STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official title: Efficacy and mechanism of action of methenamine hippurate (Hiprex™) in the management of recurrent urinary tract infections in women

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1. Purpose:

Urinary tract infections (UTIs) are the most common bacterial infection in women. According to current guidelines, typical treatment consists of a short course of culture-proven antibiotics[1]; however, UTIs can relapse. Recurrent UTIs (RUTIs) can be detrimental to the patient's health by increasing the chance of a UTI complication, such as potentially life-threatening sepsis (urosepsis) or permanent kidney damage from pyelonephritis. In addition, every time a UTI occurs, the patient will experience bothersome symptoms of urinary frequency and burning on urination and require prompt antibiotic treatment to eliminate the infection. Such repeated exposure to antibiotics can lead to adverse side-effects from the antibiotics used as well as an increase the development of antibiotic resistance. For patients with RUTIs, **there is a need to consider a prophylactic approach to lessen the frequency and severity of these recurrent episodes.**

Various prophylactic agents against UTIs have been studied, such as estrogen, cranberry juice/tablets, acupuncture, probiotics, and antiseptics, which differ from antibiotics in that they can affect a variety of microorganisms as opposed to just bacteria[2]. Hiprex (methenamine hippurate) is one of the most popular antiseptics prescribed for RUTIs. Hiprex has been around for nearly a century, yet limited information is known on its exact mechanism of action in the urine and there are no strategies in place to target its use to each treated individual. Furthermore, despite several trials against different daily antibiotic prophylaxis, its role in the management of RUTIs has not been solidly established. Methenanime hippurate functions by producing formaldehyde, which inhibits bacterial growth [cit, Validus]. Previous studies have shown a modest decrease in the rate of re-infection in women with RUTIs after beginning Hiprex compared to the rate prior to initiating the medication; however, these studies have failed to produce meaningful results regarding women with RUTIs who do not have a complicated infection involving urinary catheters for example. Hiprex metabolism and excretion in the urine has been studied in the 60's, 70's and 80's. Although it is known that **formaldehyde production is enhanced by an acid environment**, urinary pH has not been routinely tested in patients who take this medication, a regrettable oversight which might affect the efficacy of this compound in vivo.

Therefore, the purpose of this study is to measure the concentration of formaldehyde in the urine of women with RUTIs on Hiprex; and then, assuming its urinary presence is confirmed at the

proper acid urinary pH, evaluate if such a therapy has favorable effects in decreasing the rate of RUTIs over time.

2. Background:

UTIs contribute to about 25% of all infections worldwide[2]. In the US alone, UTIs incurred an estimated societal cost, including health care costs and productivity losses, of $3.5 billion in 2015[3]. Women are especially likely to contract UTIs compared to men at a ratio of 8:1 and have a 50-60% chance of experiencing at least one UTI in their lifetime[2,4]. Almost a quarter of these UTIs will recur within the next 6 months[5]. Risk factors of RUTIs include medical conditions, such as pregnancy, diabetes, immunosuppression, and abnormal urinary tracts[2]; however, women without any of these comorbidities are still at risk of RUTIs. While recurrence is already a complication of UTIs, other complications can arise including acute renal failure or multi-organ system involvement with progression into bacteremia and then life-threatening sepsis and shock[6].

Treatment of RUTIs does not differ greatly from the treatment of their uncomplicated counterparts in that both still require culture-based antibiotics. However, patients with frequent UTIs will require repeated courses of antibiotics, a process which in time exposes to adverse side-effects from the medication and enhances the risk of antibiotic resistance[7]. Thus, in patients with a history of RUTIs, prophylaxis should be considered once the treatment of the active UTI has been completed.

RUTI prevention has been attempted via lifestyle modifications and/or supplements. By drinking at least 2-3 liters of fluids a day and voiding frequently, patients will be able to eliminate bacteria from their urinary tracts. Reducing the number of new sexual partners will also decrease the risk of UTIs as will proper hygiene around the time of intercourse[2]. Women are encouraged to wipe from front to back to limit the number of bacteria tracking from the anal and genital areas to the urethra[2]. Prophylactic agents can take many forms. Aside from treatment of active infections, low-dose antibiotics (most commonly taken daily or post-coitally) have been shown to be effective at preventing UTIs[8]; however, they still expose to the risks of antibiotic resistance and/or allergies. Vaginal estrogens have been shown to decrease the rate of UTIs by decreasing vaginal wall atrophy and conferring an unfavorable environment for the colonization of uropathogens with little worry of systemic absorption[2]. Estrogen, along with probiotics, also increase the proliferation of beneficial bacteria like *Lactobacillus*, an important aspect of the natural genito-urinary microbiome protective mechanism. In animal models, D-mannose and cranberry juice/tablets have been found to block receptors that allow bacteria to attach to the bladder wall thus limiting colonization. However, despite having moderate efficacy against RUTIs, long-term adherence to D-mannose treatment was found to be questionable[9] and the amount of D-mannose or cranberry realistically reaching the urinary bladder after an oral intake remains questionable and under study.

Hiprex (methenamine hippurate) is one of the most widely prescribed antiseptics for the prophylaxis of RUTIs[cit]. Earliest recorded usage of methenamine as a urinary antiseptic was described in 1899[10]. Methenamine is hydrolyzed to ammonia and formaldehyde, which is bacteriostatic or bactericidal depending on its concentration[11,12]. The rate of this hydrolysis was found to be greater in solutions with lower pHs, achieving formaldehyde concentrations of clinical use at prescription dosage when the pH was less than 6.0[10,13,14]. Although the urine pH range varies from 5 to 8, a recent study from our group in post-menopausal women identified an average urine pH around 6.0 with daily variability in several patients, possibly related to diet intake[15]. Furthermore, as methenamine is hydrolyzed, the production of ammonia alkalinizes the solution, slowing down the reaction. Thus, in 1961, in order to increase efficacy, its formulation with adding hippurate, which increases the acidity of urine, as Hiprex, was developed[13].

A literature review on the metabolism and urinary excretion of methenamine produced sparse results. Studies by Gollamudi and Strom detailed the use of silica cartridges to determine the concentration of methenamine and formaldehyde in a solution and test this new method on human urine after administration of Hiprex to subjects[16,17]. However, this work has limitations, since only 11 participants were tested and the urine samples were only taken after the first dose of Hiprex rather than after several days or months of Hiprex intake. Strom also analyzed the kinetics of methenamine, finding that a methenamine concentration of 750 µg/mL at pH 6.0 could produce bactericidal concentrations of formaldehyde after 3 hr and that the hydrolysis of methenamine occurred 20 times faster at a pH of 5.0 versus 6.0[14]. Another study determined that the concentration of formaldehyde in urine adequate to produce a bacteriostatic effect (25 µg/mL) could be attained by adding 0.6 mg/mL of methenamine to urine at a pH ≤ 5.7 or 1 mg/mL at pH ≤ 5.85[18]. Because the two latter experiments produced their results *ex vivo,* the rate of excretion of methenamine into the urine and the proof of its metabolism of conversion to formaldehyde in a patient's urine after an oral intake of 1 gm twice a day as currently recommended remains untested.

There are **several studies reporting on the efficacy of Hiprex**; however, most only include patients with specific comorbid conditions. After excluding men, Table 1 includes a review of these studies. A case series found that the administration of Hiprex resulted in a decrease in catheter changes among patients with indwelling catheters[19]. Another long-term study reported that, after starting Hiprex, a decrease in the rate of reinfection of UTIs in geriatric patients hospitalized was noted at two years, especially in those who were supervised while taking their medications[20]; however, the rate of UTIs prior to starting Hiprex was not given. A review of Hiprex, containing 2032 patients across 13 studies, indicated that short-term treatment (1 week or less) of Hiprex in women without renal tract abnormalities led to a significant decline in UTIs[21]. However, the authors admitted that given the heterogeneity in the populations studied and the outcomes measured, there was no clear consensus amongst these studies.

Although the literature offers no recommendation for the length of time to prescribe Hiprex, the current knowledge on Hiprex suggests a possible role of longer courses to fully evaluate its

efficacy in the management of RUTIs. Fortunately, many women evaluated for RUTIs will not have any of the examined comorbid conditions as listed in Table 1, and many will present without an overt risk factor for UTIs; thus, it is important to include these women in future studies.

Hiprex can cause various mild side-effects, such as rashes, diarrhea, sore throat, abdominal pain, and bladder "stinging"[20,21]. Although the side-effects are typically self-limited and non-severe, hemorrhagic cystitis was found in the case of an accidental ingestion of 8 g of methenamine mandelate by a 2½-year-old[22]. Despite the side-effect being due to a different formulation of methenamine from Hiprex as well as the dose being much higher than the recommended maximum of 500 mg, this rare case still underlines the importance of ensuring that Hiprex is effective enough so that its benefit outweighs its potential risks.

In summary, RUTIs can be debilitating in a large proportion of women. Despite the popularity of Hiprex as an RUTI prophylactic agent, there are still **large gaps of knowledge** regarding its use. Among them are its *in vivo* metabolism, including the measurements of urinary excretion of both methenamine and formaldehyde, the optimization of urine pH under 6.0 to enhance Hiprex presumed mechanism of action, and on the clinical side, the effectiveness and safety of Hiprex in geriatric patients as well as those with comorbidities, along with its cost-effectiveness over other long-term prophylactic agents for RUTI management.

Table 1: Literature review on the effect of Hiprex (HX) in women

Author and Year	Study Design	Number of Subjects	Indication	Hiprex dose	Outcome Measures	Findings
Furness 1975[23]	RCT	226	Pregnant with asymptomatic bacteriuria	1 g BID for ≥7 days vs methenamine mandelate 1 g QID vs no treatment	Symptomatic UTI, adverse events	• No significant difference in symptomatic UTI • No relevant adverse events
Gundersen 1986[24]	RCT	50	Post-menopausal	1 g BID for 6 months vs placebo	Symptomatic UTI, bacteriuria, adverse events	• Decrease in symptomatic UTI with HX • Side-effects of nausea and rash
Hoivik 1984[25]	RCT	52	Pre-menopausal	1 g QD or BID vs placebo (duration of treatment unknown)	Symptomatic UTI, adverse events	• Decrease in symptomatic UTI with HX • Side-effect of bladder stinging
Knoff 1985[26]	RCT	64	After gynecological operations requiring short-	2 g BID for 7 days vs placebo	Symptomatic UTI, bacteriuria, adverse events	• Decrease in symptomatic UTI with HX (3.2% of HX vs

			term catheterization			27.6% of placebo) • Decrease in bacteriuria with HX (3.2% of HX vs 41.4% of placebo) • Side-effects of sore throat
Ladehoff 1984[27]	RCT	300	After gynecological operations requiring short-term catheterization	500 mg BID for 8-13 days vs no treatment	Bacteriuria, adverse events	• Decrease in bacteriuria with HX (30.3% of HX vs 45.6% of no treatment) • Side-effect of nausea
Parvio 1976[20]	Case series	52	Post-menopausal with RUTIs who are hospitalized > 2 years	No treatment for 6 months then HX 1 g BID for 6 months	Symptomatic UTI, side-effects	• Decrease in UTI reinfections with HX (17 cases without HX vs unknown number while on HX) • Side-effects of "soreness of the mouth" and abdominal pain
Schiotz 2002[28]	RCT	150	After gynecological operations requiring short-term catheterization	1 g BID for 5 days vs placebo	Symptomatic UTI, bacteriuria	• Decrease in symptomatic UTI with HX (2.7% of HX vs 13.9% of placebo) • No significant difference in bacteriuria • Side-effects of nausea and rash
Thomlinson 1968[29]	Quasi-RCT	130	After gynecological operations requiring catheterization	1 g BID with sodium acid phosphate 2g TID for 7 days vs no treatment	Bacteriuria, side-effects	• No significant difference in bacteriuria • No side-effects
Tyreman 1986[30]	RCT	109	After gynecological operations requiring short-term catheterization	1 g TID for 5 days vs no treatment	Symptomatic UTI, bacteriuria, adverse events	• Decrease in symptomatic UTI (2.2% of HX vs 28.6% of no treatment) • Decrease in bacteriuria with HX (9.8% of HX

						vs 37.9% of no treatment) • No side-effects

3. Concise Summary of Project:

Study Design:

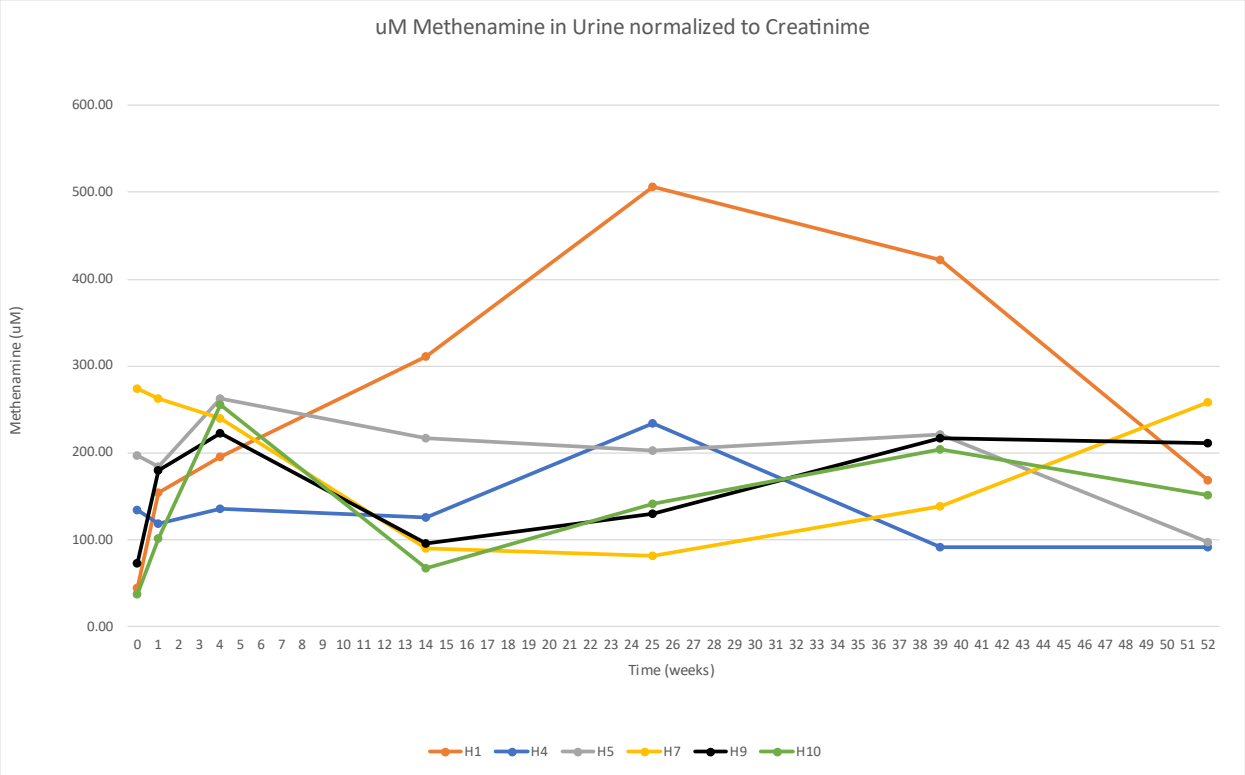
There will be two parts to this project. Part 1 will be a prospective assessment of the urinary effects of Hiprex in women with RUTIs, such as acidity and concentration of formaldehyde, to determine the best oral concentration to inhibit bacterial growth in urine for each enrolled patient. Part 2 will be a long-term assessment of Hiprex on RUTI evolution in women with a longstanding history of RUTIs, with Hiprex taken at a proper dosing (as established in Part 1) and with regular urine checks at subsequent visits up to one year of follow-up.

Part 1: Women between age 50 and 85 presenting to the outpatient UTSW Urology clinic who are currently taking Hiprex (or any methenamine-containing product) or are willing to be started on Hiprex and do not meet exclusion criteria (see below) will be invited to participate in the study. During their first study visit (V1), they will be consented for this long-term prospective study. The documentation of RUTI based on the current definition of 3 documented and treated UTIs in the past year or 2 in the past 6 months will be recorded. A 3 day diary with recordings of urine pH before each meal and before bed will be obtained to document that the patient has consistent low urine pH (<6), a critical condition for methenamine conversion to formaldehyde. Then the patient will be asked to take a tablet of Hiprex 1gm and to provide one subsequent urine samples at 1 hour after the intake of Hiprex. These midstream urine samples will be processed and transferred to our Lab on campus (K2 building) before being sent to our partnering lab at UTD run by Dr. Larry Reitzer for testing, including urine pH testing, and the measurements of methenamine and aldehyde produced in these urine samples.

Part 2:

Patients already on a methenamine drug will be queried on the history of their exposure (duration of intake, effect on RUTI, current urine pHs, etc). Patients will provide a urine sample. If the urine culture is negative, the patient will begin their prescription of Hiprex by manufacturer recommendations (if not already doing so). If the urine culture returns positive, they will be treated with a course of culture-based antibiotics and will begin Hiprex once their urine results confirm a negative culture.

Based on our preliminary data on seven women followed for one year, the levels in urine (ratioed to urinary creatinine to account for urine dilution) remain fairly stable over time.



Therefore, we don't need to follow women on Hiprex as closely as we intended to at the start of this project. Now we suggest that patients return to clinic at 3 months, 6 months, and 12 months after the onset of their Hiprex treatment to provide urine samples. During these follow-up visits, patients will also fill out a standardized, validated questionnaire regarding UTI symptoms[31]. Data concerning common Hiprex side-effects and compliance with self-administration of Hiprex will also be collected. Hiprex will be stopped when clinically indicated based on the onset of such side-effects. Urine acidity and concentration of methenamine and formaldehyde will be analyzed in these urine samples. Hiprex dosage will be increased if concentration of formaldehyde is not above the threshold for bacteriostatic effects.

<u>Outcome variables and endpoints</u>:

Primary outcome:

To confirm that the use of Hiprex will decrease the recurrence of symptomatic UTIs, our primary outcome will be the number of UTI events (namely UTI symptoms with positive urine cultures, or UTI-like episodes requiring antibiotic treatment) during the 1 year of the study period.

Secondary outcomes:

- Severity of UTI symptoms based on validated questionnaire, with the expectation of a decrease in UTI symptom severity while on Hiprex.

- Duration of intervals between UTI episodes considering that Hiprex may lessen the frequency of oral antibiotics usage to treat symptomatic UTI episodes.
- Reduction in number of hospital admission and IV antibiotics (PICC line) for urosepsis and/or pyelonephritis compared to the pre-Hiprex situation.
- Frequency and severity of adverse effects related to Hiprex, requiring treatment interruption, dose adjustment, or discontinuation.
- Evaluation of possible gradual increase in bacterial resistance to formaldehyde by testing its concentration in the urine at each visit, urine acidity, and ability to kill established bacterial strains (UTI89, CFT073)

Recruitment:

All patients will be recruited from the urology practice of the PI (WCB3 4th floor).

As part of a pilot study, we plan on enrolling 100 patients over 18 months to gather sufficient data to power a more extensive randomized control trial later on, using either a placebo or a competitive low-dose antibiotic, such as Macrobid or Septra, based on the efficacy of Hiprex given at the proper dosing and in the right urinary pH milieu in this small cohort of RUTI women. The PI is raising the enrollment goal due to COVID being tentatively over and the team is able to recruit more subjects due to this.

4. Study Procedures:

Initial visit:

Patients will be consented for the study. Patients will also be advised to return to clinic sooner than scheduled follow-up visits if they start to develop symptoms of a UTI (pain with urination, cloudy or bloody urine, etc.) or experience any side-effects that may be attributed to Hiprex. Patients who have previously been on a methenamine-containing substance will be asked about their medication history, including the length of time on the medication, the dosage and any changes to dosage, and side-effects experienced while taking the medication. Patients will be prescribed Hiprex 1 g PO BID for 3 months with 4 refills (total of 1 year). Urine samples will be obtained from patients prior to their first dose intake. Those will be mid-stream urine sample, with a collection volume around 30 ml.

Those with negative cultures (after 48-72 hours) will begin their first dose of Hiprex. Those with positive cultures will be treated with a course of culture-based antibiotics and will start their first dose of Hiprex when they can produce negative urine cultures. Using a formaldehyde assay, the concentration of formaldehyde in the urine samples with negative cultures of each patient will be measured. A pH meter will be used to determine the acidity of the urine samples.

In the lab of Dr Reitzer, the following studies will be performed on de-identified urine samples:

Sample preparation: The urine samples will be frozen at -80°C. Upon thawing for assay, the urine will be adjusted to pH 8.0 by adding 50 mM HEPES buffer and checked with pH paper in order to prevent methenamine breakdown, which occurs rapidly at pH < 6. The sample will be centrifuged to remove particulates and the soluble portion filter sterilized.

Formaldehyde assay: A variety of formaldehyde assays are available (summarized in X1). We will use a glutathione-dependent formaldehyde dehydrogenase (FDH)-based assay (X2). The urine sample will be diluted 10- to 1000-fold and placed in a microtiter dish well. The reaction will also contain 50 mM HEPES buffer (pH 8), 0.5 mM reduced glutathione, 0.5 mM NAD, and FDH (the FrmA protein) purified from *Escherichia coli.* The increase in NADH is proportional to the amount of formaldehyde. The mM extinction coefficient for NADH is 6.27. To ensure the linearity of the reaction, a known amount of formaldehyde will be added to this reaction in a separate reaction.

Methenamine assay: Methenamine breaks down to formaldehyde at acidic pHs (i.e., less than pH6). Standard assays for methenamine involve conversion of methenamine to formaldehyde. This becomes a problem when methenamine and formaldehyde are present together. To prevent this problem, we brought the pH of the urine sample to 8.0, which prevents methenamine degradation. To measure methenamine, the sample will be treated with acid (0.25 N HCl at 60° C for 10 minutes), which will completely convert the methenamine to formaldehyde. This solution will be neutralized, brought to pH 8.0, and assayed for formaldehyde as described above. The assay measures the sum of methenamine and formaldehyde, and the methenamine concentration is obtained by subtracting the formaldehyde from the first assay.

<u>Follow-up:</u>

Patients will be asked to return to clinic for 3 follow-up visits at: 3 months (V1), 6 months (V2), and 12 months (V3) after starting Hiprex. This research visit timing corresponds to standard-of-care clinic visits include those at 3 months, 6 months, and 12 months.

At each of these visits, patients will be asked to withhold their first dose of Hiprex until they arrive to clinic. Urine samples will be collected at baseline, then 1 hours after the dose intake. These samples will be tested according to the same protocol in the lab of Dr. Reitzer. In addition, patients will be administered a UTI Symptom Assessment (UTISA) questionnaire and queried about any UTI symptoms and Hiprex-associated symptoms since their last visit. If the concentration of methenamine/formaldehyde in the urine sample is found to be inadequate for bacteriostatic or bactericidal activity, the dosage might be increased to 1.5 PO BID, and/or diet-adjustment might be considered to decrease urine pH if found to be not sufficiently acidic. For patients unable to reach appropriate low pH urinary levels after the above modifications, they will be removed from the study and will not continue with the longitudinal portion.

Data:

The dataset will be extracted from EPIC and will include demographic data (age, gender, race, height, weight) as well as culture data from prior urinary tract infections, comorbid conditions (i.e. diabetes, renal insufficiency, etc.), hormonal status, methenamine usage history, and urological history (including possible surgical interventions). See Excel document.

5. Sub-Study Procedures:

None.

6. Criteria for Inclusion of Subjects:

a. Female
b. Age 50 – 85
c. Have RUTIs (at least 2 UTIs within the past 6 months or 3 within the past year)[32]

7. Criteria for Exclusion of Subjects:

a. Being on antibiotics at baseline (i.e. suppressive therapy or antibiotic therapy for urinary or non-urinary infections)
b. Neurogenic bladder condition
c. Using urinary catheters (including Foley catheter, intermittent catheterization, and suprapubic catheter)
d. Uncontrolled diabetes (HbA1c > 9)
e. Chronic renal failure defined as serum creatinine > 1.5 mg/dL
f. History of liver disease
g. Patients from out of town, in whom follow-up will not be possible
h. Pregnancy
i. Allergy to Hiprex
j. Inability to take Hiprex reliably at home, such as having psychosis, dementia, or swallowing disorders
k. Non-English speakers

8. Sources of Research Material:

Information from medical records will be included in a de-identified fashion. Confidential information will initially be recorded to facilitate returning to the medical record if needed for more information until the end of the study, at which time the confidential information will be destroyed.

9. Recruitment Methods and Consenting Process:

Once a potential patient is identified, consent will be obtained in the office. All data will be de-identified for analysis. An information sheet will be provided to all subjects.

10. Potential Risks:

a. To the participants who were already taking Hiprex, there are no direct risks because this study would not change their routines.
b. To the participants who were not initially taking Hiprex, risks include any adverse side effects associated with the medication; however, patients will be monitored very closely after the start on Hiprex therapy.
c. Tracked information will be used from patient records (EMR in EPIC), and identifiable information will be removed prior to analysis to ensure anonymity. Thus, all possible efforts will be made to protect patient confidentiality. Potential breach of PHI will be minimized with use of encrypted data devices stored in locked and protected areas.

11. Subject Safety and Data Monitoring:

Patients will be offered Hiprex, which, based on available literature since the 1960s, has not been known to lead to any serious or long-lasting side effects. However, patients will be given all relevant warnings and queried about new symptoms at each follow-up visit. Hiprex will be stopped when clinically indicated. Once the patient data has been analyzed, the data will be archived in a password-protected repository.

12. Procedures to Maintain Confidentiality:

Prior to data analysis, all patient names, ID numbers, or any other identifiable characteristics belonging to the patient will be removed from the dataset. Only the principal investigator and listed researchers will have access to the dataset, and the data will be stored electronically on a password-protected and secured UTSW computer.

13. Potential Benefits:

a. To the participants who were already taking Hiprex, there are direct benefits because this study will confirm that they are either taking Hiprex with adequate urinary dosing or need adjustments in Hiprex dose and/or urine pH to improve Hiprex efficacy.
b. To the participants who were not initially taking Hiprex, benefits would include a possible reduction in the frequency and severity of RUTIs over time if Hiprex is shown to be metabolized in their urine to produce adequate formaldehyde levels.
c. To the individuals not involved in this study, the study will provide directions for future randomized control trials involving Hiprex and another comparator.

14. Biostatistics:

Since this is a pilot study, our goal is to enroll 100 patients with RUTIs willing to be closely monitored for 1 year on Hiprex once the appropriate dosing has been confirmed. The acquired data will inform power calculations for a future randomized control trial.

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