The following includes all aspects of the protocol entitled, "*The Impact of Self-Management with Probiotics on Urinary Symptoms and the Urine Microbiome in Individuals with Spinal Cord Injury and Spina Bifida"* in which we propose use of intravesicular *Lactobacillus* to reduce urinary symptoms in people with neuropathic bladder.

Specific Aim 2 Methods and Procedures: **Develop and pilot a UTI Self-Management Protocol using Probiotics (SMP_{PRO}) to reduce the severity, frequency and impact of urinary symptoms.** The validated Urinary Symptom Questionnaire for people with Neuropathic Bladder (USQ-NB; developed in Aim 1 of this study, currently in progress IRB # 2012-187) will be the linchpin of the SMP_{PRO} protocol. The principal utility of the SMP_{PRO} will be in helping individuals identify the symptoms that signal recourse to increase fluid intake to reduce symptoms, initiate probiotic treatment, or seek medical attention.

Development of the SMP_{PRO} has been an iterative participatory design process. The multidisciplinary team began refining the decision tree underlying the protocol, concurrent with development of the USQ-NB, informed by patient focus groups, and later will be assessed through large scale national testing in conjunction with United Spinal Association. The prototype SMP_{PRO} will be shared with a diverse sample of individuals with Spina Bifida (SB), Spinal Cord Injury (SCI), and Multiple Sclerosis to develop use cases and scenarios of use to further inform the protocol. These patients further informing the SMP_{PRO} will include adults, youth, and children, both genders, persons who reuse catheters and those who don't, among individuals with SCI, SB, and MS who use intermittent catheterization for their bladder management. Participants will engage in semi-structured interviews in which they will be asked to recall the onset and progression of symptoms in their most recent episode of urinary symptoms. The interviewer will then ask participants to mentally step through the experience of urinary symptoms: 1) to confirm that the prototype SMP_{PRO} can be triggered by the items composing the USQ-NB and 2) to elucidate the mechanics of implementing the protocol across the diversity of individuals with neuropathic bladder due to SB, SCI or MS. Interview data will be managed, coded, and thematically analyzed using NVivo 10 qualitative software. Use cases and scenarios of use will be developed from themes. The interdisciplinary team will refine and expand the SMP_{PRO} to accommodate patient experience augmented by use cases and scenarios of use.

THE FOLLOWING SECTIONS REPRESENT ASPECTS OF THE PROPOSED STUDY IN WHICH THE *LACTOBACILLUS* PROBIOTIC PROTOCOL WILL BE IMPLEMENTED BY PATIENTS.

Once the SMP_{PRO} has been revised and tailored to the patient experience, we will conduct a multi phase pilot study with 120 individuals with neuropathic bladder due to SB or SCI/ Multiple Scleorosis to study its safety, acceptability and perceived usefulness. Pilot data will include weekly USQ-NB and records of any urinary symptoms and UTIs that are experienced.

Phase Ia: Safety study of a single bladder instillation of probiotic in adults. A subsample of healthy adults with neuropathic bladder who do not have urinary symptoms meeting the criteria for antibiotic treatment of urinary tract infection or initiation of the protocol will undergo a single intravesical instillation of *Lactobacillus* to determine the tolerability of the instillation, safety of a single instillation, and whether the instilled *Lactobacillus* persists in the urine.
Study Design: We will recruit N=5 adult subjects with neuropathic bladder due to SCI or SB.
Clinical Setting: MedStar National Rehabilitation Hospital

Target Enrollment: 5 subjects at least 18 years of age with SCI or SB and at least 1-year post-injury (if SCI).
Informed Consent: Consent will by obtained by the PI, Study Coordinator, or Research Assistant.
Inclusion Criteria: Inclusion criteria for safety study participants are: 1) age 18-50 years; 2) if SCI, at least 1-year duration; 3) neuropathic bladder, as determined by the attending physician; 4) utilizing intermittent catheterization for bladder management; and 5) community dwelling.
Exclusion Criteria: Study candidates with the following will be excluded: 1) known genitourinary pathology beyond neuropathic bladder (i.e., vesicoureteral reflux, bladder or kidney stones, etc.); 2) use of prophylactic antibiotics; 3) instillation of other intravesicular agents to reduce urinary symptoms or UTI (i.e., gentamycin); 4) psychologic or psychiatric conditions influencing the ability to follow instructions; 5) participation in another study in which results would be confounded; 6) pregnant or breastfeeding women; 7) individuals with a history of acquired or genetic immunodeficiencies; active, acute or chronic serious infections (e.g., viral hepatitides, HIV/AIDs); 8) individuals with cancer/autoimmune disorders; 9) serious allergy to any component or excipients in the live bacterial combination product; 10) no change in neurologic status in the previous 2 weeks; 11) taken antibiotic for any reason in the previous 2 weeks; 12) any patient with history of sensitivity or allergy to ampicillin or tetracycline; and 13) current urinary tract infection or urinary tract infection within the previous 2 weeks; (as defined by Infectious Diseases Society of America CAUTI Guidelines, 2010. According to the Infectious Disease Society of America Guidelines for Diagnosis of Catheter-Associated UTI, the presence of the following urinary symptoms is required for UTI diagnosis in patients using intermittent catheterization: new onset or worsening fever, rigors, altered mental status, malaise, lethargy with no other identified cause, flank pain, costovertebral angle tenderness, acute hematuria, pelvic discomfort, increased spasticity, autonomic dysreflexia or sense of unease. The Guidelines specifically state that "the presence or absence of odorous or cloudy urine should NOT be used as an indication for urine culture or antimicrobial therapy" and "the presence, absence or degree of pyuria should NOT be interpreted as an indication for antimicrobial treatment." If a subject presents with any of these symptoms and there are $\geq 10^3$ cfu/hpf bacterial growth on urine culture, the subject will be excluded and treated for a UTI according to clinical best practice.
Baseline Data Collection: At baseline, subjects who do not report urinary symptoms (see below) other than cloudy or foul-smelling urine will complete the validated USQ-NB. Urine will be collected for urinalysis and urine culture (see below for methods of urine collection), however urinalysis results will be used for post hoc data collection only and not inclusion/exclusion in keeping with the recommendations of the IDSA.
Intervention: Eligible patients will undergo 1 *Lactobacillus* (Culturelle GG, 20 Billion live organisms) instillation by study personnel at the study site. For the intravesicular *Lactobacillus* instillation, the contents of 1 *Lactobacillus* capsule will be mixed into 45 cc sterile 0.9% saline (adult bladder volume is 450cc, so this amount represents 10% of adult bladder volume). After mixing, study personnel will draw up the 45 cc of the liquid *Lactobacillus* mixture into a 60cc catheter tip syringe and instill via the intermittent catheter. Participants will be observed in the clinic for 30 minutes after instillation to assure that no adverse reactions occur.
Post-Intervention Follow-Up: After completion of the one *Lactobacillus* instillation and 30 minute observation period, participants will be asked to monitor for any symptoms using the USQ-NB daily for one week. They will be instructed to contact study personnel in the event of any urinary symptoms. They will then return to the clinic 5-10 days after instillation for repeat urinalysis and urine culture.

Phase 1b: Safety study of single bladder instillation probiotic in children with SB or SCI. After Phase 1a is complete, accumulated safety data (inclusive of adverse events and laboratory results)

will be presented to the DSMB. Phase 1b will be initiated only after review and approval of the Phase 1a safety data by the DSMB. Once approval demonstrates that a single dose of intravesiclur Lactobacillus has been determined to be safe in adults, the same safety study will be repeated in children. A subsample of healthy children with neuropathic bladder due to SB who do not have urinary symptoms meeting the criteria for antibiotic treatment or initiation of the protocol will undergo a single intravesical instillation of *Lactobacillus* to determine the tolerability of the instillation, safety of a single instillation, and whether the instilled *Lactobacillus* persists in the urine.

Study Design: We will recruit N=5 children with neuropathic bladder due to SB or SCI.

Clinical Setting: MedStar National Rehabilitation Hospital and Children's National Medical Center.

Target Enrollment: 5 subjects at least 6 years of age with SB or SCI, at least 1-year post-injury (if SCI).

Informed Consent: Consent and assent will by obtained by the PI, Study Coordinator, or Research Assistant.

Inclusion Criteria: Inclusion criteria for safety study participants are: 1) age >=6 years; 2) if SCI, at least 1-year duration; 3) neuropathic bladder, as determined by the attending physician; 4) utilizing intermittent catheterization for bladder management; and 5) community dwelling.

Exclusion Criteria: Study candidates with the following will be excluded: 1) known genitourinary pathology beyond neuropathic bladder (i.e., vesicoureteral reflux, bladder or kidney stones, etc.); 2) use of prophylactic antibiotics; 3) instillation of intravesicular agents to reduce UTI (i.e., gentamycin); 4) psychologic or psychiatric conditions influencing the ability to follow instructions; 5) participation in another study in which results would be confounded; 6) pregnant or breastfeeding; 7) individuals with a history of acquired or genetic immunodeficiencies; active, acute or chronic serious infections (i.g., viral hepatitides, HIV/AIDs); 8) individuals with cancer/autoimmune disorders; 9) serious allergy to any component or excipients in the live bacterial combination product; 10) no change in neurologic status in the previous 2 weeks; 11) taken antibiotic for any reason in the previous 2 weeks; and 12) current urinary tract infection or urinary tract infection within the previous 2 weeks (as defined by Infectious Diseases Society of America CAUTI Guidelines, 2010. According to the Infectious Disease Society of America Guidelines for Diagnosis of Catheter-Associated UTI, the presence of the following urinary symptoms is required for UTI diagnosis in patients using intermittent catheterization: new onset or worsening fever, rigors, altered mental status, malaise, lethargy with no other identified cause, flank pain, costovertebral angle tenderness, acute hematuria, pelvic discomfort, increased spasticity, autonomic dysreflexia or sense of unease. The Guidelines specifically state that "the presence or absence of odorous or cloudy urine should NOT be used as an indication for urine culture or antimicrobial therapy" and "the presence, absence or degree of pyuria should NOT be interpreted as an indication for antimicrobial treatment." If a subject presents with any of these symptoms and there are $\geq 10^3$ cfu/hpf bacterial growth on urine culture, the subject will be excluded and treated for a UTI according to clinical best practice.

Baseline Data Collection: Same as for Phase Ia Safety Study

Intervention: Eligible patients will undergo 1 *Lactobacillus* pediatric dose (Culturelle GG 10 Billion live organisms) instillation by study personnel at the study site. For the intravesicular *Lactobacillus* instillation, the contents of 1 *Lactobacillus* capsule will be mixed into sterile 0.9% saline using an age-based estimate of bladder capacity (see Table below). Similar to adults, this bladder instillation will represent 10% of the estimated maximum bladder volume based on age.

Age (yrs)	Estimated Bladder Capacity (ml)	Volume Culturelle mixture infused (ml)	# of particles delivered per dose (billions)
6	270	27	12.0
7	285	28.5	12.7
8	300	30	13.3
9	315	31.5	14.0
10	330	33	14.7
11	345	34.5	15.3
12	360	36	16.0
13	375	37.5	16.7
14	390	39	17.3
15	405	40.5	18.0
16	420	42	18.7
17	435	43.5	19.3
18	450	45	20.0

*Reference: Kaefer M1, Zurakowski D, Bauer SB, Retik AB, Peters CA, Atala A, Treves ST. Estimating normal bladder capacity in children. J Urol. 1997 Dec;158(6):2261-4.

After mixing, study personnel will draw up the liquid *Lactobacillus* mixture into a 60cc catheter tip syringe and instill via the intermittent catheter. Participants will be observed in the clinic for 30 minutes after instillation to assure that no adverse reactions occur.

Post-intervention Follow-Up: Same as for Phase Ia Safety Study

Phase II: Pilot study assessing the USQ-NB and SMP_{PRO}

We will recruit up to 120 persons from a national sample in who will use the SMP_{PRO} in order to **estimate the strength of the associations between successful implementation of the probiotic self-management program and urinary symptoms.** We will conduct a 18-month study in which each participant will serve as his/her own control through 3 phases of study: 6-months usual care (baseline), 6-months probiotic intervention, and 6-months follow-up.

Study Design: We will recruit N=up to 120 subjects with neuropathic bladder who will utilize the SMP_{PRO} as directed, and we will follow patient satisfaction and urinary symptom outcomes, but not utilize urinalysis or urine culture (as this is the intended use of the SMP_{PRO} in practice).

Clinical Setting: Enrollment through a national database

Target Enrollment: Adult subjects at least 1-year post-injury and 18 years of age with SCI/Multiple Sclerosis (MS) or at least 18 years of age with SB or pediatric subjects at least 6 years of age with SB or SCI.

Recruitment Strategy: SCI/MS and SB subjects will be enrolled with assistance from United Spinal Association. Additionally, we will query the MedStar National Rehabilitation Hospital inpatient service, the 40+ MedStar National Rehabilitation Network outpatient sites, various MedStar Network databases, and Children's National Medical Center (CNMC) SB database. Databases will be queried for participants who meet the eligibility criteria and have already

provided HIPAA authorization and consent to be contacted for future research. Announcements and IRB-approved flyers will be posted on the United Spinal Association and study websites and at participating institutions.
Informed Consent: Informed consent will be emailed or mailed (if not in person at MedStar NRH or CNMC) out to participants interested in the study. Paid return envelopes will be included for participants to return signed informed consent to Study Coordinator. At MedStar NRH and CNMC, consent will by obtained by the PI, Study Coordinator, or Research Assistant.
Inclusion Criteria: Inclusion criteria for participants with SCI/ MS includes: 1) age≥18 years; 2) SCI/MS; 3) neuropathic bladder at least 1 year duration, as determined by the attending physician; 4) utilizing intermittent catheterization for bladder management; 5) a history of 2 or more UTIs in the past year; and 6) community dwelling. Inclusion criteria for participants with SB adults includes: 1) age≥18 years; 2) SB; 3) neuropathic bladder as determined by the attending physician; 4) utilizing intermittent catheterization for bladder management; 5) a history of 2 or more UTIs in the past year; and 6) community dwelling. Inclusion criteria for pediatric subjects includes: 1) age≥6 years; 2) SB or SCI; 3) neuropathic bladder at least 1 year duration (for SCI), as determined by the attending physician; 4) utilizing intermittent catheterization for bladder management; 5) a history of 2 or more UTIs in the past year; and 6) community dwelling.

Exclusion Criteria: Study candidates with the following will be excluded: 1) known genitourinary pathology beyond neuropathic bladder (i.e., vesicoureteral reflux, bladder or kidney stones, etc.); 2) use of prophylactic antibiotics; 3) instillation of intravesicular agents to reduce UTI (i.e., gentamycin); 4) psychologic or psychiatric conditions influencing the ability to follow instructions; 5) participation in another study in which results would be confounded; 6) pregnant or breastfeeding; 7) individuals with a history of acquired or genetic immunodeficiencies; active, acute or chronic serious infections (i.g., viral hepatitides, HIV/AIDs); 8) individuals with cancer/autoimmune disorders; 9) serious allergy to any component or excipients in the live bacterial combination product; 10) no change in neurologic status in the previous 2 weeks; 11) taken antibiotic for any reason in the previous 2 weeks; 12) any patient with history of sensitivity or allergy to ampicillin or tetracycline; and 13) current urinary tract infection or urinary tract infection within the previous 2 weeks (as defined by Infectious Diseases Society of America CAUTI Guidelines, 2010. According to the Infectious Disease Society of America Guidelines for Diagnosis of Catheter-Associated UTI, the presence of the following urinary symptoms is required for UTI diagnosis in patients using intermittent catheterization: new onset or worsening fever, rigors, altered mental status, malaise, lethargy with no other identified cause, flank pain, costovertebral angle tenderness, acute hematuria, pelvic discomfort, increased spasticity, autonomic dysreflexia or sense of unease. The Guidelines specifically state that "the presence or absence of odorous or cloudy urine should NOT be used as an indication for urine culture or antimicrobial therapy" and "the presence, absence or degree of pyuria should NOT be interpreted as an indication for antimicrobial treatment." If a subject presents with any of these symptoms and there are $\geq 10^3$ cfu/hpf bacterial growth on urine culture, the subject will be excluded and treated for a UTI according to clinical best practice.
Baseline Data Collection: After consent, participants will complete the Health History Questionnaire Thereafter, participants will complete the Urinary Symptom Questionnaire (USQ-NB) weekly.
Intervention: After 6 months of baseline data collection, participants will receive the *Lactobacillus* (Culturelle) kits. Participants will be instructed on preparation and intravesicular instillation of the *Lactobacillus*, and will have a tutorial with a fellow consumer on use of the patient-initiated SMP_{PRO}. Participants will receive 10 Culturelle tablets the beginning of the

treatment phase. At the end of each week, the coordinator or RA will ask how many remaining tablets the participant has and will instruct them to complete the USQ-NB weekly.
If/when urinary symptoms occur, they will be instructed to follow the SMP_{PRO} protocol to determine whether to initiate intravesicular *Lactobacillus* instillation or to seek medical attention. The self-management protocol will also direct them to discontinue *Lactobacillus* instillation or to seek medical attention if symptoms remit, persist or worsen. If participants are directed by the self-management protocol to seek medical attention or s/he feels the need for medical evaluation, s/he will be advised to obtain care as they typically would by their health care provider. Participants will be supplied with letters to be brought to their health care provider notifying them of the study and requesting sharing of urinalysis and urine culture results with the research team. A verified UTI will include those that resulted in antibiotic treatment prescribed by a health care professional.

For the intravesicular *Lactobacillus* instillation, the contents of 1 *Lactobacillus* capsule will be mixed into 45 cc sterile 0.9% saline (adult bladder volume is 450cc, representing 10% of adult bladder volume). After mixing, study participants will draw up the 45 cc of the liquid *Lactobacillus* mixture into a 60cc catheter tip syringe and instill via the intermittent catheter using sterile technique. For children volume is based on age (see table on page 4 of protocol).

Study participant will recheck symptoms after 24 hrs (valid range 22-30 hrs) following the SMP_{PRO} protocol and either perform another *Lactobacillus* instillation or seek medical attention, per the subjects choice. If symptoms still persist 4 hrs after second instillation, study participants are instructed to seek medical attention.

Post-Intervention Follow-Up: After completion of the 6-month patient-initiated self-management protocol intervention period, participants will monitor symptoms weekly using the USQ-NB.

Measurements:

Health History Questionnaire: After subjects give consent, a brief medical history will be conducted using a structured questionnaire, to obtain demographic information and relevant family history (these items will be collected only at baseline), and genitourinary history

Urinary Symptom Questionnaire (USQ-NB): A preliminary urinary symptom questionnaire has been developed with consumer input based on the most common typical and SCI/MS-specific urinary symptoms. The final version (developed in Specific Aim 1 IRB # 2014-187) will be used in this phase of the study. USQ-NB will be completed weekly. All participants will receive a link to the questionnaire every Sunday morning. If they have not responded by Monday morning, they will receive another link. If they have not responded by Tuesday, the study participant will receive a phone call. If there is no response by noon Wednesday, it is considered a missed event.

Patient satisfaction: We have developed a simple four item patient satisfaction "survey" that will be requested at the end of each timeframe. Participants will be asked to rate their satisfaction, thinking only about the preceding time period, with changes in the *frequency* of their symptoms; the amelioration of the *severity* of their symptoms; and changes in the *impact* of their symptoms. A 5-point Likert scale will be used for each of these items (not at all satisfied; not very satisfied; no perceived difference; satisfied; and very satisfied). A fourth item, "Can you estimate, using a scale from 0% <would absolutely never do this>, through 50% <might do this> to 100% <would absolutely always do this>, whether or not you would seek this intervention out and pay for it yourself if insurance did not pay for it".

Medical Information:

If participants instill *lactobacillus* at any time during the intervention phase, and after Lactobacillus instillation reports a UTI as an Adverse Event (AE) we will request their urinalysis and urine culture results to attempt to determine if *Lactobacillus* might have been the cause of the UTI. If determined necessary by the PI, Co-PI and/or DSMB other AEs reported could require retrieval of medical records.

Phase III: **Pilot Study of Multiple (up to 2) *Lactobacillus* Bladder Instillations**
We will recruit 30 persons in whom we will collect urine up to 18 timepoints (up to monthly for the first year when not experiencing symptoms, up to two times when subject experience urinary symptoms, follow-up after either lactobacillus treatment or antibiotics, and three (3) months into the six (6) months follow-up) in order to **estimate the strength of the associations between successful implementation of the probiotic self-management program and urinary symptoms, bladder inflammation, and the urine microbiome.** We will conduct an 18-month study in which each participant will serve as his/her own control through 3 phases of study: 6-months usual care (baseline), 6-months probiotic intervention, and 6-months follow-up
Study Design: We will recruit N=30 subjects with neuropathic bladder who will contribute urine samples for analyses.
Clinical Setting: MedStar National Rehabilitation Hospital and Children's National Medical Center.
Target Enrollment: 30 adult subjects at least 1-year post-injury and 18 years of age with SCI/MS or at least 18 years of age with SB
Recruitment Strategy: SCI/MS and SB subjects will be enrolled with assistance from United Spinal Association. Additionally, we will query the MedStar National Rehabilitation Hospital inpatient service, the 40+ MedStar National Rehabilitation Network outpatient sites, various MedStar Network databases, and the CNMC Spina Bifida database. Databases will be queried for participants who meet the eligibility criteria and have already provided HIPAA authorization and consent to be contacted for future research. Announcements and IRB-approved flyers will be posted on the United Spinal Association and study websites and at participating institutions.
Informed Consent: Consent will by obtained by the PI, Study Coordinator, or Research Assistant. All project staff are currently CITI certified and have passed all requirements for HIPAA certifications.
Inclusion criteria for participants with SCI/SB/MS includes: 1) age≥18 years; 2) SCI/SB/MS; 3) neuropathic bladder at least 1-year duration, as determined by the attending physician; 4) utilizing intermittent catheterization for bladder management; 5) a history of 2 or more UTIs in the past year; 6) community dwelling, 7) Enrollment in Phase II
Exclusion Criteria: Study candidates with the following will be excluded: 1) known genitourinary pathology beyond neuropathic bladder (i.e., vesicoureteral reflux, bladder or kidney stones, etc.); 2) use of prophylactic antibiotics; 3) instillation of intravesicular agents to reduce UTI (i.e., gentamycin); 4) psychologic or psychiatric conditions influencing the ability to follow instructions; 5) participation in another study in which results would be confounded; 6) pregnant or breastfeeding; 7) individuals with a history of acquired or genetic immunodeficiencies; active, acute or chronic serious infections (i.e., viral hepatitides, HIV/AIDs); 8) individuals with cancer/autoimmune disorders; 9) serious allergy to any component or excipients in the live bacterial combination product; 10) no change in neurologic status in the previous 2 weeks; 11) taken antibiotic for any reason in the previous 2 weeks; 12) any patient with history of sensitivity or allergy to ampicillin or tetracycline; and 13) current urinary tract infection or urinary tract infection within the previous 2 weeks (as defined by Infectious Diseases Society of America CAUTI Guidelines, 2010 14) Not enrolled in Phase II. According to the Infectious Disease Society of America Guidelines for Diagnosis of Catheter-Associated UTI, the presence of the

following urinary symptoms is required for UTI diagnosis in patients using intermittent catheterization: new onset or worsening fever, rigors, altered mental status, malaise, lethargy with no other identified cause, flank pain, costovertebral angle tenderness, acute hematuria, pelvic discomfort, increased spasticity, autonomic dysreflexia or sense of unease. The Guidelines specifically state that "the presence or absence of odorous or cloudy urine should NOT be used as an indication for urine culture or antimicrobial therapy" and "the presence, absence or degree of pyuria should NOT be interpreted as an indication for antimicrobial treatment." If a subject presents with any of these symptoms and there are $\geq 10^3$ cfu/hpf bacterial growth on urine culture, the subject will be excluded and treated for a UTI according to clinical best practice.

Participant Burden:
Urine samples for urinalysis, urine culture and metagenomic sequencing will be obtained at up to monthly for the first year of study participation when not experiencing symptoms, at up to 2 occurrences of urinary symptoms during SMP_{PRO} intervention phase, and follow-up after either lactobacillus treatment or antibiotics and at follow-up, for a total of up to 18 samples per patient depending on the frequency with which urinary symptoms are experienced. When possible, our Research Assistant will meet with the patient at a mutually convenient location.

Baseline Data Collection:
Per IRB protocol # 2014-211 phase II, after consent, participants will complete the Health History Questionnaire, Urinary Symptom Questionnaire, In addition to questionnaires, all participants will provide a urine sample using sterile catheterization techniques. Thereafter, participants will complete the Urinary Symptom Questionnaire weekly.

Intervention:

After 6 months of baseline data collection the intervention phase will begin. Prior to implementing the intervention protocol, participants will provide study staff with a urine sample either at MedStar NRH, CNMC, or when possible, the study RA will meet with the participant at a mutually convenient location. After urine sample is collected, study participants will follow the study protocol and complete USQ-NB weekly. If/when urinary symptoms occur, they will be instructed to follow the SMP_{PRO} protocol to determine whether to initiate intravesicular *Lactobacillus* instillation or be evaluated by a physician. The self-management protocol (SMP_{PRO}) will also direct them to discontinue *Lactobacillus* instillation or be evaluated by a physician if symptoms remit, persist or worsen. If participants are directed by the self-management protocol to seek medical attention or s/he feels the need for medical evaluation, s/he will be advised to obtain care as they typically would by their health care provider. Participants will be supplied with letters to be brought to their health care provider notifying them of the study and requesting sharing of urinalysis and urine culture results with the research team. A verified UTI will include those that resulted in antibiotic treatment by a health care professional. An additional urine sample for metagenomics will either be left with the health care provider for pick up by the research team, brought to the research site, or obtained by the RA at a mutually convenient site. If participant is treating urinary symptoms at home with *Lactobacillus,* a urine sample will be dropped off either at MedStar NRH, CNMC or when possible, the study RA will meet with the participant at a mutually convenient location.

For the intravesicular *Lactobacillus* instillation, the contents of 1 *Lactobacillus* capsule will be mixed into 45 cc sterile 0.9% saline (adult bladder volume is 450cc, so this amount represents 10% of adult bladder volume). After mixing, study personnel will draw up the 45 cc of the liquid *Lactobacillus* mixture into a 60cc catheter tip syringe and instill via the intermittent catheter. For children volume is based on age (see table on page 4 of protocol). Study participant will recheck symptoms after 24 hrs (valid range 22-30 hrs) following the SMP_{PRO} protocol and either complete another *Lactobacillus* instillation or seek medical attention. If symptoms still persist 4 hrs after second instillation, study participants should seek medical attention.

Post-Intervention Follow-Up:
After completion of the 6-month patient-initiated self-management protocol intervention period, participants will monitor symptoms weekly using the USQ-NB. At the end of follow-up, we will obtain another urine sample from all participants.

Measurements:
In addition to the measurements described in Phase II the following measurements will occur for phase III;

Urine Acquisition: A 50-100 ml urine sample will be collected from each subject for (1) urinalysis and cultivation, and (2) urine microbiome by SMRT sequencing. Urine will be collected from a new, unused intermittent catheter.

Urine Preparation: Collected urine will be immediately placed at 4°C. Within 6 hours, samples will be taken to CNMC for sequencing preparation. At CNMC, urine samples will be centrifuged at 4°C, 5,000 x g for 20 minutes. The supernatant will be aliquoted in 2 ml cryotubes and frozen at -20°C. 10ml PBS will be added to the pellet with the remaining supernatant and then centrifuged (5,000xg) at 4°C for 20 minutes. The pellets and aspirated PBS wash solution will be stored at -20°C. Pellets will later be transferred for DNA isolation and SMRT sequencing.

Urinalysis**:** Urine samples will be assessed utilizing standard clinical microbiology semiquantitative chemical testing using commercial disposable test strips for glucose, bilirubin, ketone, specific gravity, blood pH, protein, urobilinogen, nitrite, and leukocyte esterase. After centrifuging for 5 minutes, microscopic examination for and quantification of WBCs, RBCs, epithelial cells, yeast, bacteria, *Trichomonas vaginalis*, sperm cells, mucous filaments and crystals will be performed using standard microbial techniques.

Urine Culture**:** Standard urine culture microbiology will be performed. A measured amount is inoculated to each of the appropriate media. A calibrated loop designed to deliver a known volume (0.0 I ml per loopful) of urine is used. The sample will be mixed the thoroughly and the top of the container removed. A calibrated wire-inoculating loop is flamed and allowed to cool without coming in contact with any surface. The sterile loop is inserted into the urine sample vertically and urine is allowed to adhere to the loop. The loopful of urine is inoculated over MacConkey agar plates using standard methods. Similarly, a second loopful is collected and inoculated on a blood agar plate. The plates are incubated aerobically at 35-37ºC for at least 24 hours. The colonies and colony forming units (CFUs) are counted by multiplying with 100 (since 0.01 ml loop was used).

SMRT Sequencing Methods*:* Thawed urine samples will be clarified by low-speed centrifugation and bacterial genomic DNA will be isolated from pelleted bacteria using a DNeasy tissue extraction kit (Qiagen) according to manufacturer's instruction. 16S rRNA genes will be amplified using universal primers 28F 5'- AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-ACCTTGTTACGACTT-3'. These primers will amplify sequences that correspond to the sequence between positions 27 and 1492 in *E. coli* 16S rRNA and produce amplification products of approximately 1500 bp. These amplification products will be used for sequencing template

preparation using DNA Template Prep Kit 2.0 (Pacific Biosciences) followed by DNA Polymerase binding (DNA Polymerase Binding Kit P4, Pacific Biosciences). Briefly, ends of the 16S rRNA will be repaired with the Klenow DNA polymerase, SMRT Bell adapters will be ligated with the PCR products, followed by annealing of sequencing primers and ternary complex formation with the P4 DNA polymerase. Ternary complexes will be loaded onto SMRT cells and sequenced on the Pacific Biosciences RSII instrument using DNA Sequencing Reagent 2.0 and one 90 min movie. Resulting circular consensus sequence (CCS) reads will be used for taxonomic classification and diversity measurements. The amount of ternary complex needed for the optimal loading of the zero molecular waveguides (ZMW) will be optimized by loading different amounts of ternary complex and measuring number of productive ZMWs. Sequenced rRNA genes will be analyzed and classified using Pathoscope. We will use the following formula to determine the number of SMRT cells needed to detect most/all bacterial species in our samples: # of SMRT cells = (189 * 1 * 87/30,000). Our preliminary data from 454 sequencing indicated the presence of approximately 189 bacterial species in samples from patients with neuropathic bladder. The ratio between most and least abundant bacterial species was 87. CCS protocol yields one accurate consensus read per sequenced rRNA gene, representing a member of the microbiome. Therefore, to ensure detection of most/all bacterial species in the sample and accurate sequence determination 1 SMRT cell per patient sample will be sufficient.

Patient satisfaction: We have developed a simple four item patient satisfaction "survey" that will be requested at the end of each timeframe. Participants will be asked to rate their satisfaction, thinking only about the preceding time period, with changes in the *frequency* of their symptoms; the amelioration of the *severity* of their symptoms; and changes in the *impact* of their symptoms. A 5-point Likert scale will be used for each of these items (not at all satisfied; not very satisfied; no perceived difference; satisfied; and very satisfied). A fourth item, "Can you estimate, using a scale from 0% <would absolutely never do this>, through 50% <might do this> to 100% <would absolutely always do this>, whether or not you would seek this intervention out and pay for it yourself if insurance did not pay for it".

Data and Safety Monitoring Plan

A Data Safety Monitoring Board has been established. Data and Safety Monitoring Board (DSMB) reports will be prepared monthly during the Phase I "Baseline: Safety Evaluation of a Single Instillation in Adults" period, and then two times a year (or as requested by the DSMB) for Phase II. Each report will consist of five sections, including: 1) recruitment, 2) adverse effects of the investigational product (i.e. the intravesicular Lactobacillus instillation), 3) completion of the Urinary Symptom Questionnaire (USQ-NB), 4) completion of, and adherence to, the Self-Management Protocol (SMP_{PRO}), and 5) data accrual and quality. The recruitment section will include counts for screening (eligible v. ineligible), enrollment (consented v. declined), and retention (completion of phase milestones and transitions v. withdrawal or lost-to-follow-up). For reporting purposes, subgroups will include age (adult v. child), neuropathic bladder etiology (Spinal Cord Injury v. Spina Bifida), and standard demographics (i.e. gender, race, and ethnicity). The adverse effects section will report all adverse outcomes that appear to be associated with the investigational product, and will include specific event details, relevant tests and laboratory results, relevant histories (including pre-existing and co-existing medical conditions), use of concomitant medications, and investigational product dose, frequency, route of instillation, timing of instillation, manufacturer's lot number, and manufacturer's expiration date (as appropriate for completion of the MedWatch FDA Form 3500A). The USQ-NB completion section will report the frequency of on-line survey completion, with and without the need for study staff reminders. The SMP_{PRO} completion/adherence section will report the frequency of on-line survey completion, with and without reminders, as well as the appropriateness/correctness of protocol

implementation, investigational product instillation, and medical care seeking. The data accrual and quality section will report counts of completed surveys and case report forms at phase-specific time points, as well as counts of specific responses to individual questions.

H1. **Compared with usual management, self-management of urinary symptoms with probiotics will reduce the frequency, severity and impact of urinary symptoms and UTIs (PRIMARY OUTCOMES)**. The goal of aim 2 is to assess the effect of *Lactobacillus* supplementation on the frequency, severity and impact of urinary symptoms and UTIs. This aim can be evaluated with all 100 participants in the SMP pilot study. We expect to find a significant difference in these primary outcomes between our three experimental timeframes (6 months prior to intervention, 6 months of SMP_{PRO} intervention, and 6 month follow-up period). Frequency of UTI will be defined as the number of verified UTIs occurring during the timeframe. Urinary symptoms will be assessed using the USQ-NB where frequency of symptoms, degree of severity, and impact on the individual, will be considered.

We will estimate the average number of symptoms on the USQ-NB endorsed (irrespective of frequency, severity and impact) across the weeks during that time frame, for each participant. Thus, each participant will have a continuous outcome (average number of USQ-NB items endorsed). We will also compute the number of verified UTIs experienced by each participant. These two outcomes are the primary outcomes for our study and analysis; they depend solely on the face validity of the USQ-NB,and on clinically verified UTIs, and so will not be affected by a failure to find or establish formal measurement properties for the USQ-NB, should any occur. They will also be less subject to any variability in participant perceptions of frequency, severity or impact, which can complicate analytic assumptions and limit power and interpretability of change that might be perceived. We will describe these features of the sample over time but will not conduct inference tests on these ratings.

We propose linear mixed-effects modeling to explore the effect of introduction to/use of the SMP_{PRO} on the number of USQ-NB symptoms (averaged over the entire time frame) and the incidence of UTIs. Each model will include gender as a fixed effect, and time (baseline; SMP_{PRO} use; post-SMP_{PRO} observation) as the explanatory variable, and random effects for intercept (subject) only. This method allows for missing data and the inclusion of covariates where appropriate; however we plan post-hoc analyses of the modeling in order to pinpoint potential interaction effects (e.g., between age or sex and time). The focus of the analyses is to determine whether (irrespective of individual starting values or specific slopes) mean USQ-NB item endorsement and UTI counts go down in the SMP_{PRO} time frame relative to the baseline values (generally negative associations in each regression between the outcome and time) and whether these values stay flat (zero slope), continue to decrease (negative slope) or increase (positive slope) during the post-SMP_{PRO} follow up.

Power calculation: We plan to use mixed effects regression modeling for the most precision in our estimated effects of the SMP_{PRO} on the USQ-NB symptom rate and UTI rate. However, it is difficult to predict the exact form of the final models since our understanding of the USQ-NB symptom makeup, and the univariate associations between gender or age (or any other of the demographic variables we will collect) and these two outcomes. Therefore, for the most conservative estimates, we use the most basic analytic method to estimate the worst-case scenario power calculation, a t-test. In this pair of primary outcomes, alpha is set at 0.025 for each power calculation (and for each inference test). We will have data from 100 participants; however, to protect against a possible 20% drop out rate over the course of the 18-month study, we will recruit 120 persons for this arm, hoping for a final N of 100. If we observe an average of 25 items on the USQ-NB endorsed over the baseline period, then with alpha = 0.025 we would have 81% power to detect a reduction in this average number of items endorsed as low as five

(i.e., to detect a change from an average of 25 to an average of 20) during the SMP_{PRO} period, assuming that the standard deviation of the average number of endorsed USQ-NB items is as high as 16. If we observe an average of one UTI per month in the initial 6-month period (for an average of six UTIs across this sample of 100 persons), then, with the alpha of 0.025 we would have 81% power to detect a reduction in this average number of UTIs as low as 1.25 (i.e., to detect a change from an average of 6 to an average of 4.75) during the SMP_{PRO} period, assuming that the standard deviation of the number of UTIs is as high as 4. We will explore the consistency of the UTI variable with the assumption of a normal distribution, and if appropriate we may utilize Poisson modeling if the assumption of normality is violated.

Additionally, because we have the potential to carefully describe the symptom profile for neuropathic bladder generally and for SCI/MS and SB specifically, we also plan secondary analyses based on the USQ-NB items. Frequency, severity, and impact of each of the USQ-NB symptoms will be estimated for each participant, within each of the study timeframes. Each of these symptom characteristics will be rated on an ordinal scale, so f*or each symptom* on the USQ-NB, the median frequency rating from their weekly USQ-NBs will be computed. These ordinal outcomes will be explored for similarities and differences across sex, age, injury (SB/SCI/MS), and other demographic variables that could be useful in diagnosis or management of USQ-NB symptoms by us and other investigators in future studies. Descriptive statistics for each primary outcome (frequency, duration, and severity of each UTI) and their change from baseline, will be produced for each time frame. We will generate preliminary (underpowered and not corrected for multiple comparisons) estimates of the strengths of associations between item frequency, severity, and impact with time, sex, age, satisfaction with the SMP-UTI, and etiology (SCI/MS/SB). If the reliability and validity evidence from Aim 1 and that national sample supports the estimation of latent factor scores on the USQ-NB factors, we will also compute preliminary estimates of association between these covariates and the factor scores, as well as the association between the SMP-UTI and changes in these factor scores. This estimation will only be appropriate if measurement invariance across groups is obtained in Aim 1, but since we would not yet have evidence of invariance over time, these estimates would be considered preliminary.

H2. **Compared with usual management, self-management of urinary symptoms with probiotics will reduce bladder inflammation (Secondary outcome)**. For those 20 participants in the subsample giving urine samples, bladder inflammation will be measured from each urine sample using a standard white blood count (WBC) from the urinalysis. WBC counts will be obtained only at the start of each timeframe, and so can be analyzed longitudinally as continuous variables representing the 6-month pre-treatment period and the 6 month-SMP_{PRO} period. As such we plan a mixed effects linear regression model of WBC counts with time treated as the factor of interest. This analysis will assess whether WBC is significantly different from the SMP_{PRO}, and post-SMP_{PRO}, relative to the baseline pre-treatment periods.

H3. **Compared with usual management, self-management of urinary symptoms with probiotics will reduce the need for antibiotics to treat UTI (Secondary outcome)**. The number of times antibiotics are used for each patient will be obtained from their medical records or self report. This value will be analyzed with the same three-point longitudinal mixed effects regression model with time as the factor of interest. Depending on the distribution of the outcome (i.e. given the count nature of this outcome, it may be Poisson distributed), we will utilize either linear or Poisson regression models. This analysis will assess whether there is an association between UTI rate during use of the SMP_{PRO}, and post-SMP_{PRO} exposure, relative to UTI rate during the baseline pre-treatment period.

H4. Compared with usual management, self-management of urinary symptoms with probiotics will return the microbiome to baseline (Secondary outcome). For analysis of the microbiome, the fasta and fastq files of each sample's CCS reads will be retrieved and processed using LUCY[54] to filter out reads with low quality segments, as well as remove sequences that are shorter than the full length 16S rRNA (approximately 1500 bp). The sequences will then be aligned against the SILVA 16S rRNA database followed by subsequent implementation of Mothur's Chimera Slayer to filter out chimeric reads from sequences in each sample. The remaining sequences will undergo taxonomic classification. Bowtie2 will align each sequence read to a reference genome derived from the SILVA 16S database and output a SAM file. With the resulting SAM file, Pathoscope [51] is capable of characterizing the biological sample to the subspecies-level, generating an excel file with the proportions of each classified organism within the sample tabled.

To determine whether bacterial diversity differs between study groups the Shannon Diversity Index will be used. The Shannon Index is a measure of sample diversity that takes into account total number of bacterial types in the sample and relative proportion of the bacterial types. From the subsample of 50 participants giving urine samples, subjects who did in fact utilize the SMP at least once during the 6-month SMP time frame will be stratified into two groups, those who responded to SMP and those who did not; we will define "response" as the observation of a 10% or more decrease in the average number of USQ items endorsed within any time frame, so that each of the 50 persons in this sub-sample will be characterized as either a responder (10% or more decrease) or a non-responder (0-9.99% decrease). A scatter plot of response group versus Shannon Index will be produced, and a mixed effects linear regression model will test the individual (main) and interaction effects of response group and time on Shannon Index. A positive time coefficient is indicative of increased microbiome diversity and a negative time coefficient indicates reduces diversity. The magnitude of difference between the response groups will be determined using the Yue-Clayton theta similarity index ( ). The index measures the difference in the number and relative abundance of bacterial species between samples[4,5] when =1 two samples have identical community structure and when =0 indicates complete dissimilarity between samples. Pathoscope will be used to calculate abundance of different taxonomic groups at each time point.

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