COVER PAGE FOR PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Study Title: Randomized clinical trial using next generation microbial sequencing to direct antibiotic selection before kidney stone lithotripsy using an interprofessional model

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UT Health San Antonio

Antibiotic Selection Using Next Generation Sequencing Versus Urine Culture For Stewardship Prior to Surgery using an Interprofessional Model

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IND Exempt

PROTOCOL 4.0

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ABBREVIATIONS

AE	Adverse Event
DSM	Data Safety Monitoring
DSMB	Data Safety Monitoring Board
DSMC	Data Safety Monitoring Committee
DSMP	Data Safety and Monitoring Plan
DSO	Data and Safety Officer
DQA	Director of Quality Assurance
GCP	Good Clinical Practice
ID	Infectious Disease
IIS	Investigator Initiated Protocol
IRB	Institutional Review Board
NGS	Next Generation Sequencing
OSP	Office of Sponsored Programs (UT HEALTH)
QAD	Quality Assurance Division
PI	Principal Investigator
SAE	Serious Adverse Event
SOC	Standard of Care
UPIRSO	Unanticipated Problem Involving Risks to Subjects or Others
UT Health	University of Texas Health Science Center at San Antonio

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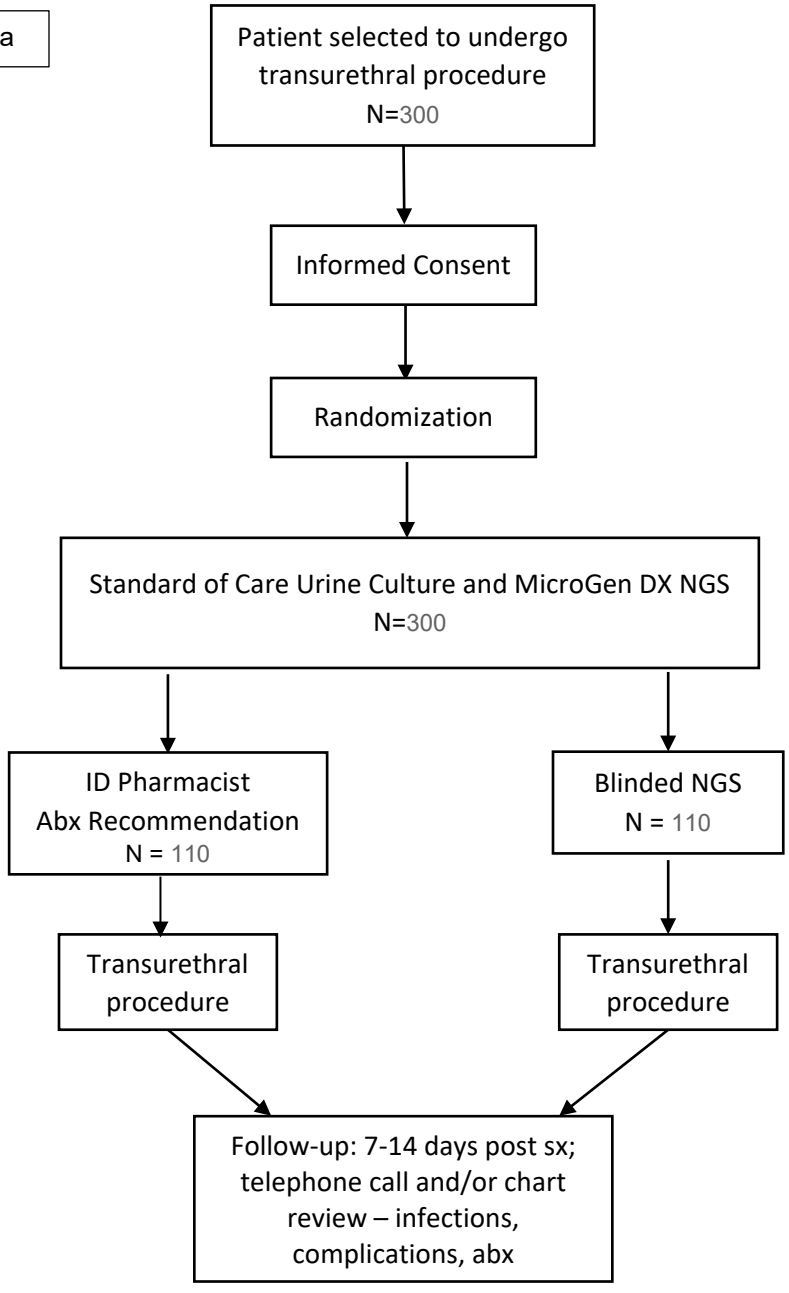
1.0 STUDY SYNOPSIS

This is a randomized controlled clinical study evaluating the use of next-generation sequencing (NGS) to improve antibiotic prescribing before ureteroscopy or percutaneous nephrolithotomy. NGS will be performed on voided urine collected as routine care approximately 30 days prior to surgery. Results will be presented to ID pharmacist within 48-72 hours to help select the most appropriate antibiotics, and independently as part of routine care, surgeons will choose the antibiotic that they would use in each case, while the ID pharmacists would select their optimum choice. Pharmacists will account for site-specific antibiotic stewardship practices, allergies, medication adjustments, and C. difficile rates. Approximately 300 subjects will be randomly assigned in a 1:1 ratio to receive either NGS antibiotic recommendation or standard of care (SOC) prophylaxis prescribed treatment. All providers within the Department of Urology who perform urologic stone procedures (n=5) will also be approached in person with an information sheet. Subjects assigned to standard of care will have urine cultures sent for analysis, and the physician will choose antibiotics based on results as per usual practice. Subjects assigned to the NGS group, in addition to routine urine culture results, will have NGS urine culture results sent to an ID pharmacist, and recommendations will be shared with the physician to determine the antibiotic selection. The physician will ultimately decide the appropriate antibiotics to prescribe. Approximately 7-14 days after surgery, the research staff will conduct a telephone call to ask about any post-operative infections, complications, and any additional antibiotics that were prescribed.

Figure 1: Study Schema



2.0 BACKGROUND AND SIGNIFICANCE

Antibiotic selection before surgery is usually left to the surgeon to make the best selection; however, there is considerable variation noted in antibiotic prophylaxis. The American Urological Association published a Best Practices white paper; however, a large proportion of surgeons continue to not abide by recommendations.[1] Efforts are needed to reduce variation from recommended antimicrobial prophylaxis for common urological procedures to reduce antibiotic adverse events and complications.[2]

The benefit of antimicrobial prophylaxis has been shown in multiple studies; however, some countries do not use antibiotic prophylaxis and have comparable infection rates. Prophylactic antibiotics should be effective against an organism's characteristic of the operative site with excellent safety profiles in short duration. The most common antibiotics utilized in urologic endoscopic surgery are cephalosporins, fluoroquinolones, and aminoglycosides. These antibiotics are usually used in short duration, but recently a rise in antibiotic resistance has led to increased risk of infection. The escalation of one or multiple broad-spectrum could be related to health policy changes indicating that insurance companies would not pay for readmission for infectious complications after surgical procedures. Surgeons are then utilizing more antibiotics or antibiotics that should be reserved for significant infections and not necessarily prophylaxis.

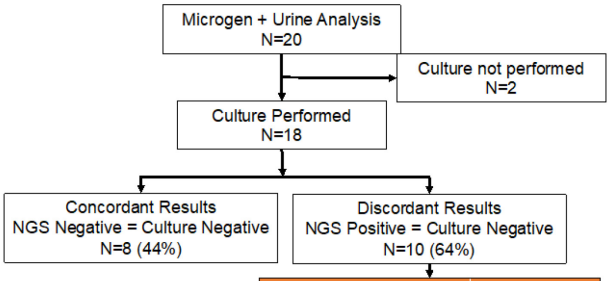
Ureteroscopy for urinary tract urolithiasis is a common procedure with an estimated 9,200 cases in the US per year.[3] However, the incidence of systemic inflammatory response syndrome (SIRS) after flexible ureteroscopic (URS) lithotripsy is 7.1%, despite the use of recommended antibiotic prophylaxis.[4] Obtaining a urine culture prior to surgery is standard of care, and physicians start culture-specific antibiotic treatment on patients with a positive culture— and preferably found to be negative on repeat culture— before their urologic stone procedure. One of the challenges when choosing a prophylactic agent is that preoperative urine cultures often show no growth for patients who later develop SIRS.[5-7] Singh *et al.* found no significant association between pelvic urine cultures or stone cultures and the occurrence or SIRS.[8] We hypothesize the stone or previously placed stent may allow bacteria to form biofilms or not detected by standard cultures.

Next Generation Sequencing (NGS) technology could assist physicians in selecting antibiotic prophylaxis therapy before surgery in patients that have a normal urine culture. Urine culture is a technique that utilizes specialized agar to select the bacterial presence in a fluid. Issues with standard culture are that one must use a priori select particular groups of bacteria and assume they would grow on the plate, which requires the bacteria to be at a certain concentration. NGS testing can use the combination of PCR and 16sRNA to identify the bacterial presence and does not discriminate by growth patterns and are not limited by bacterial counts. NGS can also identify fungal species, which require a separate technique when using standard culture. Our proposal focuses on the identification of high-risk cases and antibiotic prophylaxis patterns and how NGS can assist with antibiotic prophylaxis decision making. Anticipating the use of NGS may have unintended consequences, we address issues with implementation and how to establish an interpersonal healthcare approach to abide by antibiotic stewardship programs.

We performed NGS and PCR for common resistance genes in subjects prior to ureteroscopy. Of the urine being sent for culture, approximately 5-10mL was sent to Microgen Dx for the analysis. NGS results were blinded to the physician, and analysis was done after surgery. Surgery, as scheduled per the physician and prophylaxis antibiotics, were prescribed as the standard of care. After the surgery, we made 3 case studies that were reviewed by board-certified Urologists to determine if they would change their prophylactic antibiotic regimen and what antibiotic they would prescribe. Two infectious disease pharmacists also reviewed the case reports and provided the “ideal” antibiotic regimen to be prescribed.

After IRB approval, we obtained samples from urine specimens already provided as a preoperative urine culture from twenty patients scheduled for urologic stone surgery. Two cultures returned positive with Enterococcus, and the NGS result was the same. One sample had two species (enterococcus and coagulase-negative Staphylococcus), in which NGS was able to find both organisms and speciated the staphylococcus to

be *Staphylococcus epidermidis*. Of the 18 cultures with no growth, 56% (10/18) did show a dominant-bacteria on NGS. Eight urologists returned the three case scenarios, and in each of the 3 cases 100%, 88%, and 88% of the physicians would have prescribed a different prophylaxis antibiotic given these results. The ideal antibiotic of choice based on the ID Pharmacist is oral Bactrim for all cases, and only 50%, 0%, and 0% selected this option, and 38%, 25%, and 63% chose to escalate the antibiotic to vancomycin, Zosyn, or amikacin in conjunction with a second antibiotic. Overall on the exit survey urologists feel it is moderately important (n=2, 25%), important (n=4, 50%), or very important (n=2, 25%) to test this technology in this context and 38% have concerns about using this technology for antibiotic prophylaxis.



Microgen – Primary Detected Bacteria	Number of subjects
Escherichia coli	3
Enterococcus faecalis	2
Streptococcus pneumoniae	1
Staphylococcus hominis	1
Staphylococcus epidermidis	1
Citrobacter freundii	1
Lactobacillus crispatus	1

Figure 2: Preliminary results from NGS compared to standard urine culture prior to urologic surgery.

NGS may be more effective and specific than those predicated upon SOC preoperative urine cultures mostly negative before surgery. For example, we identified several culture negative patients with dominant bacterial types that may influence a physician to prescribe a 1st generation cephalosporin compared to a gram-negative dominant group that may need a fluoroquinolone or 3rd generation cephalosporin. Importantly, urologists do not typically send a urine specimen specifically for fungi (*Candida sp*.). The PCR/NGS test readily makes this information available and in one patient that was positive did have sepsis caused by *Candida*, which was later cultured from the patient's blood. Many studies have reported the low sensitivity of preoperative urine cultures for predicting infectious complications of urologic stone procedures.[5-7, 9] Eswara et al. (2013) compared sensitivities among preoperative and perioperative pelvic urine and stone cultures for pathogen detection, reporting that of the patients who develop urosepsis, 0% had positive midstream preoperative urine cultures, while 73% had positive stone cultures.[10] However, they report a 64% concordance rate in urosepsis patients between stone cultures and readmission cultures, indicating that stone cultures did not always appropriately guide antibiotic selection in up to 36% of cases. Moreover, an antibiotic selection from stone culture rarely is provided in a timely fashion to alter postoperative antibiotics, and many of our patients go home the same day of the procedure. Our study, while small, found that NGS was able to detect pathogens and provide alternate antimicrobial prophylaxis, especially in those who did have an infection.

Our findings are consistent with those of Long et al. (2016) and Grumaz et al. (2016), who reported that NGS is more sensitive than blood cultures for detecting pathogens in ICU patients.[9, 10] Notably, their studies observe the clinical utility of NGS to guide therapy in a high-risk population of ICU patients. Many studies have reported the increased sensitivity of NGS when compared to cultures, but further research is needed to establish cases where the improved sensitivity delivers cost-effective, clinically applicable data.[11, 12] The results of this study suggest that NGS may improve the standard of care in patients undergoing invasive urologic stone procedures.

Infectious complications are the most common cause of death following urologic stone procedures and present a sizeable economic drain on the American healthcare system.[3, 13] Other studies have found that up to 17% of urosepsis cases follow urologic interventions.[14-18] Koras *et al.* (2014) observed signs of SIRS in up to 27% of patients who underwent PCNL, 7.6% of which were diagnosed with sepsis.[14] Given the mortality rates and economic costs of urosepsis, SOC practices must be optimized to reduce the risk of infectious complications following urologic procedures.

NGS identifies a targetable bacterium in up to 50% of negative urine cultures prior to urologic stone surgery. Many physicians chose to escalate IV antibiotics rather than utilized oral medications. Therefore, working with an infectious disease pharmacist may be helpful in guiding the selection and operating room availability of

antibiotics based on NGS results to improve antibiotic stewardship. Larger long-term studies are needed to determine if this strategy will lead to reduced infection rates.

3.0 SPECIFIC AIMS

Specific Aim 1: To perform a randomized clinical trial investigating the use of NGS for antibiotic selection to lower post-operative infection rates over the standard of care.
Hypothesis: utilization of NGS for the selection of preoperative antibiotics reduces postoperative infection rates by 75%. Our baseline rate of infection was 11-15% in our pilot study and nearly 5-10% in our investigation of those ureteroscopies (not PCNL, which has a higher rate) of infections at a university hospital. We expect with the correct antibiotics that the infection rate may drop from 11% to 1% or less. The control arm will be utilized to determine if NGS may have predicted infections or high-risk groups. Retrospective databases include anywhere from 2-15% of patients will present with a postoperative infection after transurethral surgery. Current prophylaxis strategies do not always follow guidelines, and with concerns over current non-reimbursement for infectious complications, surgeons may be using more antibiotics.

Specific Aim 2: Accuracy of NGS and PCR compared to standard urine culture prior to transurethral procedures. *Hypothesis: NGS can identify 50% of negative urine cultures that could harbor uropathogenic bacteria.* We anticipate a substantial number of patients have uropathogenic bacteria present yet are not detected by traditional approaches. We seek to expand our investigation on this topic in order to understand how best to interpret the results and present them to physicians. All participants will have both a standard of care urine culture and NGS testing/ID Pharmacy review for comparative analysis. Only subjects assigned to the NGS group (n=110) will have NGS urine culture results and ID Pharmacist recommendations shared with the physician to determine antibiotic selection. Operating characteristics to include sensitivity, specificity, positive, and negative predictive value will then be performed and area under the receiver operative curve calculated. We will enroll a minimum of 250 patients and 5 urology provider-participants into this study. We anticipate that 20 of the patients will have positive urine cultures and 200 will have negative cultures. Of the 200 negative cultures we anticipate that 100 (50%) will harbor uropathogenic bacteria which will be identified via NGS.

Specific Aim 3: To investigate the influence of Next Generation Sequencing (NGS) on physician prophylactic prescribing habits in patients with a negative preoperative urine culture. *Hypothesis: NGS will cause any antibiotic change in prophylaxis in 50% of cases.* Based on preliminary data, we identified that over 80% of physicians would change their antibiotic for surgery if given this information. However, we anticipate surgeons will use an antibiotic escalation approach compared to a pharmacist's selected optimum prophylaxis antibiotic approach. Therefore, the control arm will receive standard of care antibiotics compared to the intervention group. The intervention group will get three levels of information: (1) standard of care culture, (2) full NGS report, and (3) infectious disease pharmacist recommendations based on NGS. The physician will then utilize these tools to select the best antibiotic for the case.

4.0 RESEARCH PLAN AND DATA COLLECTION

The goal of this proposal is to evaluate if the use of NGS on preoperative antibiotic selection reduces postoperative infection rates over the standard of care. Patients scheduled to receive a transurethral procedure, such as a ureteroscopy or percutaneous nephrolithotomy (PCNL), will be approached for enrollment prior to their procedure. After informed consent has been obtained, approximately 5-10 mL of left-over urine, collected for a standard of care urine culture, will be obtained for research and sent to MicroGen Dx for NGS. Patients will be randomized to receive either standard of care culture results (only), versus NGS results and Infectious Disease (ID) Pharmacist antibiotic recommendation. The physician will utilize these results to select the best antibiotic for the case but is not required to abide by the ID pharmacist's recommendation. The standard of care group will also have a urine sample sent to MicroGen Dx, but the results will not be provided to the physician. All subjects enrolled will received a standard of care culture as part of routine care. A chart review and/or telephone follow up will be completed approximately 7-14 days post urologic procedure to assess for complications, infections related to the procedure, and antibiotics prescribed.

The treating physicians will have control over antibiotic administration. Their determination, interaction, and ultimately patient outcome will be documented and evaluated. As a result, the treating physicians will be considered participants in this study. A waiver of documentation of informed consent for the treating physicians will be included. In addition, an information sheet will be provided to treating physicians specifically.

Subjects will be recruited from approved Urology clinics at time of their preoperative appointment, approximately 30 days, but up to 90 days, prior to the planned transurethral procedure. Patients with ureteral stents will not be excluded; therefore, essentially any patient going to surgery for percutaneous nephrolithotomy, ureteroscopy or other transurethral procedure, within two to four weeks will be screened for eligibility and approached for enrollment. In the event the standard of care urine culture was performed more than 45 days before the urologic procedure; the treating physician will ask the patient to provide an additional urine sample as part of normal practice. Research staff will obtain a second sample (5-10 mL) of left-over urine, which will then be sent to MicroGen Dx for analysis. If this occurs, the second sample will be used for analysis.

Next Generation Sequencing: Approximately 30-50 mL voided whole urine will be collected as per routine care. Approximately 5-10mL of left-over urine will be obtained for research, placed in special vacutainers supplied by MicroGen DX, a CAP accredited and CLIA licensed clinical diagnostic lab, and sent for analysis. MicroGen DX will perform rapid PCR for common resistance genes prior to transurethral procedure, such as ureteroscopy or percutaneous nephrolithotomy. NGS results will be available within 48-72 hours. Additionally, NGS analysis will be performed on available or leftover urinary stones for a proportion of the subjects.

Target bacterial or fungi DNA on the quantitative PCR (qPCR) panel assay on the Roche LightCycler 480 II instrument and sequencing of 16S ribosomal RNA hypervariable regions V1-V2 using Ion Torrent (Ion Torrent PGM) will be run.

Data Collection: Prior to and after surgery, patient medical history, demographics, pre and post-surgical antibiotic treatment plans, and speciation results from the urine culture will be collected by research staff. Results obtained from the NGS/PCR analysis report, such as resistance gene data, will be recorded. Recommendations from the ID pharmacist will also be recorded.

Case Study Creation: ID pharmacist will be provided with the NGS report and relevant de-identified clinical data, such as weight and allergy history, to decide the appropriate antibiotic treatment course for the intervention group. The treating physician will be provided with both NGS report and ID pharmacist recommendations to utilize these results to select the best antibiotic for the case, but is not required to abide by the ID pharmacist's recommendation. Subjects assigned to the standard of care group will have urine cultures sent for analysis, and the treating physician will choose antibiotics based on results as per usual practice. The treating physician will not receive NGS report or ID pharmacist recommendations for standard of care group.

5.0 INCLUSION AND EXCLUSION CRITERIA

Patients:

Eligibility Criteria

5.1 Patients planning to undergo kidney or bladder stone removal surgery using endoscopy including ureteroscopy and percutaneous nephrolithotomy or any other transurethral procedure

5.2 Be age 18 or older

5.3 Be able to give informed consent

Exclusion Criteria

5.4 Unable or unwilling to provide informed consent

5.5 Age <18

5.6 Does not meet inclusion criteria

Physicians:
Eligibility Criteria

5.1 Treating Physician within the Urology Clinic
5.2 Has a patient that is eligible to participate in this research study

6.0 STUDY CALENDAR

Study Visit	Visit 1 Baseline	Visit 2 Urine collection	Visit 3 Transurethral Procedure (day of surgery)	Visit 4 Follow-up
Timeline	Day 1	Up to 90 days from V1	W/in 45 days of urine collection	Approx. 7-14 days post-surgery
Informed Consent	R			
Demographics	R			
Medical History	R	R		R
SOC Urine culture	--------SOC[1, 2]---------			
Whole Urine collection	----------R[1, 2]----------			
Randomization	----------R[1, 2]----------			
NGS Analysis of Urine Culture	----------R[1, 2]----------			
Results of NGS to Physician	----------R[1, 2]----------			
Antibiotic Prophylaxis	--------SOC[1, 2]---------			
Additional Antibiotics as needed	--------SOC[1, 2]---------			
Provider-Participant Questionnaire/Survey	----------R[1, 2]----------			
Urologic Stone Procedure			SOC	
Urologic stone collection			R*	
Chart Review and/or telephone follow up	R[3]	R[3]	R[3]	R[3]
Adverse Events	R	R	R	R

[1] Indicated procedures may be completed at time of baseline or visit 2, up to 90 days from enrollment
[2]Indicated procedures may be repeated if SOC urine culture is not completed within 45 days of procedure
[3]Patient assessments may be completed over the phone
*Any extra or leftover stone(s) available will be collected and sent to MicroGen DX for analysis

7.0 SCREENING AND RECRUITMENT

Patients will be identified and recruited through Urology outpatient clinics located at the Medical Arts and Research Center (MARC), University Hospital System (UHS), and the South Texas Veterans Healthcare System Audie L. Murphy Hospital in the genitourinary (GU) clinic. Eligible patients will be approached at the time of their standard of care visit. The inclusion of subjects, or refusal to participate in this study, will not affect the health care they will receive.

Once a subject is identified who may meet eligibility criteria, the provider and/or approved research staff will explain to each potential subject the research purpose, voluntary nature of participation, study procedures, risks and benefits of participation, alternatives available and the subjects' rights and responsibilities. The informed consent process will be carried out by personnel who are IRB trained and designated for that role. There is no waiting period required before enrolling. The participant may decline to

participate, decide to participate, or take the consent home to think about participating. Subjects will consent in a private room, and usual privacy policy practices followed. Study procedures and objectives will be described to them, and they will be given a copy of the informed consent document. Subjects will also be given contact information for someone they can call with any questions. No study procedures will occur until the patient has signed the informed consent.

Remote Consent/Waiver of documentation of informed consent: Eligible subjects undergoing standard of care visits via telephone, video conference or telemedicine will be consented remotely in the absence of face-to-face contact. The purpose of remote consent is to allow the study investigator/designee and potential subject to engage in the informed consent process similar to what would be conducted face-to-face under normal circumstances. Initially, subjects will be introduced to the study by urology provider and explain the consent process. Subjects will be informed that a consent form will be sent via e-mail, mail or fax. Following delivery of the consent form, approved research staff will call subject and conduct the consent interview via telephone when the subject can read the consent form during the discussion. Research staff will confirm subject's name and date of birth from subject before discussion to ensure the identity of the subject. If agreed to participate, subject will provide verbal consent. No study procedures will occur until the subject and research staff have provided verbal consent.

Each participant will be assigned a unique subject identifier (subject ID) that has no meaning external to the study database. The participant identifiers will be further masked and password protected, and stored only on a secure server behind a "firewall." Only the PI of the IRB approved protocol (Dr. Michael Liss) and approved research staff will have access to these identifiers. A research study file will be kept for each participant accrued. This file will include the participant's consent, the assigned unique subject identifier, research records, and copies of relevant source documents. All participant records will be securely stored in the Urology research offices. A data entry system will be prepared in RedCap, Excel, or equivalent software interface for the study. Both the subject identifier and sequence number for each patient will be entered into the database together with clinical outcome data collected across visits.

For the providers, all providers within the Department of Urology who perform urologic stone procedures (n=5) will be sent an email, informing them about the research study. These providers will also be approached in person with an information sheet. Prior to study entry, approved study staff will explain to each potential provider-participant the research purpose, voluntary nature of participation, study procedures, risks and benefits of participation, and their rights and responsibilities as participants. The informed consent process will be carried out by personnel who are IRB trained and designated for that role. No study procedures will occur until the provider-participant has provided verbal informed consent, which will be documented with a research note by a member of the research team.

No identifiable information will be collected about or from provider-participants. Surveys completed by provider participants will be stored within the specific subject's (i.e. patient's) research record.

8.0 STUDY PROCEDURES

After signing informed consent, participants will complete approximately 4 study visits. The study will enroll 250 completers (and 5 Urology provider-participants). Data collected will include demographics, medical history, laboratory values pre, and post-operative procedure, medications, and treatment history. Data collected from the Urology provider-participants will include information from the provider-participant questionnaire. The following procedures will be completed:

8.1 Enrollment (research)

After shared decision making with their physician, if patients have elected to undergo a urologic stone procedure that will require transurethral surgery and access to the urinary system, the study team will be alerted. After screening the chart to confirm inclusion and exclusion criteria are met, the approved study team will approach for enrollment and engage in informed consent process.

8.2 Urine Collection (standard of care)

Approximately 30-50 mL of whole urine will be collected for a standard of care urine culture corresponding to the patients' pre-operative visit. Approximately 5-10 mL of left-over urine will be obtained for research and sent to MicroGen Dx, a CAP-accredited and CLIA licensed clinical diagnostic lab, for analysis. Urine will be collected by research staff, placed in collection containers supplied by MicroGen Dx and labeled with unique subject ID. De-identified samples will be sent to MicroGen Dx in accordance with their guidelines. In the event, the urine culture was performed more than 45 days before the urologic procedure; the treating physician will ask the patient to provide an additional urine sample. Research staff will obtain an additional 5-10 mL of urine, which will then be sent to MicroGen Dx for analysis.

8.2a Urinary Stone Analysis

During the patients' surgical procedure, the treating physician will remove the stone (s) and send to a laboratory/pathologist for analysis as part of routine care. The removed stone specimens are diluted in a saline solution, and the laboratory will analyze for the stone's chemical composition. Any extra or leftover stone (s) available that will not be sent for routine analysis will be sent to MicroGen DX for analysis and bacteria detection. Urinary stone analysis completed by MicroGen DX will be done only for a proportion of the samples (n=20). Samples will be de-identified with the same study ID as the subject's research urine sample.

8.3 Medical History/Demographics (research)

Data collected will include demographics, medical history, to include but not limited to, medication and treatment history, laboratory and vitals results, diagnosis results, and procedure and surgical history.

8.4 Randomization (research)

A randomization list will be generated using a computer-based-random number generator www.randomizer.org. Randomization will be created 1:1, n=110 per group. Once created, individual envelopes will be created, and study staff will pull an envelope after the participant signs consent. The research staff will not be blinded to the randomization groups. The standard of care group will have urine culture results directed to treating physician. The intervention group will have NGS results sent to Infectious Disease pharmacist, Dr. Kelly Reveles to provide an antibiotic recommendation. The antibiotic recommendation, along with NGS results, will then be provided to treating physician. The physician will then utilize these tools to select the best antibiotic for the case but is not required to abide by the ID pharmacist's recommendation. The standard of care group will have a urine sample sent to MicroGen Dx, but the results will not be provided to the physician.

8.5 The patient is scheduled for surgery (standard of care)

8.6 Presentation of data to the primary surgeon (research)

Antibiotics are standard of care prior to urologic stone surgery, yet generic recommendations allow for variable prescribing practices. In order to provide precise, individualized prophylaxis antibiotics, we will provide the primary surgery and prescriber the following information without mandating a specific antibiotic:

1. Standard of care urine culture.
2. Next-generation sequencing report – contains bacteria presence and resistance gene profiles.
3. Infectious disease pharmacist first and second choice antibiotics based on NGS report.
4. When NGS report and ID pharmacist recommendations are provided, enrolled provider-participants will be asked to complete a survey (research only) regarding their antibiotic selection with rationale.

8.7 Follow-up phone call contact (research)

The study staff will conduct a chart review and/or telephone follow up approximately 7-14 days post urologic procedure to assess for complications, infections related to the procedure, and antibiotics prescribed. In the event an infection or complication related to the procedure is identified by study staff before patient's clinical follow up visit, study staff will alert medical team and provider if patient has not done so.

8.8 Clinic follow up (standard of care)

The chart will be reviewed for infections missed on phone calls.

9.0 RISKS TO HUMAN SUBJECTS

Each patient will be consented prior to treatment and appropriate IRB and HIPAA guidelines will be followed. The selection of antibiotics can vary with treating physician if the urine test is negative. In some cases during the study, the treating physician will receive additional information and choose an antibiotic based on the data rather than their personal preference. Alternatively, the treating physician may choose to select their personal preference despite the additional information provided, as the treating physician will select the best antibiotics for the subject. The American Urological Association best practice statement will be included to the ID Pharmacist and treating physician to ensure that guidelines are met.

No adverse events are anticipated as this is a minimal risk study. Participation in this study is completely voluntary and will not affect their healthcare in any way. The alternative to this study is to not participate. Separately, any risks associated with the standard of care transurethral procedure will be discussed and outlined with the treating physician and medical team.

Each provider will consent before initiation of research procedures. Providers will be informed that the data collected from them will not be used for purposes outside of the research and that their decision to participate or not participate will not positively or negatively affect their standing as UTHSA employees and their participation is voluntary. No identifiable information will be collected from providers. Providers are to follow best practices when prescribing and select what they believe is best for the subject.

10.0 METHOD OF ANALYSIS/STATISTICS

Next Generation Sequencing: Standard of care whole urine will be collected (approximately 30-50 mL) utilizing special vacutainers supplied by MicroGen Dx. Approximately 5-10mL of urine taken for culture will be sent to MicroGen Dx, a CAP-accredited and CLIA licensed clinical diagnostic lab, for analysis. MicroGen Dx will perform rapid PCR for common resistance genes prior to ureteroscopy or percutaneous nephrolithotomy. The plan will be to run target bacterial or fungi DNA on the quantitative PCR (qPCR) panel assay on the Roche LightCycler 480 II instrument and sequencing of 16S ribosomal RNA hypervariable regions V1-V2 using Ion Torrent (Ion Torrent PGM).

Microbiome: Sequencing reads were demultiplexed using QIIME2 software and denoised using dada2 software, using a phred score threshold of 28. Alpha diversity will be measured using the Shannon index, and a Wilcoxon test was used to determine whether the Shannon index differed between patients with stents and those without stents at the time of urine collection. Principle coordinates analysis (PCoA) of Bray-Curtis dissimilarity will be performed to visualize differences in species composition between patients with and without stents, and Linear discriminant analysis Effect Size (LEfSe) analysis was conducted to identify differentially abundant taxa between stent and non-stent groups.

Sample size: This will be a randomized trial of Microgen Dx versus standard of care. The primary endpoint is infection within 14 days of surgery. The primary objective of the study is to evaluate whether Microgen Dx reduces an infection rate compared to the standard of care. The null hypothesis is that infection rates within 14 days of surgery are the same between the randomization arms. The alternative hypothesis is that infection

rates within 14 days of surgery are not equal between the randomization arms. The infection rates are assumed to be 11% in the control arm and 1% in the experimental arm based on our preliminary data. A sample size of 85 per group will give 80% power to detect a 10% difference in infection rate when the two-sided Z-test with alpha=0.05 is used. Demographics and potential confounding factors will be summarized by randomization arm. A logistic regression model will be used to measure the association between the primary outcome and treatment arm with adjustment for the observed covariates.

Statistical analysis: Our primary outcome is infection after surgery as a dichotomous outcome. We will utilize a chi-squared analysis to determine the difference between intervention and control groups. We will assess demographics between groups using chi-squared for dichotomous variables and Student's t-test for continuous variables. Additionally, we will investigate the utilization of antibiotic in a mixed-model regarding a number of antibiotics and antibiotic class utilization.

11.0 DATA REPORTING / REGULATORY REQUIREMENTS

Data and Safety Monitoring Oversight

A Data and Safety Monitoring Plan (DSMP) is required for all individual protocols conducted at UT Health San Antonio. All protocols conducted at UT Health San Antonio are covered under the auspices of the UT Health San Antonio Institutional Data Safety Monitoring Plan.

The UT Health San Antonio Institutional DSMP global policies provide individual trials with:

- institutional policies and procedures for institutional data safety and monitoring,
- an institutional guide to follow,
- monitoring of protocol accrual by the UT Health San Antonio Protocol Review Committee,
- review of study forms and orders by the Forms Committee,
- tools for monitoring safety events,
- monitoring of UPIRSO's by the Director of Quality Assurance and DSMC,
- determining the level of risk (Priority of Audit Level Score – PALS) ,
- oversight by the Data Safety Monitoring Committee (DSMC), and
- verification of protocol adherence via annual audit for all Investigator Initiated Studies by the UT Health San Antonio Quality Assurance Division.

Monitoring Safety

Due to the low risk associated with participation in this protocol, The Principal Investigator will conduct independent *quarterly* (*every 3 months*) review and report any findings to the Data Safety Monitoring Board (DSMB) and the UT Health IRB. It is not anticipated that any safety issues will arise from this study because it is an observational study.

Reporting Requirements

As per our UT health San Antonio Data Safety Monitoring Board, any protocol modifications, problematic safety reports, unanticipated problems, and suspension or early termination of a trial must be reported to all members of the research team and study site research offices *(*UTHSCSA). Suspension and early termination of a trial must also be reported immediately to the Director of Quality Assurance (DQA) who will promptly notify the sponsor and the UT Health IRB.

Assuring Compliance with Protocol and Data Accuracy

As with all studies conducted at UT Health San Antonio, the PI has ultimate responsibility for ensuring protocol compliance, data accuracy/integrity, and responding to recommendations that emanate from monitoring activities. **Source verification of data will be performed every <u>six months</u>**. Protocol compliance, data accuracy and reporting of events is further ensured by an annual audit conducted by the Data Safety Officer, whose audit report is shared with the PI, the research team, and will be reviewed by the data safety monitoring committee (DSMC).

UT HEALTH UPRISO REPORTING REQUIREMENTS		
Type Event	Report to	Timeframe
UPIRSO - life-threatening	UT Health IRB and QA Director	within 48 hours of the PI determining a UPIRSO exists
UPIRSO - non-life threatening	UT Health IRB and QA Director	within 7 days of the PI determining a UPIRSO exists

Safety Definitions:

For this study, the following safety definitions will be applicable:

Unanticipated Problems Involving Risks to Subjects or Others Definition: Unanticipated problem involving risk to subjects or others includes any incident, experience, or outcome that meets all of the following criteria:

A. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as “anticipated” constitutes serious non-compliance);
B. definitely related or probably related to participation in the research; and
C. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

All UPRISO’s will be reported following UT HEALTH institutional guidelines.

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