A Perspective on the Metabolic Potential for Microbial Contributions to Urolithiasis

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Introduction
The prevalence of urolithiasis has been steadily rising with an increasingly earlier onset of the disorder (1). Current knowledge about the pathogenesis of urolithiasis is scarce, and clinical strategies to correct urine chemistry have only been partially effective in preventing stone recurrence (2). Kidney stones are complex structures composed of a mineralized scaffolding within a biologic matrix that contains proteins, metabolites, sugars, and cellular components, such as lipids and extracellular vesicles (3). There are numerous potential sources of lithogenic compounds that include the diet, host metabolism, and microbial metabolism. The microbial contribution to these compounds is perhaps the most variable given the immense diversity and interindividual variability of microbes inhabiting hosts (microbiome) and the plasticity of host-associated microbial communities over time (4). Although studies focused on the role of single bacteria species in the development of urolithiasis have produced conflicting data in animal and clinical trials (5), the advance of modern, multiomics technologies has renewed the interest in the contribution of the microbiome for the onset of this condition. Recent analyses have found significant associations between antibiotic use and the onset of urolithiasis (6–8) as well as between urolithiasis and microbiome composition (9), which suggest that the microbiome influences the onset of urolithiasis. The microbiome affects a number of factors important for health, which include epithelial barrier integrity, adaptive immune responses, and toxin metabolism among others (10). Disruption of these microbial services through antibiotic use, diet, or other factors can lead to chronic inflammation, recurrent infections, autoimmune disease, or other disorders (11). Given recent advances in microbiome research for urolithiasis, it is imperative to understand the ways in which the microbiome can contribute to this disorder, which will aid in designing future mechanistic studies. Thus, in this perspective, we discuss the potential factors that link the microbiome to the onset of urolithiasis using a few examples to contextualize these interactions, and we conclude with the most promising areas of future research into the microbiome and urolithiasis.

Categories of Microbial Involvement in the Onset of Stone Formation
Any microbial contribution to urolithiasis can be defined by three distinct categories and collectively frame the metabolic potential of the microbiome for lithogenesis. To illustrate the three categories of interactions, we will focus on two diurnally opposing examples: that of Proteus mirabilis in the urinary tract associated with struvite and apatite stones and Oxalobacter formigenes in the gut negatively associated with calcium oxalate (CaOx) stones.

First, microbes can exert either a prolithogenic or antilithogenic influence on stone formation. Infection by P. mirabilis is a well-known prolithogenic interaction that causes the formation of struvite and apatite stones (12). Oxalate metabolism by O. formigenes, which eliminates some fraction of intestinal oxalate prior to absorption (13), is an example of an antilithogenic interaction. However, the relationship between O. formigenes and urolithiasis is currently inconclusive (14).

The second aspect that needs to be considered is that the prolithogenic or antilithogenic effects of the microbiome can be exerted in an adjacent location to lithogenesis or at a location physiologically upstream of lithogenesis, such as in the gut. In our example above, the urease-producing P. mirabilis in the urinary tract is exerting an adjacent influence over stone formation given its location relative to the site of formation. Conversely, the oxalate-degrading O. formigenes in the gut has an influence upstream of where stones form.

Finally, the microbiome can contribute to stone formation through direct or indirect contributions. A direct microbial contribution to the onset of stones is one in which the microbes in question modify the stone-forming elements directly. Such is the case for the oxalate-degrading O. formigenes that reduces levels of oxalate in the body. An indirect microbial contribution is one in which the microbes in question affect the host or microbial environment rather than the stone-forming elements. The urease-producing P. mirabilis is an example of an indirect contribution. Urease hydrolyzes urea into ammonia and carbon dioxide, increasing the local pH within the urinary tract, and changes the thermodynamics of phosphate crystal growth (15). This change in host environment, rather

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than direct microbial by-products, favors the precipitation of calcium and magnesium ions to form struvite and apatite stones (15). However, the ammonia produced by these reactions is also incorporated into the resulting stone, which is a direct contribution (16). Additionally, permutations from each of the three categories can be found in the literature as referenced (Table 1).

**Other Factors That Influence the Microbial Contribution to Urolithiasis**

The total metabolic potential of the human microbiome for stone formation is controlled by several key factors, driven fundamentally by the diversity of the microbiome (4). With increasing genetic diversity comes an increase in the number of ways that microbes can interact with their host environment to produce pro- or antilithogenic effects. The density and diversity of the human microbiome are highest in the distal colon and provide a strong potential for influence over host physiology overall (17). In contrast, the microbiome of the lower urinary tract harbors a density about seven orders of magnitude lower than the distal colon, with about 1/20th of the overall diversity (18). Recent studies consistently detect bacteria in kidney stones through both culture-based and molecular means (6), which suggests that a resident microbiome must also be present in the upper urinary tract and contribute to lithogenesis. In fact, recent biogeochemical studies on kidney stones have revealed active sites of dissolution and recalcification in stones, indicative of biofilm activity (19–21). Additionally, in vitro studies have shown that the flagella from strains of *Escherichia coli* in the urinary tract promote CaOx crystallization (22). These data suggest a complex, direct involvement of kidney bacteria in lithogenesis. However, no studies have directly looked for the presence of a resident kidney microbiome. Thus, knowledge about how the upper urinary tract microbiome influences host physiology, whether pertaining to urolithiasis or not, is limited.

The fulfillment of the microbial metabolic potential is driven in large part by the diet consumed by the host, which provides much of the substrates required for growth by the microbes and can thus either promote or inhibit stone formation. *O. formigenes*, for instance, increases in abundance when oxalate is supplemented in the diet (23). In contrast, a high-fat, high-sugar diet elicits an acute loss of bacterial oxalate metabolism in animal models (24). An exhaustive review of nutritional factors that can modify lithogenesis through the microbiome has recently been published (25). In addition to the effect of diet on the microbial metabolic potential, certain pharmaceuticals can also alter the microbiome in ways that may influence urolithiasis. For example, three independent studies have linked antibiotic use to urolithiasis and the microbiome (6–8). However, other medications, such as diuretics, antacids, immunosuppressives, and others, may also affect the microbiome in ways that influence urolithiasis. Moreover, the specific metabolites that link the microbiome to urolithiasis can be quantified through metabolomic techniques as have been demonstrated previously (6,26).

Along with the microbial metabolic potential and the diet, the host also influences potential microbial contributions to urolithiasis in key ways. First, the host’s immune system and the microbiome normally interact to produce a stable homeostasis, which helps to control the composition and biomass of the microbiome overall and thus, the type of by-products produced by the microbiome (27). Additionally, the host harbors a number of barriers between the gut and the urinary tract where stones form, thus limiting the potential pro- or antilithogenic compounds produced or modified by gut microbes from getting to the site of stone formation. For instance, the intestinal epithelium prevents compounds, such as insoluble CaOx crystals, from entering the bloodstream (28). The integrity of the intestinal epithelial barrier can also be modified (improved or reduced) by the gut microbiome (29). After a pro- or antilithogenic compound is in circulation, it can accumulate in other tissues before reaching the kidneys. As an example, oxalate, which is the most frequent element of urinary stones, can also be integrated into coronary calcifications in patients receiving dialysis (30). Finally, many pro- or antilithogenic compounds may be filtered out in the glomeruli before reaching the

| Category 1 | Category 2 | Category 3 | Example | Reference |
|------------|------------|------------|---------|-----------|
| Prolithogenic | Upstream | Direct | Microbial production of lithogenic compounds in the gut | NA |
| Prolithogenic | Adjacent | Direct | Flagella of nonurease *Escherichia coli* promote calcium oxalate crystal growth | 22 |
| Antilithogenic | Adjacent | Direct | *L. crispatus* colonization of urinary tract is negatively associated with USD* and has reduced recurrent urinary tract infections | 6 |
| Antilithogenic | Upstream | Direct | Oxalate degradation in the gut | 13 |
| Prolithogenic | Adjacent | Indirect | Urease production in the urinary tract | 15 |
| Antilithogenic | Adjacent | Indirect | *L. crispatus* colonization of urinary tract* or Lactobacillus casei* outcompeting inflammatory pathogens in the urinary tract | 6 |
| Antilithogenic | Upstream | Indirect | Gut bacteria produce butyrate, which decreases the permeability of the intestinal epithelium | 29 |
| Prolithogenic | Upstream | Indirect | Intestinal pathogens causing intestinal barrier disruption | 29 |
| Prolithogenic | Adjacent | Direct | Biofilm formation causing biomineralization | 19–21 |

USD, urinary stone disease; NA, not available.

*The roles of Lactobacillus crispatus and urolithiasis are associative, and no mechanistic studies have been done to determine direct or indirect contribution to stone formation.*
tubules where kidney stones often originate (31). For instance, reduced GFRs can lead to elevated plasma levels of oxalate (32). Thus, the host modulates the pro- and antilithogenic activities of the microbiome by shaping the overall composition of the microbiome and through the accumulation or excretion of compounds produced or modified by the microbiome before they reach the kidneys. The potential ways in which the microbiome can influence the onset of urolithiasis are summarized in Figure 1.

Future Directions for the Microbial Contributions to the Onset of Urolithiasis

The two most likely sites of microbial activity that promote or inhibit stone formation are in the gastrointestinal and urinary tracts. The gut microbiome harbors far greater microbial density and diversity than the urinary tract and is directly modified by the diet. However, the metabolic output of the gut microbiome must go through several barriers prior to reaching the kidneys. In contrast, the urinary tract microbiome harbors much less diversity and biomass but resides at or near the site of lithogenesis. Therefore, any pro- or antilithogenic activities produced by the urinary tract microbiome would have a more direct influence on lithogenesis overall. In fact, a comparative analysis suggests that the urinary tract microbiome may influence lithogenesis more than the gut microbiome (6).

Although there is considerable evidence that the microbiome influences urolithiasis, the types of interactions that potentially link the microbiome to urolithiasis are complex. Future mechanistic studies will resolve important details, such as the specific bacteria and microbe-microbe interactions that produce pro- or antilithogenic compounds and the identity of those compounds. Additionally, the effect of bacteria on the gut or kidney epithelial barriers is an important unresolved topic along with the processes of biofilm-induced biomineralization in the kidneys. Finally, how the diet and pharmaceuticals shape the metabolic output of the microbiome in ways that influence lithogenesis will be important to more rationally design dietary or pharmaceutical interventions to prevent recurrent episodes of stone formation.

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Author Contributions

J. Agudelo and A.W. Miller conceptualized the study; A.W. Miller was responsible for funding acquisition and provided supervision; and J. Agudelo and A.W. Miller wrote the original draft and reviewed and edited the manuscript.
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