Bead-Beating Demonstrates Enhanced Bacterial Detection in Kidney Stone Culture Compared to Traditional Mortar-Pestle

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Introduction

Percutaneous nephrolithotomy (PCNL) is a standard surgical treatment for the removal of large (>2cm) kidney stones,[1] yet it continues to be associated with a risk for post-operative sepsis.[2,3] Renal calculi can harbor infectious organisms, which can lead to the development of infectious complications despite negative preoperative urine cultures.[4,5] Increasing evidence suggests that intraoperative kidney urine or stone cultures more accurately identify the causative organisms of postoperative infections following PCNL.[4,6] This is reflected in the recent 2023 update to the European Association of Urology (EAU) Urolithiasis Guidelines which recommend that "urine taken directly from the renal pelvis, or a stone culture be performed at the time of PCNL as they are more predictive of post-PCNL sepsis than pre-operative midstream urine culture."[7]

Despite this guideline recommendation, there remains a lack of a standardized microbiology protocol nor standard policy recommendation for how to best perform clinical stone cultures. Our group previously demonstrated that amongst fellowship-trained endourologists, there is a large variability not only in practice patterns, but also in how stone cultures are performed by microbiology labs at multiple institutions.[8] The American Society for Microbiology (ASM) provided a preliminary protocol in their 2022 edition of their Clinical Microbiology Procedures Handbook, stating to 'grind the stone to a homogeneous consistency using a sterile mortar and pestle (MP)'.[9] Grinding for how long and to what extent to produce a 'homogenous consistency' remains unclear.

Bead beating (BB), a method for tissue/specimen homogenization involving rapid shaking of glass or metal beads in a reinforced vial, is generally more standardized and unbiased than other mechanical grinding methods.[10] While BB is commonly used in microbiological preparation in fields of medicine,[11-13] its application to stone cultures is relatively novel. This study aims to determine whether kidney stone fragmentation using BB versus traditional MP results in differences in microbial identification and quantification from stone cultures.

Methods

<u>Patient and Sample Collection</u>

The University of Arizona is a site for ReSKU (Registry for Stones of the Kidney and Ureter), which is an institutional review board-approved prospectively collected database (#1910099958). ReSKU allows for clinical data to be tracked and combined with bio-banked stone and urine samples.[14]

At our institution a preoperative urine culture is obtained from all patients prior to undergoing PCNL. Patients with a positive urine culture were treated with an appropriate course of culture specific antibiotics. This pre-operative culture directs what IV antibiotic the patient receives at the time of surgery. If this culture showed no growth, patients generally receive a 2nd-generation IV cephalosporin (Cefoxitin) immediately prior to the start of their PCNL.

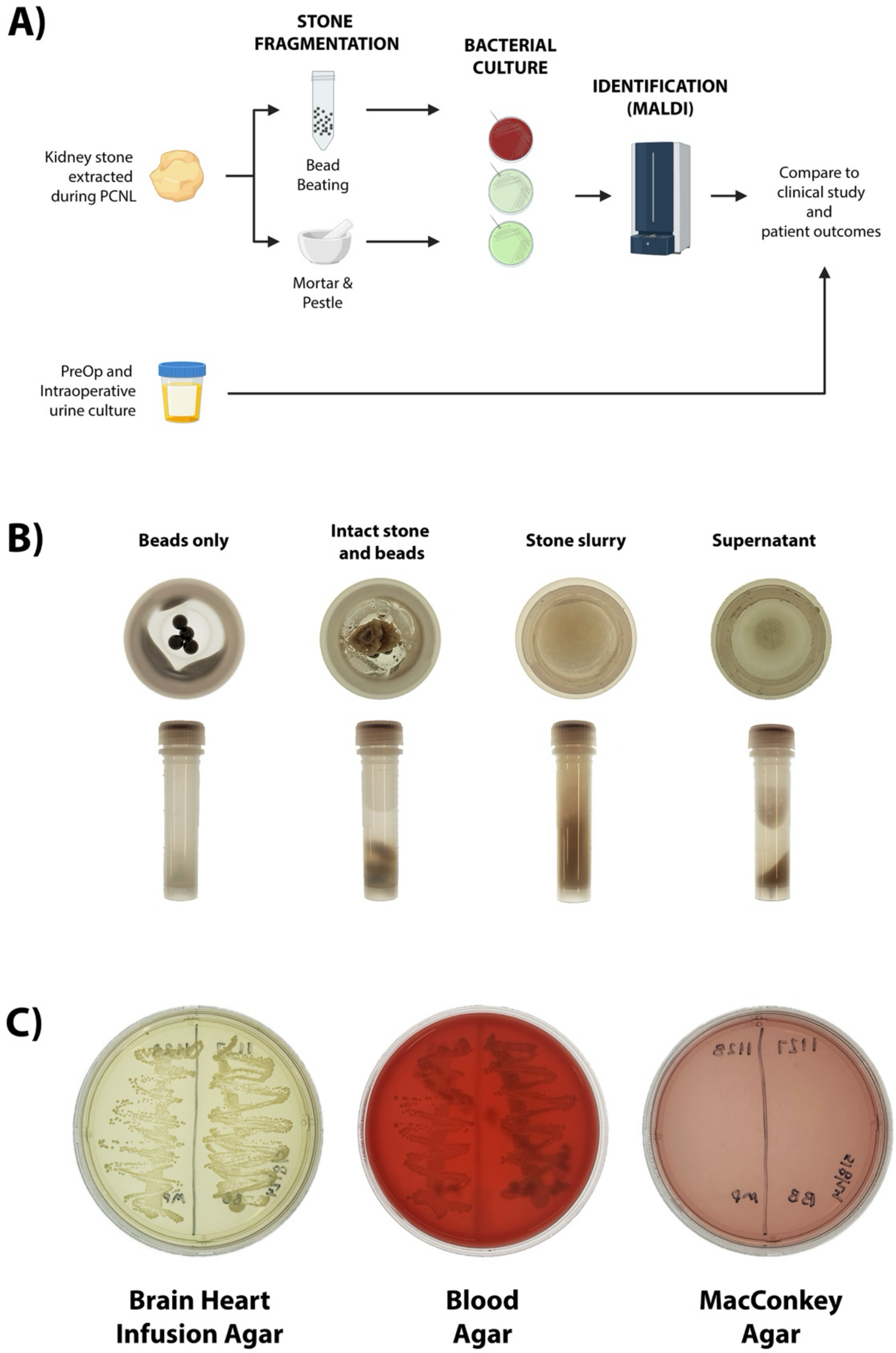
Biospecimens are taken from every PCNL that is performed by a single surgeon (DTT). A 24-French balloon dilator is used, and every effort is made to fragment stones with a lithotrite such that the largest fragments can be removed from a 26-French sheath. Both intact stones and fragments removed at the time of surgery are placed directly into individual vials containing 1 mL of 30% glycerol/PBS solution which are then stored at -80°C. For this study, kidney stone fragments were collected from 60 unique PCNL patients taken from PCNL operations that occurred between August 2023 and August 2024.

Sample Preparation

Fragments from each patient were thawed, weighed, and prepared for bacterial culture. Kidney stone fragments were placed in bead ruptor prefilled bead tubes with 1 mL of 30% glycerol/PBS solution. The samples were homogenized for one cycle at 8000 rpm for 15 seconds with an Omni Internation Bead Ruptor 24 (SKU: 19-070). After the slurry was created, the entire tube was then transferred and chilled on ice for 10 minutes and then spun at 7000 rpm for 1 minute to pellet the kidney stone debris. Similarly for mortar-pestle, samples were thawed, weighed, and ground for 15 seconds. The kidney stone slurry was then transferred to a 1.5 mL centrifuge tube and then spun at 7000 rpm for 1 minute to pellet the kidney stone debris. 5% blood agar and McConkey agar plates were inoculated with 10μL of the supernatant. Additionally, Brain Heart Infusion (BHI) agar plates were inoculated with 10μL of the supernatant to non-selectively promote the growth of organisms. The plates were streaked for qualification and incubated in 5% CO2 at 37°C for up to 72 hrs. Individual colonies were identified with Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry.

Discrepancies in the organisms grown were reported, and appropriate Chi-Square tests were performed using R version 4.1.0 (R Core Team, Vienna, Austria) to determine statistical significance. **Figure 1** provides a schematic of the experimental workflow and examples of both bead-beating and the bacterial culture plating protocol.

Figure 1



A) Schematic of experiment workflow.
B) Example of Bead-Beating tubes containing (from left to right): beads only, beads + stone with glycerol/PBS solution, resultant stone slurry, supernatant
C) Examples of Bacterial Culture using agar plates

Results

The demographic features and stone characteristics of the 60 patients are summarized in **Table 1.**

Table 1 – Summary of Patient and Stone Characteristics

Parameter	Value
Age (Yrs)	
Mean	59.3
Median	63
Age	18 - 83
Sex	
Male	29/60 (60.4%)
Female	31/60 (60.1%)
PreOp Urethral Stent or Nephrostomy Tube	
Stent	17/60
Nephrostomy tube	8/60
Both	2/60
Predominant Composition > 50%	
Calcium Oxalate	29/60 (48.3%)
Carbonate Apatite	16/60 (26.7%)
Struvite	9/60 (15.0%)
Uric Acid	3/60 (5.0%)
Unknown*	2/60 (3.3%)
Cystine	2/60 (2.7%)
Staghorn Calculi	
Staghorn	8/60 (13.3%)
Partial Staghorn	16/60 (26.7%)
Aggregate Linear Stone Burden (mm)	
Mean	53.3
Median	44.5
Range	12 - 195

* After repeated analysis, we were unable to identify the constituents of stone

A total of 60 kidney stones were cultured and 25 (41.7%) harbored microbes (Gram-positive bacteria, Gram-negative bacteria, and yeast) with titers ranging from 100 to >100,000 CFU/mL. The average time to detect growth in stone cultures was 27.4 hours, with a median of 24 hours. No growth was observed beyond 48 hours. The organisms recovered on stone culture, Pre-Op Urine culture, and Intra-Op Kidney Urine culture are depicted in **Figure 2**.

All patients underwent a preoperative urine culture prior to PCNL, with 40/60 patients (66.7%) having a positive culture result. A total of 27/60 (45%) patients had a recent history of either a ureteral stent or a nephrostomy tube placed prior to PCNL. Among these 27 patients with a preoperative stent or nephrostomy tube, 23 (85.2%) had a positive preop urine culture. Additionally, 15 (55.5%) of these 27 patients had a positive stone culture with 8 stones having the same culture results as the preop urine culture.

Figure 2. Comparison of Organisms Identified on Urine vs Stone Cultures

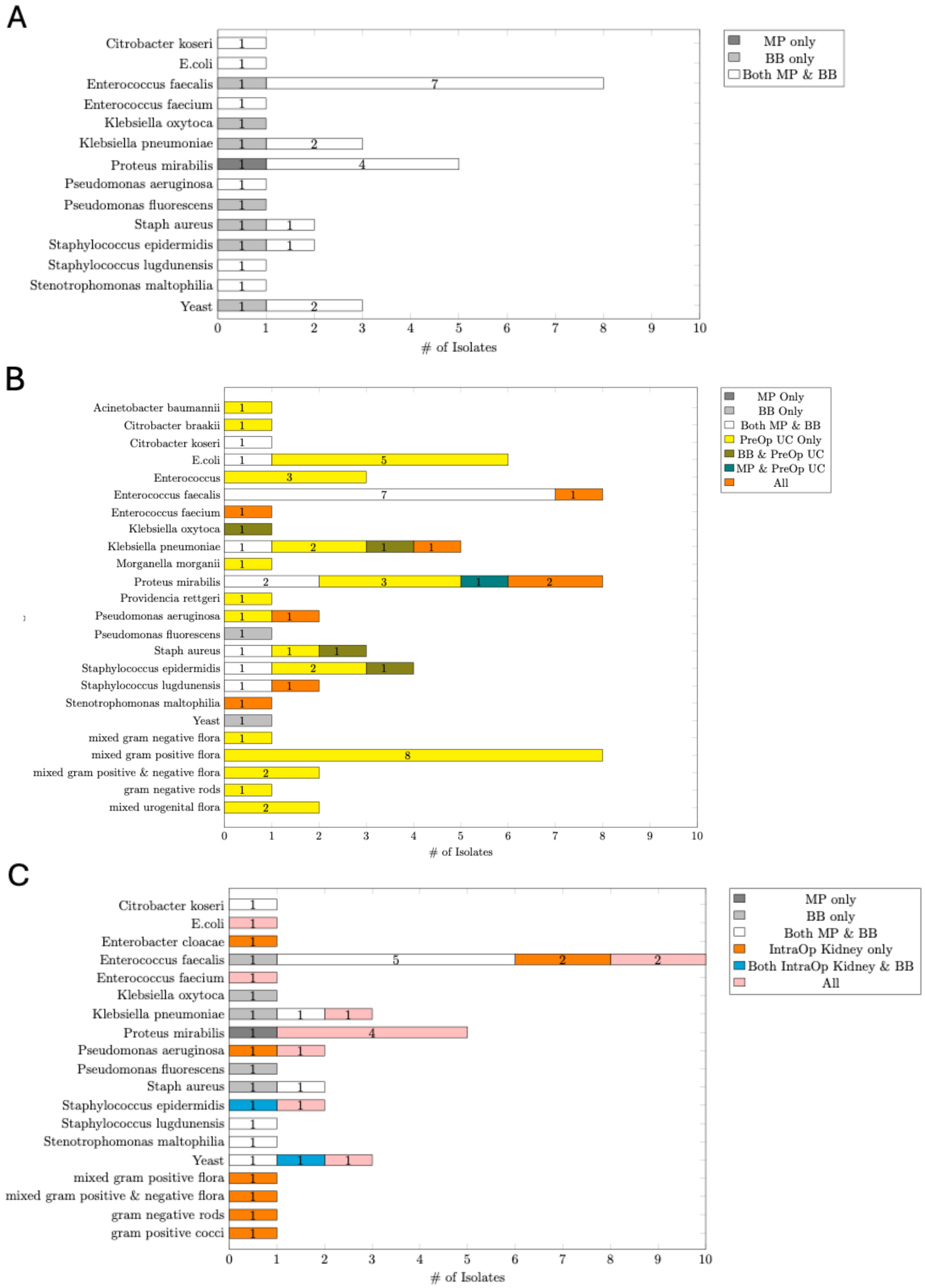


Figure 2A: Comparison of Organisms Identified on Bead-Beating Stone Culture (BB) versus Mortar-Pestle Stone Culture (MP) - of the organisms identified, 1 was exclusive to MP, 7 exclusive to BB, and 23 present on both.
Figure 2B: Comparison of PreOp Urine Culture (UC) versus BB & MP - of the organisms identified, 35 were exclusive to UC, 2 were exclusive to BB, and none were exclusive to MP. Additionally, 15 organisms were present in both BB and MP, 4 were present on BB and UC, 1 organism was present on MP and UC, and 10 were present on all culture methods.
Figure 2C: Comparison IntraOp Kidney Urine Culture (KUC) versus BB & MP - of the organisms identified, 1 was exclusive to MP, 5 exclusive to BB, 8 exclusive to KUC, 11 were on both BB and MP but were absent on KUC, and 12 were present on all culture methods.

All patients underwent a preoperative urine culture prior to PCNL, with 40/60 patients (66.7%) having a positive culture result. A total of 27/60 (45%) patients had a recent history of either a ureteral stent or a nephrostomy tube placed prior to PCNL. Among these 27 patients with a preoperative stent or nephrostomy tube, 23 (85.2%) had a positive preop urine culture. Additionally, 15 (55.5%) of these 27 patients had a positive stone culture with 8 stones having the same culture results as the preop urine culture.

Bead-beating successfully recovered microbial growth from 25 stones, compared to 18 stones with MP ($p = 0.0096$). Both methods identified the same microorganisms in 17 stones. BB demonstrated higher sensitivity for 7 stones, where it exclusively detected organism growth. For one stone, while both BB and MP detected yeast, MP additionally recovered *Proteus mirabilis*.

Meanwhile of the 25 stones that harbored microbes on stone culture, 19 (76%) had received a preoperative course of antibiotics. Of these 25 stones, the same microbe identified on stone culture was also present in the preoperative urine culture in 12 (48%) cases. Moreover, 16 of the 25 stones (64.0%) harboring microbes had compositions traditionally considered infectious (primarily Struvite and Carbonate apatite), while 6 (24.0%) were calcium oxalate stones, and 1(4.0%) was cystine. Unsurprisingly, there was a statically significant association between possessing a predominant infectious composition and a positive stone culture ($p = 0.0011$). Moreover, 7 of the 24 (29.1%) stones with growth were staghorn calculi or partial staghorn.

Discussion:

In this study, we demonstrated that BB provided a significantly higher rate of microbial detection from kidney stone cultures obtained during PCNL compared to the traditional MP method. Specifically, BB was able to recover growth in 25 stones, while MP recovered growth in 18 stones. This suggests that BB offers an improved microbial recovery and superior sensitivity relative to MP which is likely due to enhanced homogenization of stones prior to conducting stone culture. Further study is needed to explore whether BB can be adopted clinically as a preferred method for kidney stone cultures.

The use of a beadmill to homogenize tissue specimens is a well-established technique in microbiology, which has been extensively studied to aid in diagnosing periprosthetic joint infections (PJI). This method has proven to be superior at facilitating bacterial release in PJI compared to other homogenization techniques.[11,13-14] In the context of kidney stone cultures, however, BB is a relatively novel technique. To our knowledge, it has only been applied to assess kidney stone formation in artificial urine inoculated with bacteria.[15]

A significant challenge in kidney stone microbiology is the lack of standardized protocols for stone culture processing.[8] Variability in methodologies—including differences in stone homogenization techniques, culture media used, incubation times, and microbial identification methods—can lead to inconsistent microbial detection rates. This lack of standardization not only hampers the comparison of results across different studies but also affects clinical decision-making based on culture outcomes. The inconsistency underscores the urgent need for developing and adopting standardized, optimized methodologies to improve the reliability and reproducibility of microbial detection from kidney stones.

To our knowledge, this is the first study to compare bead beating (BB) to the traditional mortar-pestle (MP) technique for kidney stone cultures. By providing a direct comparison, we offer evidence that BB is not only more efficient but also potentially more sensitive in detecting microbes from stone cultures. Additionally, the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) allowed for fast and highly specific identification of microorganisms, ensuring accurate results in a clinically relevant timeframe. This approach provided a robust means for microbial recovery and identification, making the findings both applicable and potentially useful for clinical microbiology.

Despite these strengths, our study has some limitations. The sample size of 60 stones may not fully capture the range of microbial diversity encountered in all cases of nephrolithiasis. Additionally, while we demonstrated an advantage of BB over MP in microbial recovery, further studies are needed to assess the clinical implications of these findings. Specifically, future work should evaluate whether BB-detected microbes correlate with clinical outcomes, such as postoperative infections or systemic inflammatory response syndrome (SIRS). Moreover, the impact of antibiotic use prior to surgery may have influenced microbial recovery, as 19 out of 25 patients who had positive stone cultures had already received antibiotics preoperatively. This factor needs to be explored in further studies to better understand the relationship between preoperative antibiotic use and microbial detection.

Conclusions

Bead-beating is a more sensitive stone culture protocol in detecting bacteria within kidney stones when compared to the traditional technique of mortar-pestle. As stone cultures are now recommended in Urology Guidelines, standardizing the methods by which these tests are performed will allow for improved data quality and a better understanding of how specific bacteria impact clinical outcomes.

References:

1) Assimos D, Krambeck A, Miller NL, et al. Surgical Management of Stones: American Urological Association/Endourological Society Guideline, PART II. *J Urol*. 2016;196: 1161-1169 (2016).
2) Whitehurst L, Jones P, Somani BK. Mortality from kidney stone disease (KSD) as reported in the literature over the last two decades: a systematic review. *World J Urol*. 2019;37:759-776.
3) Zhou, G. et al. The influencing factors of infectious complications after percutaneous nephrolithotomy: a systematic review and meta-analysis. Urolithiasis. 2022; 51:17.
4) Mariappan P, Smith G, Bariol SV, et al. Stone and pelvic urine culture and sensitivity are better than bladder urine as predictors of urosepsis following percutaneous nephrolithotomy: a prospective clinical study. *J Urol*. 2005;173(5):1610–4.
5) Charton M, Vallancian G, Veillon B, et al. Urinary tract infection in percutaneous surgery for renal calculi. *J Urol*. 1986;135,15-17.
6) Korets R, Graversen JA, Kates M, et al. Post-percutaneous nephrolithotomy systemic inflammatory response: a prospective analysis of preoperative urine, renal pelvic urine and stone cultures. *J Urol.* 2011;186:1899.
7) Skolarikos A, Hung H, Neisius A, et al. EAU Guidelines on Urolithiasis – Limited Update March 2023.
8) Tzou DT, Stern KL, Duty BD, et al. Heterogeneity in stone culture protocols and endourologist practice patterns: a multi-institutional survey. *Urolithiasis*. 2023;51(1):15
9) Yarbrough ML & Potter RF. *Clinical Microbiology Procedures Handbook*, 4th Ed. ASM Press, Washington DC. 2022. Chapter 3.12.2 – Stone Cultures.
10) Redanz S, Podbielski A, Warnke P. Improved microbiological diagnostic due to utilization of a high-throughput homogenizer for routine tissue processing. *Diagn Microbiol Infect Dis*. 2015;82(3):189-193.
11) A.-L. Roux, V. Sivadon-Tardy, T. Bauer, A. Lortat-Jacob, J.-L. Herrmann, J.-L. Gaillard, M. Rottman, Diagnosis of prosthetic joint infection by beadmill processing of a periprosthetic specimen. *Clinical Microbiology and Infection*, (2011) 17(3):447-450.
12) Drago L, Clerici P, Morelli I, Ashok J, Benzakour T, Bozhkova S, Alizadeh C, del Sel H, Sharma HK, Peel T, et al. The World Association against Infection in Orthopaedics and Trauma (WAIOT) procedures for Microbiological Sampling and Processing for Periprosthetic Joint Infections (PJIs) and other Implant-Related Infections. *Journal of Clinical Medicine*. 2019; 8(7):933. https://doi.org/10.3390/jcm8070933
13) Jazmati N, Liebold C, Offerhaus C, Volkenand A, Grote S, Pöpsel J, Körber-Irrgang B, Hoppe T, Wisplinghoff H. 2024. Rapid high-throughput processing of tissue samples for microbiological diagnosis of periprosthetic joint infections using bead-beating homogenization. J Clin Microbiol 62:e01486-23.
14) Cai, Y., Fang, X., Zhang, L. *et al.* Microbial yield from infectious tissues pretreated by various methods: an invitro study. *BMC Musculoskelet Disord* **22**, 209 (2021).
15) Wallace B, Chmiel JA, Al KF, Bjazevic J, Burton JP, Goldberg HA, Razvi H. The Role of Urinary Modulators in the Development of Infectious Kidney Stones. J Endourol. 2023 Mar;37(3):358-366. doi: 10.1089/end.2022.0458. Epub 2023 Jan 20. PMID: 36562270.