provides robust evidence that both gut and urinary microbiota are intimately involved in the pathogenesis and recurrence of calcium oxalate nephrolithiasis through modulation of intestinal and urinary oxalate homeostasis, mucosal barrier integrity, metabolic regulation, and crystal–bacteria interactions within the collecting system.<sup>[1,6,9,13]</sup> The consistent identification of a dysbiotic gut microbiome characterized by *O. formigenes* depletion and *Enterobacteriaceae* enrichment in stone formers across diverse populations represents a reproducible biological signal with strong pathomechanistic grounding. While microbiome-targeted therapeutic interventions have not yet demonstrated definitive clinical efficacy, the biological rationale is compelling and the field is rapidly advancing. Concerted efforts to standardize microbiome research methodology in urolithiasis, to conduct well-powered prospective studies, and to develop novel microbiome-modulating strategies tailored to the specific dysbiotic signatures of stone formers will be essential to translate these scientific insights into meaningful clinical advances in stone disease prevention.

#### 4.1 CONCLUSION

This systematic review demonstrates that both the gut and urinary microbiota are integral components of the pathophysiology of calcium oxalate nephrolithiasis, operating through complementary mechanisms of oxalate degradation, metabolic regulation, and crystal–microbe interactions within the urinary collecting system. Consistent gut dysbiosis characterized by depletion of *Oxalobacter formigenes*, *Lactobacillus*, *Faecalibacterium*, and *Prevotella* alongside enrichment of *Enterobacteriaceae* was reproduced across geographically diverse stone former cohorts, establishing dysbiosis as a generalizable feature of urolithiasis rather than a population-specific finding. Urinary microbiome data further demonstrate pathology-specific compositional shifts that are distinct from gut microbiome alterations, suggesting that both compartments contribute independently to stone-forming risk. Microbiome-targeted therapeutic interventions remain biologically plausible but clinically unproven, requiring larger, methodologically rigorous randomized trials with long-term follow-up and imaging-confirmed stone recurrence endpoints. As microbiome science continues to mature, integration of functional metagenomics, metabolomics, and precision therapeutic strategies into urological practice holds the potential to transform the prevention and management of this highly prevalent and recurrent disease.

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