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I. Research Strategy

A. Significance and Background

Damage to the nervous system secondary to spinal cord injury (SCI) results in a disruption of motor, sensory, and autonomic systems. Deficits to the urinary system profoundly impact quality of life and bladder dysfunction is ranked as a top priority issue in those having SCI (1-4). After injury, volitional control to all or a portion of the lower urinary tract is impaired. As a result, bladder dysfunction may manifest as a failure of the bladder to store urine, characterized by involuntary bladder contractions and an areflexic outlet, or as a failure to empty urine with an areflexic bladder and a sphincter that is unable to relax (5). Urinary retention and an inability of the bladder to store urine under appropriately low pressures can lead to infection and ultimately impact renal health. Urological consequences due to a neurogenic bladder are responsible for many clinical complications post-SCI, including recurrent urinary tract infections (UTI), autonomic dysreflexia and repeated hospitalizations. The primary goal of managing bladder dysfunction after SCI is to achieve urine storage and bladder emptying under a low-pressure system in order to protect the upper urinary tract and preserve renal function (6). The standard of care includes the use of pharmacological agents to promote bladder storage and intermittent self-catheterization for bladder evacuation (7). Given that the majority of SCIs occur in the cervical region, resulting in tetraplegia (8) and insufficient upper extremity dexterity, performing independent self-catheterization may not be a viable option for some and therefore, the use on an indwelling suprapubic catheter is recommended. However, indwelling catheters are costly, as they are associated with a high risk of complications such as bladder cancer (9), bladder stones (10), and frequent UTIs (11), impacting long-term health outcomes. Urinary complications are also the primary precipitating factor triggering dramatic increases in blood pressure, termed autonomic dysreflexia, with potentially life-threatening consequences (12). Individuals with injuries at T6 and above are at risk for developing autonomic dysreflexia as a result of bladder distention.

B. Prevalence of Urinary Tract Infections

The prevalence of UTIs in the SCI population is high, with an incidence of about 2.5 episodes per year (13). The inability to effectively empty the bladder increases the risk of UTIs (14, 15), which are the single most common secondary medical complication after SCI (16, 17). UTIs account for an increased rate of emergency department visits as well as extended hospital lengths of stay, predisposing individuals to nosocomial infections (18). The large number of UTIs also results in higher increases in antibiotic usage and a rise in multi-drug resistance (19), with the highest rates of antimicrobial resistance in those with indwelling catheters (20). Overtime, the development of a bacterial biofilm from catheter usage enables microorganisms to resist penetration by both antibiotics and the body's own immune mechanisms (21). Furthermore, the presence of bacteria in the bladder places both the upper and lower urinary tract at risk for further deterioration and systemically, can trigger uncontrolled autonomic dysreflexia. Collectively, these factors pose a prevalent medical dilemma in addressing appropriate urological treatment.

C. Etiology of Urinary Tract Infections

The etiology of UTIs in the setting of neurogenic bladder is complex and impacted by numerous factors that disrupt bladder physiology. Alterations in the intrinsic defense mechanisms of the bladder as a whole can facilitate the development of chronic UTIs. For example, a defect in the thin mucus or glycosaminoglycan layer has been implicated as a possible source for recurrent infections, which may be exacerbated by inflammation and bladder over-distention (22). The abnormal bladder urothelium can allow bacteria to persist in underlying cells and thereby avoid being washed out with bladder emptying. High intravesical pressure and bladder over distention resulting in mucosal tearing decreases blood supply to the bladder, resulting in an increased risk of infection (23). Chronic ischemia of the bladder wall can lead to a replacement of normal muscle fibers with fibrotic scar tissue, resulting in bladder wall trabeculation and overall poor bladder compliance, further exacerbating the existing ischemia (24). Altered genital skin flora has also been noted, with an over-representation of Gram-negative uropathogens constituting a reservoir for nosocomial UTI in SCI individuals (25, 26).

D. Categorizing Urinary Tract Infections in SCI

In the medical literature, the term UTI has been used when bacteriuria (presence of bacteria in the urine - with or without symptoms) is present. However, for individuals living with a SCI, this classification is not appropriate as the use of urinary catheters results in colonization of the bladder with bacteria (27). Differentiating between asymptomatic bacterial colonization and a true clinical infection can also be difficult to diagnose in persons with SCI since signs and symptoms in these individuals are often subtle and not always recognized as being related to a UTI. Diagnosis has been further challenged by a lack of consensus regarding what constitutes UTI symptoms, what combination of symptoms and laboratory findings are necessary for the diagnosis, and what symptoms require antibiotic treatment (vs being managed with alternative conservative measures, such as increasing fluid intake or catheterizations) (27). Thus, the National Institute on Disability and Rehabilitation Research (NIDRR) consensus conference and the International Clinical Practice Guidelines from the Infectious Diseases Society of America (IDSA)(28) have recommended criteria for the diagnosis of significant bacteriuria in persons with SCI and catheter-associated UTI (CA-UTI), respectively.

NIDRR Guidelines: (1) $\geq 10^2$ cfu/mL (colony forming units per milliliter) for catheter specimens from individuals on intermittent catheterization; (2) $\geq 10^4$ cfu/mL for clean-void specimens from catheter-free males using condom collection devices; and (3) any detectable concentration of uropathogens from indwelling catheters or suprapubic aspirates (29). Furthermore, investigators and clinicians have categorized UTIs as being asymptomatic or symptomatic.

An asymptomatic UTI is not considered a true infection, but rather colonization of the bladder with organisms and defined by the following criteria (30):

- No symptoms and
- Initial counts of 10^2 cfu/ml or higher (except for indwelling catheters)
- For individuals with indwelling catheters, any detectable bacteria

A symptomatic UTI in those having neurogenic bladder is defined as (31, 32):

- Increased bacterial urine colony counts,
- Increased white blood cells in the urine (pyuria), and
- New onset of symptoms [cloudy, malodorous urine, discomfort or pain over the bladder or kidney areas, dysuria (painful or difficult urination), malaise, fever, lethargy or general feeling of un-wellness, increased incontinence, or symptoms specific to the population with SCI such as increased spasticity or autonomic dysreflexia].

IDSA Guidelines: Methods of Diagnosing Catheter Associated Asymptomatic Bacteriuria (CA-ASB) and CA-UTI

- (1) CA-UTI in patients with indwelling urethral, indwelling suprapubic, or intermittent catheterization is defined by the presence of symptoms or signs compatible with UTI with no other identified source of infection along with $\geqslant 10^3$ cfu/mL of $\geqslant 1$ bacterial species in a single catheter urine specimen or in a midstream voided urine specimen from a patient whose urethral, suprapubic, or condom catheter has been removed within the previous 48 hours;

- (2) CA-ASB should not be screened for except in research studies evaluating interventions designed to reduce the incidence of CA-ASB or CA-UTI and in selected clinical situations, such as in pregnant women. CA-ASB in patients with indwelling urethral, indwelling suprapubic, or intermittent catheterization is defined by the presence of $\geqslant 10^5$ cfu/mL of $\geqslant 1$ bacterial species in a single catheter urine specimen in a patient without symptoms compatible with UTI. CA-ASB in a man with a condom catheter is defined by the presence of $\geqslant 10^5$ cfu/mL of $\geqslant 1$ bacterial species in a single urine specimen from a freshly applied condom catheter in a patient without symptoms compatible with UTI;

- (3) Signs and symptoms compatible with CA-UTI include new onset or worsening of fever, rigors, altered mental status, malaise, or lethargy with no other identified cause; flank pain; costovertebral angle tenderness; acute hematuria; pelvic discomfort; and in those whose catheters have been removed, dysuria, urgent or frequent urination, or suprapubic pain or tenderness. In patients with spinal cord injury, increased spasticity, autonomic dysreflexia, or sense of unease are also compatible with CA-UTI.

- (4) In the catheterized patient, pyuria is not diagnostic of CA-bacteriuria or CA-UTI. The presence, absence, or degree of pyuria should not be used to differentiate CA-ASB from CA-UTI. Pyuria accompanying CA-ASB should not be interpreted as an indication for antimicrobial treatment. The absence of pyuria in a symptomatic patient suggests a diagnosis other than CA-UTI (A-III);

- (5) In the catheterized patient, the presence or absence of odorous or cloudy urine alone should not be used to differentiate CA-ASB from CA-UTI or as an indication for urine culture or antimicrobial therapy (A-III).

Refer to Research Procedures Section – Urine Sample Collection and Culture for the classification of symptomatic UTI in this study.

E. Urological Effects of Bladder Irrigation

The routine use of oral prophylactic antibiotics has not been shown to prevent symptomatic UTI in individuals performing intermittent catheterization (33, 34) and is not recommended due to the lack of efficacy and the increased rate of developing antimicrobial resistance(35). One alternative approach to mitigate long-term catheter-associated complications, such as bacterial colonization, encrustations leading to catheter blockage, and bacteriuria, is performing bladder irrigation using antibiotics, acidifiers, and other antiseptics (36). Bladder instillation solutions for the prevention of UTI dates back to the early 1960s with the goal of targeting the bladder flora directly and bypassing systemic antibiotic side effects (37, 38).

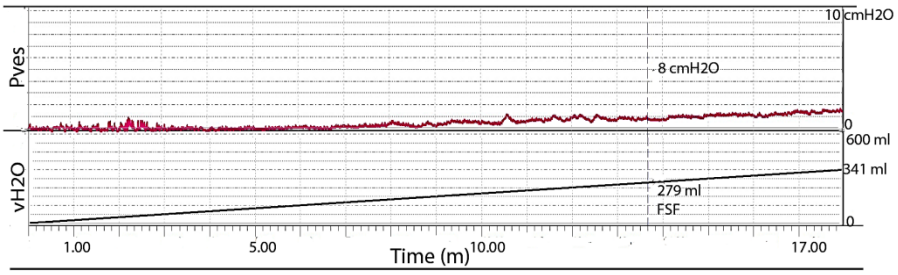

Figure 1. A cystometry recording from a 32-year-old female (AIS A, C4) with a suprapubic catheter showing a first sensation of fullness (FSF) at 279 ml and a final infused volume of 341 ml. Total capacity measured 400ml with low bladder pressure during the entire fill cycle. Note that the participant did not leak, and the fill cycle was stopped due signs and symptoms of autonomic dysreflexia.

Antimicrobial bladder washout methods have been used in individuals with SCI but have had varied results (39). Given that much of this research was conducted in small cohort groups, the effects on individual tolerability, long-term use, and any potential functional bladder changes have not been thoroughly investigated in the SCI population. Preliminary data from one of our adult research participants with a suprapubic catheter using a daily antimicrobial bladder irrigation having a bladder capacity and bladder pressure values within normative ranges (350-600ml and <40cmH_2O, respectively (40)) is demonstrated in Figure 1. This participant also reported a dramatic reduction in the incidence of UTIs in which she attributed to the use of an antimicrobial washout (from a monthly occurrence to no UTIs over the past 3 years).

The use of intravesical gentamicin sulfate as a clinical strategy to prevent UTIs has been shown to be successful and safe in treating and suppressing recurrent UTIs in both adults and children (41-43). The administration of aminoglycosides (having primarily bactericidal activity against Gram-negative aerobes and facultative anaerobic bacilli) intravesically permits the use of high concentrations of antimicrobial solution targeting a local pharmacological effect while limiting systemic adverse effects such as nephrotoxicity, ototoxicity, or impacting gastrointestinal flora. This approach helps prevent the development

of antimicrobial resistance. From the various aminoglycosides, gentamicin remains the best studied of these agents for bladder washout use and is the only intravesical treatment that has been shown to be safe and effective in both the prevention and treatment of UTIs (44, 45).

II. General Experimental Design

A. Objectives: The purpose of this feasibility study is to address the prevalence of chronic, recurrent urinary tract infections in adults living with a chronic, severe, spinal cord injury (SCI). We propose to determine if daily gentamicin bladder instillations reduce the rate of symptomatic UTIs as well as the use of oral and intravenous antibiotics in adults with chronic SCI who have recurrent UTIs. This study will test the efficacy of intravesical gentamicin delivery and provide insights into the mechanisms that underlie the benefits of this local pharmacological effect for translation into the clinic.

Overall Hypothesis: The use of a daily gentamicin instillation treatment will significantly decrease the incidence of catheter-associated complications, such as chronic symptomatic urinary tract infections as well as improve urological outcomes, thus greatly enhancing the quality of life for individuals with bladder dysfunction.

Specific Aim 1: To assess the effects of gentamicin bladder instillation (saline instillation and no instillation will serve as controls) on:1) bacteriuria (number and species, antimicrobial resistant bacteria, urinary leukocytes, and urinary pH), 2) risk factors for developing UTI (over-distention of the bladder, high-pressure voiding, large post-void residuals, detrusor sphincter dyssynergia, presence of stones, and signs of hydronephrosis resulting from vesico-ureteral reflux), and 3) individual tolerability (blood pressure outcomes).

***Hypothesis 1.1*:** Bladder instillation with a gentamicin antimicrobial solution will result in significantly decreased episodes of symptomatic UTIs, fewer courses of oral antibiotics, and a reduction of multi-drug resistant organisms on urine culture, as compared to controls.

***Hypothesis 1.2*:** Bladder instillation with a gentamicin antimicrobial solution will decrease detrusor spasms, increase bladder compliance as well as mitigate the rapid rise in systolic blood pressure (autonomic dysreflexia) evidenced during cystometry as compared to controls.

B. Study Design/Methodology

In a randomized, double-blind, prospective study, we will compare the long-term effects (80 daily sessions, 2x per day) of a bladder instillation protocol in 3 groups of individuals (n=36; 3 groups of 12) with chronic SCI receiving either:

1) gentamicin bladder instillation

2) saline installation

3) no instillation (catheter clamping only in those with suprapubic catheters for the duration of the irrigation time frame - refer to Fig. 2 timeline).

The control groups will also serve to address the question of whether bacteria already colonized in the bladder would also provide a protective role in preventing more pathogenic bacteria or other organisms, such as yeast, which also colonize the bladder. Research participants will complete the 80 sessions of twice per day instillations initially in the Urogenital and Bowel Laboratory, where blood pressure and heart rate will be continually monitored (approximately 1-2 weeks). Once safe pressures are established, the remaining instillations may be completed in the home setting. A major issue confounding validity of a clinical trial is compliance. Given that this study assesses the long-term outcomes of daily bladder instillations and allows for the twice-daily instillations to be conducted in the home-setting, it is possible that participants may not be fully

compliant with respect to study duration and frequency of instillations. In an effort to improve the applicability of the study results to individual participants and mitigate non-compliance, our research nurse will guide participants through all aspects of the instillation procedure to ensure that they are fully independent. At-home blood pressure monitoring during instillation times is automatically recorded and will be an additional step in monitoring protocol adherence.

1. **Blinding Plan**: This is a double-blind study whereby the investigators, research assistants, research nurse and participants will be blinded as to the drug treatment designation. Once participants have been assessed for eligibility and enrolled in the study, they will be randomized into 1 of 3 different groups. Participants randomized to either group I or II will be blinded to the intervention [antibiotic instillation in group I or placebo (saline alone) in group II]. Gentamicin sulfate will be prescribed for the research participants by the study physician or nurse practitioner and instilled under the direction of the research nurse. Thus, the participants in groups I and II will not be able to discern their assigned treatment. To ensure the double-blind, only the study physician or nurse practitioner as well as the pharmacy, all of whom are not associated with the data collection or data analysis, will be un-blinded. Note, the research nurse assisting participants with the instillation will be blinded. All participants will be identified by number and the instillation solutions (gentamicin or placebo) will be numbered with the pharmacy having access to the code specifying which instillations are active and which are placebo for each participant. The investigators and direct research team members will not be permitted to have knowledge of the group assignments. In the case of a drug related adverse event, the study physicians will evaluate the event and prescribe treatment that will minimize or completely resolve the event if minor or moderate in severity and it does not interfere with activities of daily living or quality of life (as determined by the participant). Should it be determined that the event has gotten worse, or if the participant can no longer tolerate the event, the study drug will be discontinued, and the participant will be terminated from study participation. There are no expected reasons to unmask the study treatment. The study physician or nurse practitioner will decide if the blind is to be broken or not. The blind will not “routinely” be broken when a serious adverse event (SAE) occurs. If an SAE is probably or definitely drug-related, the drug/placebo intervention will be stopped, and the participant treated appropriately. Emergency drug code envelopes will be created by the pharmacy at the study site. These will be labeled by participant randomization number and contain information, which will un-blind the treatment of the individual subject. These envelopes are to be used only in extreme emergencies, such as when an emergency situation exists in which the participant’s treatment would be affected by knowledge of his drug therapy, and there is no time to contact the Medical Monitor. Note, that at the end of the study, participants will be un-blinded to the intervention designation by the study physician or nurse practitioner. If a participant was randomized to the no instillation group, however, he/she may re-enroll into the study and directly receive the gentamicin intervention and are not required to wait until the end of study, as all parties are un-blinded and aware of the treatment (standard of care) in this instance. All data from participants re-enrolling into the gentamicin-only group will be kept separate from those who are enrolled in the main study who are blinded.

The following participant withdrawal criteria may be implemented in determining when and how to withdraw participants from the trial/investigational product treatment.

Drug Discontinuation Criteria:

The subject’s participation may be terminated for safety precautions based on any of the following by the study investigator. 1) Drug-related severe adverse event; 2) UTI requiring intravenous antibiotics; 3) Diagnosis of systemic absorption; 4) Diagnosis of nephrotoxicity; 5) Diagnosis of ototoxicity; 6) Diagnosis of renal failure

Temporary Discontinuation Criteria:

Situations of temporary discontinuation of study drug followed by subsequent resumption of study drug are: 1) Resolution of an adverse event, especially in cases where it is determined that the study drug was unlikely to have caused the event. 2) Resolution of a symptomatic UTI.

Withdrawal from Study:

A participant may withdraw from study participation at any time. Whatever data has been collected at the time of study termination will be evaluated by the PI for use in the final study database. If a participant is terminated from the study, the investigator will have the option to recruit an additional participant. Of withdraw from the study was due to an adverse event, participants will be followed until complete resolution of the adverse event that was possibly or definitely drug-related.

2. **Statistical Plan**: Data will first be summarized. We will use means with associated standard deviation, median with associated interquartile range and full range (min-max) to summarize continuous variables. Categorical variables will be summarized with frequency count and standard deviation. We will perform 2 types of analyses: pre-post differences within each group and between group comparisons of the differences. To evaluate the pre-post changes in each group, we will use paired t-test. To compare the changes between the 3 groups, we will use ANOVA test. If the ANOVA test yields significant results, we will perform follow-up 2 by 2 group comparisons using 2-sample t-tests. If the underlying assumptions of these tests are not satisfied, we will use the non-parametric equivalent tests instead.

   We propose to use a sample size of 36 (12 in each of the three arms). Cox et al., (46) has found that the use of intravesical gentamicin was associated with a reduction of a median 3 symptomatic UTIs in 6 months in 22 patients. Using the formulas proposed by Hozo et al., (47) we estimated the corresponding standard mean change to be 2.48 with a standard deviation of 1.7. Assuming the same standard deviation in our data, a sample size of 12 achieves 80% power to detect a mean of paired differences of as low as 1.5 symptomatic UTIs for the gentamicin group with a significance level of 0.05 using a two-sided paired t-test (48, 49). We expect to observe a drop of at least 2 symptomatic UTIs in 6 months. With the same setting, i.e. SD = 1.7, a sample size of 12 achieves 97% power to detect that. Our study will compare changes between the gentamicin instillation, the saline instillation and the no-instillation groups. We will use the comparison between the gentamicin and the non-instillation group to estimate the power. The estimated effect of gentamicin is a reduction in symptomatic UTIs of 2 with a standard deviation of 1.7 (described above) and we assume no change for the non-instillation group (mean reduction of 0 with a standard deviation of 0). With these values, sample sizes of 12 (gentamicin) and 12 (no-instillation) achieve 96% power to reject the null hypothesis of equal means with a significance level of 0.050 using a two-sided two-sample unequal-variance t-test (48-51). The power analysis was performed in PASS 14 Power Analysis and Sample Size Software (NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass).

Experimental Outcomes – Research Procedures

1. **Intravesical Gentamicin Sulfate Instillation**

   Gentamicin sulfate will be prescribed for the research participants by the study physician or nurse practitioner. A formulation derived from 480 mg gentamicin sulfate diluted in 1 L normal saline will be used for instillation. The formulation will be prepared by the pharmacy, located at the study site (first floor of Jewish Hospital), in a laminar flow hood using USP 797 regulations just prior to the scheduled assessment (within 20-30 minutes) and stored in the refrigerator at a temperature range of 2-8ºC until use in the outpatient setting. Gentamicin will be compounded for immediate use in single-dose, disposable BD 50ml Luer-Lok tip syringes. The container closure system for the diluted gentamicin sulfate solution is sterile and depyrogenated. A 60 ml syringe containing the gentamicin solution will be connected to the end of the catheter through which the solution will be slowly infused over 1-2 minutes (depending on bladder capacity – determined from initial Urodynamics) after drainage of urine is complete. The solution will remain in the participant's bladder for a least 30 minutes (unless the participant is experiencing autonomic dysreflexia, and in that case, immediately drained) with the goal of attaining 2 hours.

   Participants will undergo initial instillations in the laboratory to determine the optimal bladder instillation storage time frame while monitoring blood pressure throughout the intervention. The initial intervention

sessions will be performed in the laboratory. The time frame to retain the instillation treatment in the bladder will be incrementally increased by 15 minute intervals in the laboratory over the course of 3 - 4 weeks. Instillation retention times may begin for 30 minutes. Once 3 consecutive days of stable blood pressure values without autonomic dysreflexia are demonstrated during that time frame, the time will be increased to 45 minutes and the same process will continue by 15 minute intervals, up to 2 hours, as long as blood pressure remains stable throughout the instillation retention time frame. The instillation retention time frame will not be increased if the participant experiences autonomic dysreflexia and thus, an optimal time frame in which his/her blood pressure remains stable during the intervention will be established. Once 3 consecutive stable days are achieved with the same instillation parameters and the participant has demonstrated self-sufficient use of the instillation procedure, he/she will be sent home for one day to perform the twice daily instillations at home. For home instillations, the participant will be instructed and provided with written instructions indicating that the solution is to be stored in the refrigerator no more than 10 days and stored at room temperature for no more than 48 hours. The participant will return to the laboratory the following day for instillation with blood pressure monitoring. Provided blood pressure parameters remain stable, the participant will be allowed to complete the intervention at home for 3 days prior to returning to the laboratory for instillation and blood pressure monitoring. If blood pressure parameters remain stable, the participant will be able to begin the home intervention program of 6 days a week and 1 day in the lab. Participants will be instructed to monitor blood pressure for at least 15 minutes.

2. **Saline Instillation**
   A 50ml syringe containing saline will be connected to the end of the catheter and slowly infused over 1-2 minutes after drainage of urine is complete. The saline will remain in the participant's bladder for at least 30 minutes (unless the participant is experiencing autonomic dysreflexia, and in that case, immediately drained) with the goal of attaining 2 hours. Note that participants will follow the same instillation training paradigm as mentioned above, including the incremental progression of instillation retention time with stable blood pressure values.

3. **Bladder Clamping Protocol – no instillation (for those with suprapubic catheters)**
   Participants with suprapubic catheters will be instructed by the research urology nurse on how to perform a catheter clamping protocol. These procedures will initially be conducted in the laboratory setting until the participant can demonstrate independence with clamping and blood pressure remains stable during that time frame. To begin, the nurse will instruct the participant on how to slide the plastic piece of the suprapubic tube to the "closed" position and plug the end of the tube. This will ensure that urine is not draining from the tube. After the tube has been clamped for 15 minutes, the participant will un-clamp the suprapubic tube, take the plug off the end, and draining any additional urine into a collecting "hat" or urinal. What comes out of the suprapubic tube will be measured. The nurse will record this amount on a chart as a measurement of the participant's bladder capacity. This procedure will be repeated 4 times a day until he/she can work up to clamping for 30 minutes, increased by 15 minute intervals, up to 2 hours as indicated above in the instillation description (if appropriate). Each day the nurse will help the participant re-clamp the tube, plug the end, and continue recording residual urine that comes out of the tube. At night, the participant may keep the suprapubic tube connected to a bag in order to let the bladder drain during sleep. When the participant is able to demonstrate independence with clamping, he/she will restart bladder clamping in the home setting, clamping the suprapubic catheter 4 times a day for up to 2 hours each time. He/she will continue to clamp the suprapubic tube throughout the duration of this study.

4. **Urine Sample Collection and Culture**
   Urine collected for cultures will be collected as a clean-catch midstream technique from an immediately installed urine catheter or as a suprapubic aspiration from an installed catheter (52). For participants having a suprapubic catheter, the catheter will be clamped for 20 to 30 minutes until at least 5 mL of urine can be collected. The catheter will then be unclamped, and urine will be allowed to flow into a sterile plastic container. In those that use intermittent catheterization, participants will be required to first cleanse the urethral area with a castile soap towelette. A sterile 12 French straight catheter is inserted through the urethra into the bladder. The first portion of the urine stream should flow into the toilet in order to reduce the opportunities for contaminants to enter into the urine stream. The urine midstream is then collected into a clean container (any excess urine will be emptied into the toilet), Urine samples will be placed in

sterile storage vials and stored at 4C for less than 3 hours and sent to the Outpatient Care Center, Jewish Hospital for analysis. Symptomatic UTIs will be defined as a participant complaint of symptoms consistent with UTI (cloudy/foul smelling urine, fever, chills, increase in bladder and/or lower extremity spasms, worsening dysreflexia, leakage, or pain) combined with a positive leucocyte esterase and/or nitrates on dipstick urinalysis or a urine microscopy with > 10 WBC/HPF (white blood cells/high powered field) with a urine culture with $>10^5$ bacteria/ml (46). Note that urine smell and cloudiness alone (i.e. without other symptoms) are not considered UTI signs, given their high prevalence in this catheterizing population. All cultured microorganisms will be identified to the species level and quantified as colony-forming units per ml (cfu/ml $^{-1}$) (16). If the microorganism(s) cultured is/are resistant to three or more different antimicrobial agents to which the microorganism would normally be susceptible, it will be defined as being multi-resistant. If a participant is diagnosed having a symptomatic UTI necessitating systemic antibiotics by the study physician/nurse practitioner, he/she will follow standard of care treatment under provider oversight. The study physician/nurse practitioner will recommend whether or not continuation of either the instillation or clamping protocol is appropriate for the participant during systemic antibiotic treatment.

5. **Blood Draw**
An assessment of metabolic parameters (glucose, calcium, albumin, total protein, blood urea nitrogen, creatinine, alkaline phosphatase, alanine amino transferase, aspartate amino transferase, bilirubin, and other electrolytes), inflammatory markers (C-reactive protein, erythrocyte sedimentation rate, procalcitonin), and complete blood count (includes white blood cells, red blood cells, hematocrit, and platelets) will be performed. A trained technician will insert a butterfly catheter into an antecubital vein to allow the collection of blood. Blood samples will be sent to the Outpatient Care Center, Jewish Hospital for testing.

6. **Urodynamics**
The participant will be asked to refrain from taking any bladder medications 24-hours prior to Urodynamics. For the urodynamic study (necessary to determine bladder function/voiding pressures/degree of detrusor sphincter dyssynergia (53, 54)), a complex cystometrogram (to evaluate the filling phase of the bladder) with a pressure flow study and simultaneous abdominal pressures and flow rate (to evaluate the voiding phase of the bladder) will be performed. The cystometry evaluation is accomplished using standard procedures (55-57), measuring bladder pressure during filling, possible uninhibited bladder contractions and maximum cystometric capacity, with determination of the leak point pressure and post-void residual volume when voiding is possible at the end of the study when a second uroflow can be obtained. A 12 French straight catheter will be used to empty the bladder completely for a urine sample to be collected and assessed for the presence of blood, urobilinogen, glucose, ketones, bilirubin, protein, nitrites, leukocytes, pH, and specific gravity using DiaScreen reagent strips for urinalysis. The collected urine sample may also be sent out for culture. Then a 7 French urodynamic catheter will be placed in the bladder to fill the bladder as well as to measure and record intra-vesical pressure. Another catheter with a balloon will be placed in the rectum to record the intra-abdominal pressure. Detrusor pressures will be calculated by subtracting the intra-abdominal pressure from the intra-vesical pressure.

The bladder will be filled at a slow rate with body temperature water or physiologic saline. Each participant will be asked to cough to verify intra-abdominal catheter position and will be instructed to communicate when s/he first feels a full bladder (first sensation); when s/he first feels the desire to urinate (first urge to void); and when s/he can no longer wait to void (maximum capacity). The volume of water and bladder pressure will be recorded. Uninhibited bladder contractions will be identified. Since the majority of SCI individuals have abnormal or no sensation, filling will be stopped when the participant has an involuntary contraction, increasing blood pressure (autonomic dysreflexia) or high intravesical pressures (greater than 60 cm of water). After stopping the fill, the bladder will be completely emptied, and a residual volume obtained. A second fill/void cycle may then be performed (starting again with an empty bladder) following the same procedure. During this cycle, filling may cease prior to the participant's capacity and he/she will then be asked to attempt to empty his/her bladder voluntarily; voiding bladder pressures will be recorded. During the time when cystometric evaluation is being done, surface patch EMG electrodes will be placed on the skin at 3 and 9 o'clock positions alongside or just anterior to the anus in the lithotomy position (ground electrode placed on the hip) to capture muscle activity during the

cystometrogram. The EMG activity will evaluate coordination of the urethral and anal sphincters during the voiding phase and during possible uninhibited contraction episodes. Detrusor-sphincter dyssynergia may be evaluated and classified according to the Blaivas classification into: type 1 DSD characterized by a crescendo increase of the sphincter activity that reaches its maximum at the peak of the detrusor contraction (as the detrusor pressure begins to decline, sudden complete external sphincter relaxation occurs); type 2 DSD characterized by clonic contractions of the external urethral sphincter interspersed throughout the detrusor contraction (these Participants usually void with an interrupted spurting stream); or type 3 DSD, characterized by external urethral sphincter contraction persists throughout the entire detrusor contraction) (53, 58). Blood pressure (BP) parameters may be monitored throughout urodynamic testing using either the Dinamap Carescape V100 (GE Healthcare) or continuous arterial BP acquired from a finger cuff placed around the finger or thumb (Finapres MedicalSystems).

7. Bladder/Kidney Ultrasound

Ultrasound (US) imaging will be performed using a Phillips EPIQ 7 US scanner. Generally, no prior preparation, such as fasting, or medication cessation would be required. However, for a bladder US, participants may be asked not to empty the bladder prior to the procedure. Participants with suprapubic catheters will be asked to clamp the suprapubic tube for 30 minutes prior to the assessment to allow the bladder to fill with urine in order to be visualized during scanning. The procedure should last about 45-60 minutes and will be performed by a certified sonographer. Participants will be able to resume a usual diet and activities following the procedure.

The participant will be assisted on the examination table in the appropriate assessment position (noted below). A clear, water-based gel will be placed on the skin over the analyzed area in order to allow for smooth movement of a hand-held probe (transducer) over the skin and to eliminate air between the skin and the transducer for the best sound conduction. The organs of interest will be scanned in real-time through all tissue planes in at least two orthogonal directions. Images of both kidneys will be obtained in the longitudinal and transverse planes for purposes of comparison and to exclude absence of either kidney. The right kidney may be visualized with an anterior subcostal approach using the liver as a sonographic window. With the participant in the left lateral decubitus position he or she may be asked to take and hold a deep breath in order to extend the liver window so that it includes the inferior pole of the kidney. If parts of or the entire kidney may not be seen in this view due to interposed loops of bowel, the kidney can be imaged using an intercostal approach in the right flank between the anterior axillary line and mid axillary line. For this approach, the participant can be placed in the decubitus position with a bolster under the lower side with the arm of the upper side fully abducted, thus spreading the intercostal spaces. To obtain transverse images, the transducer is rotated 90 degrees counter-clockwise from the longitudinal plane. Once in the transverse plane, the transducer can be moved superiorly and medially, or inferiorly and laterally to locate the renal hilum. Images cephalad to the hilum represent the superior pole and those caudad represent the inferior pole. The left kidney lacks the hepatic window, necessitating an intercostal approach similar to the one described for the right flank. The kidneys will be assessed for abnormalities of the renal sinus and parenchyma such as calculi, blood flow and degrees of hydronephrosis: Grade I (Slight splitting), Grade II (Evident splitting; confined within renal border), Grade III (Wide splitting; pelvis dilated outside renal border; calices dilated) and Grade IV (Wide splitting with pelvis dilated outside renal border; calices dilated and may appear convex). Other abnormalities identified including cysts and masses may require additional diagnostic evaluation and would be referred to the participant's personal urologist. Measurements may be made of the dimensions of abnormal findings and the length and width of the kidneys. The bladder will be imaged to assess for volume, thickness, and blood flow, evidence of distal ureteral obstruction, diverticula and for calculi. The bladder will be imaged from top to bottom and from side to side, in transverse and sagittal planes, respectively.

8. International SCI Data Sets

The International SCI Urinary Tract Infection data set (32) will be used to standardize the collection and reporting of information related to possible UTIs and includes such variables as: date of data collection, length of time of sign(s)/symptom(s), results of urine dipstick test for nitrite and leukocyte esterase, urine culture results and resistance pattern. This data set will be completed by the researcher(s). It will also be

used in combination with the International SCI Lower Urinary Tract Function Basic Data Set (Version 2), which contains information on the bladder-emptying method(s), urinary incontinence and any medications usage (59). The latter data set will be conducted by the researcher(s) with the participant.

D. Limitations

A limitation of this study will be the variable nature of the host urinary microbiome. Conventional thought has been that we all are colonized with similar organisms and thus, we should have similar responses to treatment interventions. However, through the Human Microbiome Project (60), we have learned that each of us have diverse microbial communities present in the gut, skin, and vagina (bladder was not investigated) and may help explain why some individuals benefit more from a particular treatment or are more susceptible to infections than others. Our approach in this study is to investigate the effects of a focused, placebo-controlled intervention in a specific population. In doing so, the results from this project may help differentiate responders from non-responders and can be integrated with current studies in the lab assessing the urinary proteome.

III. Human Subjects

There are approximately 1,275,000 Americans with a SCI. Fifty-six percent of the injuries occur in people aged 16 to 30, with an average age of 31, and 82% of the total population are male. Minorities make up 38% of SCI cases, and while every effort will be made to recruit minorities, based on the incidence rates, their participation may be limited. Every effort will be made to recruit women, though only about 18% of SCI patients are female.
University of Louisville Hospital and Frazier Rehab Institute evaluates approximately 300 chronic SCI outpatients each year. We also have a database of over 5,000 people with SCI who have expressed interest in participating in our research programs. We will select individuals to assure that there is a minimum of 25% women to adequately represent the percentage in the SCI population.

All SCI research participants, irrespective of gender, will be selected based on the following:

Inclusion Criteria

- At least 18 years of age;
- Non-progressive spinal cord injury;
- Stable medical condition;
- Bladder dysfunction as a result of spinal cord injury
- History of recurrent UTIs (defined as 3 or more within the past year or 2 or more within the past 6 months).

Exclusion Criteria

- Signs or symptoms of serious UTI that requires the use of systemic antibiotics;
- Use of any urine-acidifying agent, bladder irrigant, or systemic antibiotic within the previous 2 weeks;
- Participants colonized with gentamicin-resistant bacteria on baseline urine culture or a gentamicin allergy/sensitivity;
- Participants with known hearing loss and/or renal disease;
- Pregnant at the time of enrollment or planning to become pregnant during the time course of the study.

A. Screening and Enrollment

Potential research participants will be identified by our research database (UofL IRB 06.0647) and contacted by research staff prioritized by the initial date they entered the database and provided with general information regarding the research study. If the individual is interested in learning more about the study, the clinical coordinator will set up an info session via teleconference or a meeting at Frazier Rehab Institute. The designated project leader and clinical coordinator will attend the info session and the project leaders (Drs. Herrity and Harkema) will describe the project including the time commitment, the general design of the study, the interventions and the randomization process. Specific questions from the potential research participant will be answered.

The potential research participant will be consented for participation and assessed for medical eligibility by the research team's Physiatrist, and/or Nurse Practitioner (as applicable). The Nurse Practitioner will conduct a medical history and physical examination and determine medical eligibility based on the clinical inclusion criteria and exclusion criteria and consideration of the risk assessment specific for the individual based on their medical history and examination. Following medical clearance for the assessments, the potential research participant will undergo urological assessments. Potential study eligibility will be determined by the investigators based on the evaluation of these assessments to be consistent with the inclusion criteria. The participant will then complete the bladder washout protocol for the study for 80 days and may continue with his/her current daily activities. Throughout the protocol, a number of assessments will be performed to assess the participant's progress in the study. Following the protocol, the participant will participate in post-instillation bladder assessments.

B. Consent and Eligibility Documentation

After the research participant signs the consent document, an Eligibility Checklist is completed by the research participant attesting to the eligibility requirements. This is placed in the research medical record along with supporting source documentation. These will be reviewed by the research manager and the principal investigator. The eligibility checklist will be signed by the person verifying eligibility and by the Principal Investigator who is responsible for final determination of eligibility. All source documentation for eligibility will be placed in the research medical report. We will request past medical records to determine eligibility. For research participants injured within a month of enrollment, an AIS examination would need to have been completed within four weeks of enrollment. For all other time points (three months, six months, one year, or 2 years post-injury) an AIS examination will need to be completed within three months of enrollment. If the individual has not had an AIS exam within the appropriate time point the research medical core staff will conduct the AIS exam. In consult with the medical core staff, the investigator will determine eligibility based on the medical history and the study inclusion/exclusion criteria and the research participants Attestation eligibility form. The regulatory research manager will do periodic internal audits of all enrolled research participants research medical report to ensure compliance of documentation.

C. Costs and Compensation

Participation in this study will require no additional study related costs to the research participant, unless they are injured. The participant or the participant's insurance company will be billed for all office visits, tests, medications, and procedures that are part of routine medical care outside of this research study. Participants will not be billed for the office visits, tests, medications, and procedures that are done for this research study. The charges for these items will be paid by the study. Participants will be responsible for paying co-pays associated with any office visit, test, medication, or procedure outside of this research study. Participants will be informed that some insurance companies will not pay for medical bills for individuals in research studies. It will be made clear that is the responsibility of the research participant to find out what costs, if any, the insurance company will cover before taking part in the study. The study

physician will be available to assist research participants should they have questions regarding what the insurance company will cover. In the case that the insurance company will not pay for bills associated with the study, the participant will be responsible for payment. Participants will be compensated for local travel to and from Frazier Rehab Institute for participation in this study based on standard mileage rate and parking fees up to $30 per day by Visa prepaid card. Because participants will be compensated to be in this study, the University of Louisville may collect name, address, social security number, and keep records of how much they are compensated. Participants may or may not be sent a Form 1099 by the University. This will only happen if they are compensated $600 or more in one year by the University. This will not include payments they receive as reimbursement, for example mileage reimbursement. We are required by the Internal Revenue Service to collect this information and you may need to report the payment as income on your taxes.

D. Risks of Assessments and Interventions

The assessments will be conducted as described in detail in the above sections (III). The frequency of assessments will be determined based on the Specific Aim and will be documented in their Research Medical Report. Each visit will include one or more assessment(s), performed by a qualified, licensed nurse, which could last up to 2 hours at the most. A maximum of 2 assessments may be performed in one day.

1. Potential Risks

Any study can have risks involved. We will try to inform you of the risks and encourage you to ask questions about anything that you wish. The risks are placed into categories based on how severe the risk is and the seriousness of the harm that could happen. The severity can depend on your age, individual physical status as well as the medication or intervention itself. Minimal risk would be the level of risk similar to those you would encounter in daily life or during routine doctor exams or tests. A minor risk is slightly increased, and you may feel some discomfort from these events. A moderate risk event is more serious and could require medical intervention, treatment, and follow up. The harm is reversible in these cases. A severe risk is much higher, and the harm may not be reversible. Risks can be potentially severe depending on the nature of the event.

The frequency is an estimated range of the likelihood that the risk will occur to you. These are general ranges. Rare (0-10%), Less likely (11-30%), Likely (more than 30%) chance that these risks may occur from you being in the study. At any time depending on your condition, a risk may increase to another level to be more severe. Again, please ask us if you are concerned about any particular risk and we will try to answer all your questions. There may also be new risks that we have not anticipated.

This study may involve the following physical risks and/or discomforts:

Risks of Bladder Assessments and Study Interventions

Note that gentamicin is a drug used to treat a variety of bacterial infections. Gentamicin is only approved by the FDA for intravenous use. This method of gentamicin delivery affects the body systemically and is not included in this study. The well-known risks for such delivery can include ototoxicity (hearing loss, dizziness, ringing in the ears) and nephrotoxicity (azotemia – insufficient filtering of the blood by the kidneys leading to abnormally high levels of urea, creatinine, and other nitrogen-rich waste compounds that can cause kidney failure) and are usually associated with higher doses.

Risks of IV Gentamicin use:
Likely

- Neurotoxicity (spinning sensation [vertigo], loss of control of bodily movements)
- Gait instability

- Ototoxicity (toxic to the auditory, vestibular nerves – can be reversible and temporary, or irreversible and permanent)
- Kidney damage (decreased CrCl)
- Kidney damage if trough concentration greater than 2 mg/L
- Swelling (edema)
- Rash
- Itching
- Stomach upset
- Injection site reactions (pain, irritation, and redness)

Less Likely
- Drowsiness
- Headache
- Pseudomotor cerebri (increased pressure in the brain that mimics symptoms of a brain tumor)
- Photosensitivity (sensitivity to light)
- Allergic reaction
- Skin redness
- Loss of appetite
- Nausea/vomiting
- Weight loss
- Increased salivation
- Enterocolitis (inflammation of the digestive tract)
- Granulocytopenia (decrease in granulocytes, a type of white blood cell involved in fighting infection)
- Agranulocytosis (drug-induced decrease in the amount of granulocytes)
- Low platelet counts (thrombocytopenia)
- Elevated liver function tests
- Burning
- Stinging
- Tremors
- Muscle cramps
- Weakness
- Shortness of breath

Rare, but serious side effects of gentamicin include:
- Ringing or roaring sounds in the ear
- Hearing loss
- Dizziness
- An unusual decrease in the amount of urine while using gentamicin injection (pediatric)

Gentamicin may also be applied topically or instilled locally into the bladder, as described in the intervention for this study. Risks for the gentamicin intervention in this study include those associated with the local application in the bladder.

Risks of Gentamicin bladder instillation and associated assessments:
Likely
- Feelings of shyness
- Significant changes in heart rate and/or blood pressure from bladder clamping or urodynamics
- Autonomic dysreflexia symptoms (sudden high blood pressure) that resolves when the cause is removed (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)
- Discomfort and/or skin irritation at the site of needle insertion for the blood draw

- Skin irritation from adhesive tape and/or pads

Less Likely
- Bleeding, bruising and/or pain from blood draw
- Lightheaded during the blood draw
- Mild discomfort, especially during urination after assessments of the bladder
- Urinary tract infection requiring oral antibiotics after urodynamics
- Discomfort from lying still for ultrasound
- Antibiotic-related vaginal yeast infection and/or diarrhea (opportunistic infection) from gentamicin instillation
- Localized allergic reaction to gentamicin (rash, redness, swelling around groin region)

Rare
- Infection from blood draw
- Autonomic dysreflexia symptoms (sudden high blood pressure) but the cause cannot be identified, and the high blood pressure does not resolve, and medical intervention is required (*individuals with an injury level above T6 and in those who have previously experienced these symptoms)
- Urinary tract infection requiring intravenous antibiotics
- Growth of organisms resistant to gentamicin
- Intravesical (bladder) absorption of gentamicin
- Excessive pain, fever, chills

E. Adequacy of Protection against Risk

1. Recruitment and Informed Consent

Recruitment of patients may be performed through our secure research database that includes over 5,000 individuals registered with SCI. In addition, University of Louisville Hospital and Frazier Rehab Hospital serves approximately 100 individuals with SCI who may be referred to our research program. All potentially eligible research participants may meet at University Hospital or Frazier Rehab Institute to discuss the complete protocol, including risks and benefits with the PIs and/or designated research staff. The informed consents will be written in language that an eighth-grade student would be able to understand and will contain information on all assessments to be performed as well as contact information if the research participant and his/her associates should have any questions. All potential research participants will be encouraged to read the informed consent and discuss the study with their physician, family and friends, before signing. The original signed informed consents will be kept in a locked cabinet in a locked room within an area of controlled access. A scanned copy will be stored on our server with password protection. Eligibility checklists will be signed by the PI with all source documentation and stored in a locked cabinet in a locked room within an area of controlled access. A scanned copy will be stored on our server with password protection.

If a participant is employed by the University of Louisville, we will review and discuss a risk management plan. The plan will be signed by the investigator and the participant and witnessed by an individual outside of the study and department to ensure there is no coercion. A copy of the plan will be provided to the participant and placed in his/her employee file.

To protect confidentiality, each research participant will be assigned a coded identifier with no association to their identity. This identifier will distinguish all evaluations and analyses. Data will be stored on computer media and video and will be secured in a locked storage area. Only members of the research team including research staff, post-doctoral students and graduate students will have access to the data for analyses. The PI will have access to the coding of the coded identifier to the research participants.

2. Assessments

a. General protection against risk

All eligible research participants will be encouraged to discuss the study with their primary physician. Participants will be continuously monitored for any signs of discomfort or risks by a designated research staff member during every assessment. Blood pressure and heart rate will be routinely measured. A research team member will be assigned to continuously observe the research participant for signs of discomfort or medical events during the assessment. If discomfort or any risks persist the recording session will be immediately discontinued, and the research nurse will be contacted immediately to assess the participant. If needed the study physician or physician assistant will be notified and will also examine the individual. Immediate medical care will be provided when necessary. Their primary care provider will be informed when necessary. If there are no medical events during the assessment this will be noted and verified with a signature by a research team member.

b. Bladder Assessments

Assessments will be performed by either a licensed registered nurse, or by a certified sonographer (for ultrasound), under the supervision of the principal investigator(s). Both during and in the days following the procedure, participants will be monitored for excessive discomfort, pain, and other adverse reactions. A nurse or specialist trained in the procedures will perform the assessment. The participant's blood pressure will be continuously monitored during the assessment. The observer will communicate with the research participant and monitor for signs of autonomic dysreflexia, risks and other signs of discomfort. If the participant displays symptoms of autonomic dysreflexia and/or with a rapid rise in systolic blood pressure and decreased HR, the following steps will be taken:

- Stop the assessment
- Identify and relieve the noxious stimulus (i.e. stop bladder filling, empty bladder, remove catheters, unclamp the suprapubic catheter)
- Move the participant to an upright position and continue to monitor blood pressure
- Monitor blood pressure and heart rate until values return to the participant's established normal limits.
- If the autonomic event is not resolved, the study physician will be called to evaluate the participant. If the autonomic event is still not resolved, immediate emergency medical care will be provided. There is an emergency room located on the first floor of the building.

There may be circumstances where a participant has been pre-prescribed nitrate medication by the physician that he/she would have available for the research nurse to administer in the case an autonomic dysreflexic event was not mitigated by means of our standard protocol. In this instance, the nurse would administer the participant's prescribed medication while monitoring his/her blood pressure. If blood pressure did not resolve in response to medication, emergency protocol would be instated, as described above.

Prior to initiating cystometry, the research participant's blood pressure and heart rate will be obtained to establish his/her pre-fill values. Once the cystometrogram has begun, continuous beat-to-beat arterial blood pressure will be recorded from a cuff placed around the finger using Finapres Human Non-Invasive Blood Pressure Medical Systems unit. Brachial arterial blood pressure measurements will be taken periodically with a Dinamap V100 (GE Medical Systems) device for calibration purposes. Throughout the fill cycle, the research team will also assess the participant for symptoms of autonomic dysreflexia (see list of symptoms below). If rapid rising of systolic pressure as well as fluctuations in heart rate are observed, the fill cycle will be stopped, and the participant's bladder will be immediately emptied, followed by the removal of both the vesical and abdominal catheters. The participant's head will be elevated while blood pressure will be continuously measured until values return to the pre-fill status.

The signs and symptoms of autonomic dysreflexia include:

- A sudden, significant increase in both the systolic and diastolic blood pressure above their usual levels, frequently associated with bradycardia
- Pounding headache
- Reflex bradycardia (note that this may be a relative slowing such that the heart rate is still within the normal range)
- Profuse sweating above the level of the lesion, especially in the face, neck, and shoulders, or possibly below the level of the lesion
- Piloerection or goosebumps above or possibly below the level of the lesion
- Flushing of the skin above the level of the lesion, especially in the face, neck, and shoulders, or possibly below the level of lesion
- Blurred vision
- Appearance of spots in the visual fields
- Nasal congestion
- Feelings of apprehension or anxiety
- Minimal or no symptoms, despite a significantly elevated blood pressure (silent autonomic dysreflexia)

Note that a research participant may have one or more of these signs and symptoms when experiencing an episode of autonomic dysreflexia. Symptoms may be minimal or even absent, despite an elevated blood pressure. An episode of autonomic dysreflexia will be documented in the research participant's medical record including the presenting signs and symptoms during the assessment, what treatment was instituted, a copy of the logged blood pressure and pulse recordings, the participant's response to treatment, and resolution of the incident (i.e. final recording demonstrating that the blood pressure and pulse rate have returned to a participant's established normal limits).

The urodynamic assessments will be conducted by our research urology nurse who is specifically trained and experienced in managing bladder dysfunction in the spinal cord injury population, including recognizing signs and symptoms of autonomic dysreflexia as well as administering appropriate treatment to resolve the event. The study physician and/or physician assistant is also present in the building during the time urodynamics is conducted. The principal investigator is present in the room during the assessment as well as other members of the research team who will also be monitoring the participant's blood pressure and response to bladder filling/stimulation.

Symptoms associated with infection will be addressed immediately and the participant's primary care provider will be notified if needed. Participants experiencing excessive pain, chills, or fever will be triaged accordingly. If needed, the study physician or physician assistant will be notified and will also examine the individual. Immediate medical care will be provided when necessary. Their primary care provider will be informed when necessary. Viscous Lidocaine will be applied through the urethra in an attempt to damper a burning sensation. Participants experiencing excessive pain, chills, fever, or other external symptoms of an infection will be treated accordingly. Both during the assessment and in the days following the procedure, participants will be monitored for excessive discomfort, pain, bleeding, and other adverse reactions. Research participants may also feel uncomfortable answering questions regarding their bladder management. A private room will be used to complete the questionnaires and it will be explained to the participant that he/she may refuse to answer any question that makes him/her feel uncomfortable.

3. Interventions

a. Bladder Instillation or Bladder Clamping Alone

Bladder instillation or bladder clamping alone will be performed in the Urogenital and Bowel Core laboratory to ensure these interventions are safe for the research participant. Participants will conduct the bladder instillation intervention or clamping protocol within the laboratory prior to being cleared for the home intervention program. Participants will be instructed to call their assigned research team member immediately if complication from bladder distention develops during the home intervention program. If serious adverse effects such as autonomic dysreflexia, sustained elevation or reduction in blood pressure or bradycardia or tachycardia have recurring onset on an individual or become present across the tested sample population, the research team will evaluate the instillation protocol. Instillation dwell times or clamping durations will be assessed initially limiting the time in bladder storage that could reduce such effects. If a serious adverse event occurs as a result of instillation or clamping alone, the research participant will be required to conduct the procedure in the laboratory for at least a week where he/she can be monitored by research staff. Participants will not be allowed to return to a home-based instillation or clamping alone program until they show stable responses to the procedures and have been cleared by a physician or nurse practitioner. If instillation or clamping parameters cannot be found to eliminate the onset of adverse effects, the study will be stopped.

4. Medical Event Reporting Unrelated to the Study

A medical event is defined within our program as any untoward or unfavorable medical occurrence in a human research participant that occurs during the enrollment period of a study. All medical events for study participants will be recorded in a log for tracking and reporting, as the team is made aware of them. Medical events that occur during a research visit will be reported to the study nurse and then elevated to the study physician if not immediately resolved. The physician will evaluate these medical events, to determine causality with relation to the research and referral for medical care. A weekly report of all medical events is generated by the research nurse and sent to the study physician (and physician assistant), the principal investigator, the regulatory core, and the study physical therapists for review of causality and follow-up. We have developed a table outlining the most common expected events in our SCI population. These events occur with this population on a daily basis. This table will allow the IRB and our staff to understand how expected and unrelated events will be documented and reported. If a physician outside the study team treats the medical event, the study physician will follow the event to assess the following:

- The medical condition affecting their medical eligibility for research
- Determination on causality of the event
- The event’s effect on research assessments or training paradigms

All conclusions reached by the study physician will be reported to the PI. If another physician treats the medical issue the study physician will follow the individual to only assess whether the medical condition affects their medical eligibility in the study or any of the research assessments or training paradigms and will notify the principal investigator. These non-study related medical events are followed by the research team and will be reported to the IRB as outlined in Medical Reporting Table. A weekly report of all medical events is generated by the research nurse and sent to the study physician (and physician assistant), the principal investigator, and the study physical therapists. In this study, a medical event will only be reported if it occurs on the same day that the assessment associated with this study also occurs.

5. Data Safety and Monitoring Plan

Day-to-day oversight of the trial is provided by the Principal Investigators (PIs), Drs. Herrity and Harkema. They will review all study data and any adverse events, and report all adverse events to the Medical Monitor, sponsor, and University of Louisville IRB. Monitoring for the study will be provided by a Medical Monitor who will review study conduct including accrual, drop-outs, protocol deviations, and AEs in aggregate on an annual basis. The Medical Monitor will review serious adverse events (SAEs) in real-time. All data analyses (Labs, urodynamics, ultrasound) will be conducted as assessed with annual summary reports generated. Study data will be provided to the Medical Monitor to each annual review

one month prior. Data reports will be prepared by the principal investigators and the study statistician. The Medical Monitor will provide a written report to the study team with recommendations for study modification, study continuation/discontinuation as relevant. The study team is responsible for forwarding the report to the IRB.

Figure 2. Study Timeline.

Screening Phase		**Intervention**			**Midpoint**		**Intervention**			**Post Intervention**	
Assessments		**Groups**			**Assessments**		**Groups**			**Assessments**	
Labs	**Fxnl*	*Gent (n=12)*	*Saline (n=12)*	*None (n=12)*	*Labs*	**Fxnl*	*Gent (n=12)*	*Saline (n=12)*	*None (n=12)*	*Labs*	**Fxnl*
UA Culture + Sensitivity CBC CMP HCG	UDS US ~Intake forms	IC (n=6) 28.8 mg in 60 ml NS	IC (n=6) 60 ml NS	IC (n=6) No procedure	UA Culture + Sensitivity CBC CMP HCG	UDS US ~Intake forms	IC (n=6) 28.8 mg in 60 ml NS	IC (n=6) 60 ml NS	IC (n=6) No procedure	UA Culture + Sensitivity CBC CMP HCG	UDS US ~Intake forms
		SP (n=6) 28.8 mg in 60 ml NS	SP (n=6) 60 ml NS	SP (n=6) Catheter clamping for duration of instillation			SP (n=6) 28.8 mg in 60 ml NS	SP (n=6) 60 ml NS	SP (n=6) Catheter clamping for duration of instillation.		
5 days		**80 instillations - 7x/wk - 2x/d**			**5 days**		**80 instillations 7x/week – 2/d**			**5 days**	

CBC, Complete Blood Count; CMP, Comprehensive Metabolic Panel; Gent, Gentamicin sulfate; IC, Intermittent Catheterization; Fxnl, Functional; HCG, Human Chorionic Gonadotropin (Pregnancy Test); NS, Normal Saline; SP, Suprapubic Catheter; UDS, Urodynamic Study; US, Ultrasound.

*****The term "functional", abbreviated fxnl in this study, signifies an objective assessment of bladder functioning using urodynamic testing and ultrasound imaging.

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