



The Role of Bacteria in Non-infection Stone Formation

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Introduction

Urinary stone disease (USD) is frequently encountered in United States healthcare with an estimated lifetime prevalence of 10.6% in men and 7.1% in women [1]. The prevalence of urinary stone disease has doubled over the last 15 years, with increases more pronounced in historically less affected groups, such as children and women [1, 2]. Moreover, the risk of recurrence is significant with 39% of first time stone formers having a second episode within 15 years of follow up [3]. The economic burden of USD in the United States is immense, resulting in over 600,000 emergency room visits and \$2 billion in annual expenditures [4].

The role of bacteria in USD has historically been limited to the association between urease-splitting organisms and magnesium-ammonium-phosphate (struvite) stones, as discussed in previous chapters. However, infection stones make up only 4% of stones with calcium-based stones (calcium oxalate (CaOx) and calcium

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phosphate (CaPhos)) constituting the majority. Other more common stones include uric acid and mixed composition stones [5]. The role of bacteria in non-infection stone disease has not been well defined, but mounting evidence indicates that bacteria may play an important role.

Evidence of Bacteria in Non-infection Urinary Stone Disease

Urinary Tract Infection and Urinary Stone Disease

The concurrent presentation of urinary tract infections with USD for both obstructing and non-obstructing stones is a common clinical occurrence. In a cohort of 1325 Scandinavian patients with USD, 28% had a positive standard urine culture. Of the 535 patients with calculi available for analysis, 31% had a positive standard urine culture at the time of presentation, regardless of stone composition [6]. This association was true in Japanese patients as well; 7% of stone forming patients had a positive standard urine culture within 1 month of surgical intervention [7]. In the pediatric population of Taiwan with newly diagnosed USD, the most commonly associated condition was a history of urinary tract infection at 34.1% (23.5% of males, 43.9% of females) [8].

Outside of clinical presentation and association, many patients with recurrent UTIs have resolution of bacteriuria on standard urine culture after stone removal. In an analysis of 120 patients with recurrent UTI and asymptomatic, non-obstructing renal calculi, Omar and colleagues found that 48% of patients were infection free at an average follow up of 14 months. The majority of stone compositions were calcium-based, while only 6 (5%) patients had struvite stones [9]. Oliver and colleagues looked at a similar cohort of 103 patients with positive preoperative standard urine culture (79%) or recurrent UTIs with negative preoperative standard urine culture (21%). Following ureteroscopy, 70.7% of patients were infection-free at 12 month with most stones (74%) being composed of calcium oxalate. Moreover, 80% of patients with stone recurrence also had recurrent infections, suggesting an association between the recurrent infection and the recurrent stone [10].

Infectious complications are a known hazard of USD management for all stone compositions. The American Urological Association and European Association of Urology recommend the routine use of preoperative standard urine culture and prophylactic antibiotics prior to any surgical stone manipulation [11, 12]. Despite this practice, sepsis occurs in 4.7% of patients undergoing percutaneous nephrolithotomy (PCNL) [13]. In reviews from the Endourological Society, 8.8% of patients with negative preoperative standard urine culture undergoing PCNL developed fever [14]. Though infectious complications are more commonly associated with infection stones [15], the development of systemic inflammatory response syndrome (SIRS) has been demonstrated in up to 5.3% of patients with non-infection stones [16]. Similarly, Rivera and colleagues reviewed their experience in 227 patients undergoing PCNL for management of USD; infectious complications (UTI/

SIRS/Fever/Sepsis) occurred in 37 patients (16%). Overall, 73% of patients experiencing infectious complications had non-struvite stone composition [17].

Bacteria Can Be Cultured from Non-infection Stones

In addition to the association with UTI and infectious complications, multiple studies have demonstrated the ability to culture bacteria from urinary stones (Table 16.1) [18–25]. Depending on the study, bacteria have been isolated from urinary stones in 7% to 75% of stone cultures. When limiting to non-infection stones, positive culture results have been obtained in 5–33% of stones. Pure calcium stones (CaOx or CaPhos) are culture positive in up to 44% of cases. Moreover, these stone cultures contain bacterial isolates of both non-urease splitting and urease splitting organisms. Figure 16.1 [18, 21, 26, 27] demonstrates the frequency of primary isolates from 455 positive urinary stone cultures. Commonly implicated urease splitting bacterial genera include *Staphylococcus*, *Proteus*, *Klebsiella*, *Pseudomonas*, and *Providencia*. However, non-urease splitting bacteria also have been isolated, including known uropathogens *Escherichia coli* and *Enterococcus spp.*

Enhanced Culture Techniques and 16S rRNA Sequencing of Urinary Stones

The previous studies relied on culture protocols similar to techniques popularized by Stamey and colleagues in the 1970s [25]. These culture protocols involve washing the stones in saline and crushing, prior to plating on standard culture media. However, these protocols are not designed to isolate the slow growing, fastidious organisms that make up a majority of urinary biomass [28]. The recent use of enhanced culture-based methods, such as enhanced quantitative urine culture (EQUC), and culture-independent methods, such as 16S rRNA gene sequencing, have demonstrated the existence of resident microbes in the urinary bladder (called the urinary microbiome or urobiome) and debunked the historical view that the bladders of women and men are sterile [28–31]. EQUC utilizes increased urine volumes, longer incubation time, multiple media types, and a variety of atmospheric conditions to isolate slower growing bacteria [29]. 16S rRNA sequencing allows for the identification of bacteria that cannot be cultured (e.g., those exposed to antibiotics prior to collection). In initial experiments utilizing 16S rRNA sequencing on five kidney stones from calcium oxalate stone-formers, our group identified members of several bacterial taxa, including *Pseudomonas*, *Gardnerella*, *Lactobacillus*, *Enterobacteriaceae*, *Bradyrhizobium*, *Phyllobacterium*, and *Brucella*. As stones represent a low biomass medium, available in limited quantities, strategies to determine which sequenced bacteria are truly stone associated are ongoing. The incorporation of EQC, a stone-relevant derivative of EQUC, allowed for the isolation of live *Pseudomonas* and *E. coli* strains in two of the stones collected. In each case, this was concordant with the dominant organism

Table 16.1 Percent of urinary stones with positive culture growth, overall and subdivided by stone composition

Study (n = stones analyzed)	Positive stone culture (%)	CaOx (%)	CaPhos (%)	CaOx + CaPhos (%)	Calcium + Uric acid (%)	Uric acid (%)	Non-infection stones (%)	Any infection comp. (%)
Paonessa et al. (n = 776) [18]	38.7	NA	NA	NA	NA	NA	32.7	69.6
Wang et al. (n = 32) [19]	18.8	18.8	NA	NA	NA	NA	18.8	NA
Shafi et al. (n = 45) [20]	22.2	14.3	NA	NA	0.0	0.0	8.8	63.6
Tavichakornrakool et al. (n = 100) [21]	36.0	13.3	0.0	35.3	75.0	35.7	32.9	53.3
Golechha et al. (n = 100) [22]	31.0	16.7	20.0	15.7	NA	NA	16.2	73.1
Gault et al. (n = 258) [23]	7.0	1.2	14.3	NA	NA	5.3	5.4	16.2
Dajani et al. (n = 130) [24]	15.4	12.5	16.0	NA	NA	29.4	15.4	NA
Thompson et al. (n = 86) [25]	76.7	44.0	16.7	NA	NA	0.0	33.3	95.9

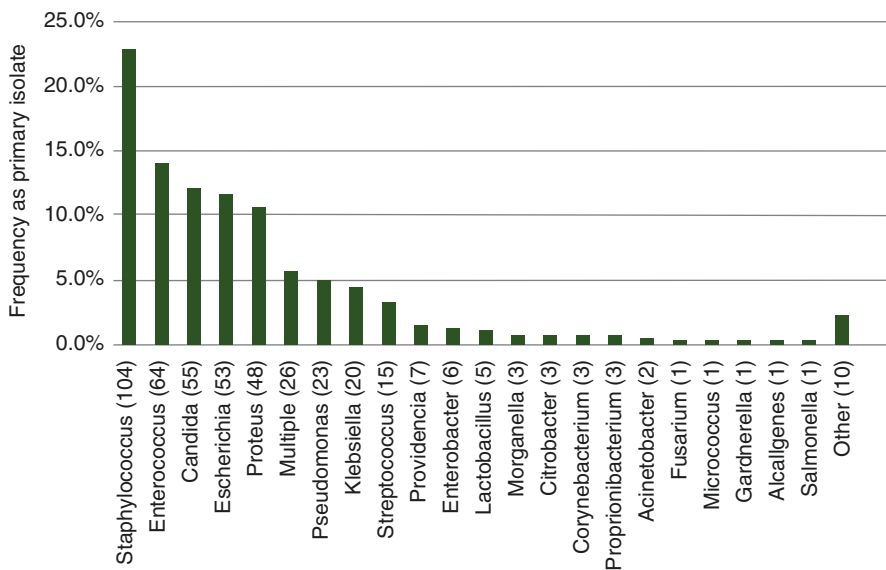


Fig. 16.1 Frequency of bacterial isolates from positive urinary stone culture (Stone samples = 1283; positive culture = 455) [18, 21, 26, 27]

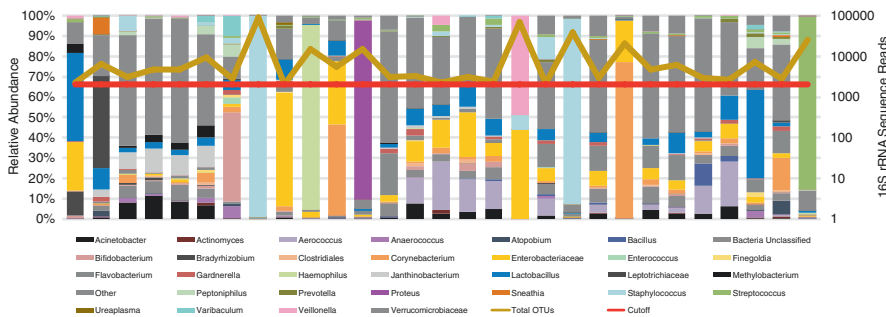


Fig. 16.2 16S rRNA gene sequencing of 29 sequence positive stones obtained via ureteroscopy

identified by 16S sequencing [32]. Analysis of 52 additional kidney stones obtained via ureteroscopy identified 29 (55.7%) sequence positive stones (Fig. 16.2). 16/29 (55%) were composed entirely of non-infection stone compositions, while only one stone contained elements of struvite. Furthermore, EQC was able to isolate bacteria from 11/29 (37.9%) of sequenced stones [33]. These results reflect the idea that live bacteria are associated with non-infection stones, regardless of antibiotic exposure.

Contribution of Bacteria to Urinary Stone Disease

It is now clear that bacteria are associated with stones of all compositions. Patients with USD often have concomitant UTI or infectious complications following intervention, and bacteria are readily sequenced and cultured from stone samples. Though the presence of bacteria in non-infection stones is apparent, it is unclear whether a causal relationship exists.

Supersaturation and Bacteria as a Modifier of Urinary Composition

Supersaturation of urinary solutes has long been recognized as a major pathophysiologic factor in USD. As the concentration of urinary components, mainly calcium and oxalate, reach their limits of solubility, crystallization can occur resulting in stone formation [34, 35]. Historically, the understanding of bacterial contribution to urinary solutes has focused on the intestinal microbiota; specifically, the role of *Oxalobacter formigenes*, which metabolizes dietary oxalate, reducing oxalate concentration of the urine, and providing a protective effect for recurrent CaOx stone formation [36, 37]. The role of *O. formigenes* in CaOx stone formation is discussed in depth in other chapters; notably, however, its role as a probiotic to protect against CaOx stone formation has been of limited success [38–40]. *Oxalobacter* does not commonly inhabit the genitourinary tract. However, other oxalate-degrading bacteria have been identified in the mammalian gut including *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, and *Streptococcus*; microbiota that are more routinely isolated from the genitourinary tract [41]. The role of these bacteria in the urine is not currently understood.

Outside of oxalate-degrading bacteria, the gut microbiome appears to have a complex relationship with urinary solute concentration. Stern and colleagues, investigated the gut microbiome of 11 stone formers and their 24 h urine collection. They found an inverse relationship between *Escherichia* and urinary citrate; as well as an inverse relationship between *Eubacterium* and urinary oxalate [42].

Though the relationship is complex, the gut microbiome may have a protective role in USD formation. However, as previously discussed, urinary bacteria appear to be strongly associated with urinary stone formation. Hypocitraturia is a known risk factor for calcium stone formation. Urinary citrate is a strong inhibitor of stone formation as it binds to free calcium, reducing urinary calcium concentration, making urinary calcium less available to complex with oxalate [34]. Urinary bacteria may contribute to stone formation by metabolizing citrate, lowering urinary citrate concentration, thereby promoting calcium oxalate supersaturation and urinary crystal formation. In a study of idiopathic calcium stone formers, De Ferrari and colleagues noted significantly decreased urinary citrate concentrations in 17 urine culture positive patients compared to standard urine culture negative patients. These standard urine cultures grew *Escherichia*, *Streptococcus*, *Staphylococcus*, *Pseudomonas* and *Citrobacter* species [43]. Moreover, using in vitro urinary models, *E. coli*, both pathogenic and non-pathogenic, decreases urine citrate levels, promoting crystallization [44, 45].

Bacteria as Crystal Aggregator

Bacterial modification of urinary solute concentrations potentiates known risk factors of nephrolithiasis. However, supersaturation of urinary solutes alone does not always result in USD, as there is considerable variation in urine chemistries between stone formers and non-stone formers [46, 47]. As such, bacteria may play a role in crystal adherence, acting as a nidus and promoting crystal deposition in patients with stone solutes. Using an in vivo murine model, our group induced a CaOx nephropathy and uropathogenic *E. coli* (UPEC) pyelonephritis in ten mice. There was a significantly higher number of CaOx deposits in the CaOx and UPEC inoculated mice compared to CaOx or UPEC inoculated mice alone [32]. Similarly, crystals have been shown to aggregate on both Gram-negative and Gram-positive bacteria. Chutipongtanate and colleagues analyzed strains of *E. coli*, *K. pneumoniae*, *S. aureus*, and *S. pneumoniae*, finding increased CaOx crystal growth and aggregation with all four bacteria compared to controls [48].

There also is the possibility that bacteria may play an indirect role in crystal aggregation. In addition to crystals, urinary stones contain a protein matrix that frequently contains innate immune proteins [49]. Urinary stones create an inflammatory response that results in the release of inflammatory proteins and cytokines. This promotes the growth and adhesion of CaOx and uric acid crystals [50–53]. In our study, mice inoculated with CaOx and UPEC had an increased expression of inflammatory and stone matrix protein genes compared to inoculation with either substance alone [32]. Therefore, it is reasonable to believe that the presence of bacteria may work synergistically with CaOx to potentiate stone formation and aggregation. This is similar to findings in vascular calcifications in which bacteria potentiate atherosclerotic plaque formation [54–56].

Conclusion

The role of bacteria in non-infection USD is complex and poorly understood relative to that of infection stones, but the association of bacteria with all stone compositions is undeniable. Patients with non-infection stones present with UTIs, experience infectious complications after stone procedures despite negative standard urine culture, and have positive stone cultures. The use of 16S rRNA sequencing and enhanced culture techniques has expanded our knowledge of stone-associated bacteria and allowed for isolation of these bacteria. The mechanism by which bacteria promote stone formation in non-infection stone formation is an area of active research. Going forward, it is important that we further investigate this association by sequencing and isolating a larger number of stone bacteria from a diverse patient population, evaluating the genomic and proteomic capacities of these bacterial isolates, and determining their effect on stone contribution via in vitro and in vivo models.

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