Urethral sexual exposures: relationship with prevalent non-gonococcal urethritis, the urethral microbiota, and resolution of symptoms

Laura C. Chambers

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

University of Washington

2019

Reading Committee:

Lisa E. Manhart, Chair

David N. Fredricks

Matthew R. Golden

Program Authorized to Offer Degree:

Public Health – Epidemiology

University of Washington

Abstract

Urethral sexual exposures: relationship with prevalent non-gonococcal urethritis, the urethral microbiota, and resolution of symptoms

Laura C. Chambers

Chair of the Supervisory Committee:

Dr. Lisa E. Manhart

Professor, Department of Epidemiology

Adjunct Professor, Department of Global Health

Non-gonococcal urethritis (NGU), the most common male genital tract syndrome, is often caused by *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG). However, up to 50% of cases are of unknown etiology, and yet-unidentified pathogens and polymicrobial communities likely play a role in some of these cases. Studies suggest that specific urethral sexual exposures influence the composition of the male urethral microbiota, in addition to facilitating transmission of known pathogens, although this hypothesis has not been tested. Clinical management of NGU is challenged by its diverse etiologies and syndromic management, and the efficacy of syndromic management may vary depending on the etiology. At NGU diagnosis, men are advised to abstain from sex for ≥7 days and until their urethral symptoms resolve, although the efficacy of and adherence to these recommendations is unknown. An

improved understanding of the risk factors and etiologies of NGU, as well as the effectiveness of current NGU management approaches, would advance prevention, diagnosis, and treatment of the syndrome.

We enrolled patients assigned male sex at birth and age ≥16 years who were attending the Public Health – Seattle and King County STD Clinic into cross-sectional and cohort studies. The cross-sectional study included patients with and without NGU. The cohort study included only patients with NGU who reported sex with exclusively with men in the past year. We defined NGU as ≥5 polymorphonuclear leukocytes (PMNs) per high-power filed (HPF) with either urethral symptoms or visible urethral discharge on examination, in the absence of *Neisseria gonorrhoeae* (GC). Cross-sectional study participants had a single study visit, while cohort study participants returned for follow-up visits every three weeks over three months, collected urine at home weekly, and completed a web-based sex diary weekly. Study visits included an examination, collection of urethral swab and urine specimens, and a computer-assisted self-interview. We tested clinic-collected urine specimens for CT, MG, and GC (Aptima, Hologic, Inc., Marlborough, Massachusetts). We applied PCR and sequencing to MG-positive specimens to detect macrolide resistance mediating mutations (MRMM). We applied broad-range 16S rRNA gene PCR with deep sequencing to all clinic- and home-collected urine specimens. Among cross-sectional study participants, we used logistic regression to estimate the association between urethral sexual exposures at last sexual episode and NGU and non-CT/non-MG NGU, separately among cisgender men and transgender women who have sex with men (MSM/TGWSM) and cisgender men who have sex with women (MSW). Among MSM in the cohort study, we estimated the association between the diversity (Shannon Index) and log10-richness (i.e., number of bacterial species) of the urethral microbiota and participants' urethral sexual exposures in seven 3-day time windows before specimen collection using generalized estimating equations, adjusting for recent antibiotics, age, race/ethnicity, HIV status, and HIV pre-exposure prophylaxis use. For each exposure category, we tested whether all

seven window coefficients equaled zero (i.e., no overall association) using a Wald test. Finally, among MSM in the cohort study who received azithromycin, we estimated median time until resolution of symptoms and resumption of sex after presumptive azithromycin therapy for NGU using the Kaplan-Meier method, overall and by etiology. We also fit a Cox proportion hazards model to estimate the association between NGU etiology and time of symptom resolution, adjusting for confounders.

From 08/2014-11/2017, we enrolled 432 patients in the cross-sectional study (118 NGU+ MSM/TGWSM, 65 NGU- MSM/TGWSM, 126 NGU+ MSW, 123 NGU- MSW). Seventy-two (30%) and 49 (20%) participants with NGU had CT and MG, respectively. Compared to MSM/TGWSM reporting only non-urethral exposures at last sex, those reporting insertive anal intercourse (IAI) alone (adjusted odds ratio [AOR]=4.46, 95% confidence interval [CI]=1.09-18.19) and IAI with insertive oral sex (IOS) (AOR=7.88, 95%CI=2.67-23.26) had higher odds of NGU, while those reporting IOS alone had similar odds of NGU. Compared to MSW whose only urethral exposure at last sex was vaginal sex (VS), MSW reporting IOS and VS had similar odds of NGU. Results were similar for non-CT/non-MG NGU. From 12/2014-5/2018, we enrolled 92 MSM with NGU in the cohort study. They contributed 1,095 person-weeks of behavioral data (median=12 diaries/man, interquartile range [IQR]=12-13). Among 894 clinic- and home-collected urine specimens (median=10 specimens/man, IQR=8-12), median diversity was 1.33 (IQR=0.76-1.99), and median richness was 14 species (IQR=9-23). Overall, diversity and log10-richness were associated with condomless IAI alone (both $P<0.01$) but not IOS alone or IOS with condomless IAI in the 21 days prior. Diversity and log10-richness were lower 1-3 days after condomless IAI alone and higher 16-18 days after condomless IAI alone. Finally, between 12/2014-7/2018, 103 MSM in the cohort study received presumptive azithromycin therapy for CT-NGU (35%), MG-NGU (21%), and non-CT/non-MG NGU (41%); 3% had CT/MG co-infection. Among MSM with MG-NGU, 94% had MRMM. Overall, median time to symptom resolution after azithromycin was seven days (95%CI=5-9), and 37% had symptoms

lasting >7 days. For CT-NGU, MG-NGU, and non-CT/non-MG NGU, median time to symptom resolution was four (95%CI=2-6, 16% >7 days), undefined (95%CI=7-undefined, 60% >7 days), and seven (95%CI=5-11, 46% >7 days) days, respectively. Men with MG-NGU (hazard ratio [HR]=0.29, 95%CI=0.13-0.68) and non-CT/non-MG NGU (HR=0.56, 95%CI=0.36-0.89) had a decreased "hazard" for symptom resolution (i.e., longer time to symptom resolution) after azithromycin therapy compared to those with CT-NGU. Median time to urethral sexual exposure after treatment was 16 days (95%CI=12-18); 27% did not avoid exposure for ≥7 days and until symptoms resolved.

Results from these studies suggest that, among MSM/TGWSM, IAI may lead to transmission of yet-unidentified rectal micro-organisms that cause non-CT/non-MG NGU, in addition to transmission of known pathogens. Among MSM with NGU at baseline, condomless IAI in the prior 21 days was independently associated with diversity and richness of the urethral microbiota during follow-up, suggesting that some urethral sexual exposures influence at least certain dimensions of the composition of the urethral microbiota. In contrast, sites of urethral sexual exposure appear less important for understanding NGU risk among MSW due to minimal variation in sexual behavior. Finally, among MSM, NGU symptoms often persist for >7 days following presumptive azithromycin, particularly for those with MG-NGU and non-CT/non-MG NGU. The very high prevalence of MRMM among men with MG-NGU likely led to their long duration of symptoms after azithromycin, while inadequate treatment of unidentified pathogens or polymicrobial communities may have contributed to the long duration of symptoms for non-CT/non-MG NGU. Counseling at NGU diagnosis should educate patients that symptoms may persist for >7 days and emphasize the rationale for the 7-day abstinence period.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. Introduction

Non-gonococcal urethritis

Non-gonococcal urethritis (NGU) is the most common male genital tract syndrome. The syndrome leads to over 200,000 clinician office visits per year in the United States.[1] Of 6,152 men attending the Public Health – Seattle and King County STD Clinic for new problem visits in 2012, 867 were diagnosed with NGU. Almost half (n=417, 48%) of these diagnoses were among men who have sex with men.

Urethritis is characterized by urethral symptoms and elevated polymorphonuclear leukocytes (PMNs) and has infectious and potentially non-infectious causes.[2] *Neisseria gonorrhoeae* causes relatively few cases of urethritis, and the remainder are considered NGU. *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) cause approximately 30-40% and 15-25% of NGU cases, respectively;[2] *Trichomonas vaginalis*, herpes simplex virus,[2] *Ureaplasma urealyticum*-biovar 2,[3,4] and adenovirus[5] also account for a small proportion of cases. However, up to 50% of NGU cases are of unknown etiology.[6] Unidentified pathogens, distinct polymicrobial communities, and/or shifts in microbial composition probably cause some of these cases of unknown etiology.

Most pathogens associated with NGU are sexually transmitted, the inflammation associated with NGU may lead to increased risk of HIV acquisition and transmission,[7,8] and in rare cases NGU can lead to sequelae such as epididymitis, urethral stricture, and prostatitis.[2,9] Moreover, some of the NGU-associated pathogens can cause serious reproductive tract sequelae in women, including pelvic inflammatory disease, ectopic pregnancy, preterm birth, and infertility.[10] Therefore, prevention and appropriate clinical management of NGU are critical for sexual and reproductive health and HIV prevention.

Clinical management of NGU

There are no point-of-care tests to differentiate between the etiologies of NGU, and the clinical presentation of the most common etiologies is similar.[2] Thus, clinicians must treat NGU syndromically with either a single dose of azithromycin (1g) or a 7-day regimen of doxycycline (100mg twice daily).[11] However, up to 20% of men experience persistent and recurrent NGU after syndromic therapy.[12] Antimicrobial resistant MG,[12,13] reinfection, and inadequate treatment of unidentified pathogens or polymicrobial communities likely all contribute to persistent and recurrent episodes. Consideration of the urethral sexual exposures associated with NGU and non-CT/non-MG NGU may identify anatomic sites from which etiologically-relevant microorganisms are acquired.

Dissertation objectives

This dissertation uses data from two studies of men attending the Public Health – Seattle and King County STD Clinic to identify urethral sexual exposures that are associated with NGU and the composition of the urethral microbiota and to evaluate resolution of urethral symptoms and resumption of sexual activity after diagnosis and treatment of NGU. We begin by presenting cross-sectional study estimates of the association between specific urethral sexual exposures and NGU and non-CT/non-MG NGU to identify anatomic sites from which etiologically-relevant micro-organisms may be acquired (Chapter 2). Subsequently, we describe the relationship between specific urethral sexual exposures and the ecological diversity and richness of bacterial species of the urethral microbiota among MSM in a prospective cohort study (Chapter 3). Finally, we present estimates of the time to resolution of urethral symptoms and time to resumption of sexual activity after syndromic treatment of NGU, overall and by etiology of NGU (Chapter 4). We conclude by summarizing the findings of this dissertation and discussing their implications (Chapter 5).

CHAPTER 2. A cross-sectional study of urethral exposures at last sexual episode associated with non-gonococcal urethritis among sexually transmitted disease clinic patients

ABSTRACT

Background: Although *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) are major causes of non-gonococcal urethritis (NGU), up to 50% of cases are of unknown etiology. We sought to identify urethral exposures at last sexual episode associated with NGU and non-CT/non-MG NGU to identify anatomic sites from which etiologically-relevant microorganisms may be acquired.

Methods: We enrolled STD clinic patients with and without NGU assigned male sex at birth age ≥16 into a cross-sectional study. NGU was defined as urethral symptoms or visible discharge plus ≥5 polymorphonuclear leukocytes without *Neisseria gonorrhoeae*. Urine was tested for CT and MG (Aptima). We used logistic regression to estimate the association between urethral exposures at last sex and NGU separately among cisgender men and transgender women who have sex with men (MSM/TGWSM) and cisgender men who have sex with women (MSW).

Results: Between 8/8/2014-11/1/2017, we enrolled 432 patients, including 183 MSM/TGWSM (118 NGU+, 65 NGU-) and 249 MSW (126 NGU+, 123 NGU-). Mean age was 34; 59% were white. CT and MG were detected in 72 (30%) and 49 (20%) NGU+ participants, respectively. Compared to MSM/TGWSM reporting only non-urethral exposures at last sex, those reporting insertive anal intercourse (IAI) only (adjusted odds ratio [AOR]=4.46, 95% confidence interval [CI]=1.09-18.19) and IAI with insertive oral sex (IOS) (AOR=7.88, 95%CI=2.67-23.26) had higher odds of NGU. MSM/TGWSM reporting IOS only had no

significant increased odds (AOR=1.67, 95%CI=0.58-4.85). Compared to MSW whose only urethral exposure at last sex was vaginal sex (VS), MSW reporting IOS and VS had similar odds of NGU (odds ratio=0.84, 95%CI=0.50-1.41). Results were similar for non-CT/non-MG NGU.

Conclusions: Among MSM/TGWSM, IAI may lead to transmission of yet-unidentified rectal microorganisms that cause non-CT/non-MG NGU, in addition to transmission of known pathogens. Sites of urethral exposure appear less important for understanding NGU risk among MSW due to minimal variation in behavior.

INTRODUCTION

Non-gonococcal urethritis (NGU) is the most common male genital tract syndrome and leads to >200,000 physician visits per year in the United States.[1] NGU is characterized by urethral symptoms and elevated polymorphonuclear leukocytes (PMNs) in the absence of *Neisseria gonorrhoeae* (NG) and has infectious and potentially non-infectious causes.[2] Although sequelae associated with NGU in men are rare, the pathogens that cause NGU are sexually transmitted and carry a high risk of sequelae in women (e.g., pelvic inflammatory disease, ectopic pregnancy, infertility).[10] Moreover, the inflammation associated with NGU may increase risk of HIV acquisition and transmission.[7,8] Thus, prevention and appropriate clinical management of NGU are critical for reproductive health and HIV prevention.

However, prevention and management of NGU is challenging. Although *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) are major causes of NGU, up to 50% of cases are of other or unknown etiology. Clinical testing for MG and less common causes (e.g., *Trichomonas vaginalis*, adenovirus, herpes simplex virus) is uncommon in many settings.[2] Thus, presumptive therapy active against CT is generally provided to patients with NGU,[11] with treatment failure rates of up to 20%.[12] An improved understanding of the etiologies of non-CT/non-MG NGU would inform targeted NGU diagnosis, treatment, and prevention.

Some evidence suggests that the etiology of NGU may differ for men who have sex with men (MSM) and men who have sex with women (MSW). MSM with NGU have more often reported urethral discharge and dysuria,[12] more often tested negative for known pathogens,[12,14,15] and had a different distribution of NGU-associated pathogens than MSW.[12,15] Different etiologic agents of NGU in MSM and MSW could result from urethral exposure to different anatomic sites during sex or different bacteria within the same anatomic site of men versus women. However, the sexual behaviors associated with NGU have been evaluated primarily in separate studies of MSM[16,17] and MSW.[18-20] One study included MSM and MSW but did not evaluate the behaviors associated with NGU separately by sex of sex

partners.[21] Moreover, published studies to date have evaluated the role of behaviors in the past one[18,21] or two[16,17,19,20] months. Empiric analyses of comparable data in both populations that examine behaviors during etiologically relevant time-frames may identify anatomic sites from which known and unknown microorganisms are acquired.

To address these knowledge gaps, we evaluated urethral exposures at last sexual episode associated with NGU and non-CT/non-MG NGU among MSM and MSW. We hypothesized that insertive anal intercourse (IAI) at last sex would be associated with NGU among MSM and MSW but that insertive oral sex (IOS) at last sex would be associated with NGU only among MSM, consistent with prior observations.[16-20]

METHODS

Study design and procedures

We recruited persons assigned male sex at birth and age ≥16 years attending the Public Health – Seattle and King County (PHSKC) Sexually Transmitted Disease (STD) Clinic into this cross-sectional study. Individuals who self-identified as cisgender men, transgender women, or non-binary were eligible. We required participants to have had exclusively male or exclusively female sex partners in the past year. Patients who had both male and female partners in the past year, no sex in the past 60 days, antibiotics in the past 30 days, known urethral contact to GC, or urethral GC diagnosed by Gram stain or nucleic acid amplification testing were not eligible. The participants included in this analysis were enrolled into either a cross-sectional (42%) or cohort (58%) study. We included only the enrollment visit data for cohort participants.

All clinic patients complete a clinical computer-assisted self-interview (CASI) providing sociobehavioral and clinical information. Potentially eligible patients saw one of two study clinicians who

confirmed eligibility and offered enrollment. Patients were offered $25 for participation. They underwent a standard clinical interview and genital examination, including collection of 30-45 mL of first-void urine and a urethral swab specimen for Gram staining. The Gram-stained slide of urethral exudates was examined to quantitate PMNs and identify Gram-negative intracellular diplococci indicative of urethral GC. We defined NGU as urethral symptoms (urethral discharge, dysuria, or other urethral symptoms) or visible urethral discharge on examination, in combination with ≥5 PMNs per high power field (HPF) assessed over the three most populated oil immersion fields. Our comparison group of patients without NGU was defined as those without urethral symptoms, without visible urethral discharge, and with <5 PMNs/HPF. Patients who did not meet our study NGU definition but had either (1) urethral symptoms or visible discharge or (2) ≥5 PMNs/HPF alone, but not both, were excluded from this analysis. The urine specimen was tested for GC and CT using the Aptima Combo 2 assay and MG using the Aptima assay with analyte-specific reagents (Hologic, Inc., Marlborough, Massachusetts). Participants also completed a study CASI developed with Research Electronic Data Capture (REDCap)[22] to collect additional sociobehavioral data, including recent sexual exposures and condom use.

Statistical analysis

We compared characteristics of patients with and without NGU using Fisher's exact tests and two-sample *t*-tests with unequal variances. To identify urethral exposures at last sex associated with NGU, we fit two separate multivariable logistic regression models with robust standard errors: one for MSM and transgender women who have sex with men (TGWSM), and one for MSW. Due to very different distributions of urethral exposures at last sex, exposure variables for MSM/TGWSM and MSW were categorized differently. For MSM/TGWSM, we created four mutually-exclusive categories of urethral exposures: (1) non-urethral exposures only (receptive oral sex, receptive anal sex, rimming,

hand-penile contact), (2) IOS only, (3) IAI only, and (4) IOS and IAI. For MSW, we created two mutually-exclusive categories of urethral exposures: (1) vaginal sex (VS) only and (2) IOS and VS only. MSW reporting other combinations of behaviors (n=16, 8%) were excluded from this analysis.

Initially, for MSM/TGWSM and MSW, we included the respective exposure variables, condom use with last sex partner (always/not always), and our hypothesized confounders (age [continuous], known HIV-positive status [MSM/TGWSM only], HIV pre-exposure prophylaxis [PrEP] use [MSM/TGWSM only], and number of sex partners with which participants engaged in each behavior [IOS, IAI, and VS (MSW only)] in the past two months). Covariates were only retained in a final model if they changed an exposure effect estimate by >10%. We also evaluated interaction terms for each exposure and condom use with last sex partner; however, there was no evidence that condom use modified the relationship between any urethral exposure and NGU for MSM/TGWSM or MSW (P>0.35), so no interaction terms were included in final models.

In a secondary analysis of non-CT/non-MG NGU, we restricted the study sample to participants with negative tests for both CT and MG. The same analysis described above was performed.

This study was approved by the University of Washington Human Subjects Division (STUDY00002106). All participants provided written, informed consent. We used Stata 13 (StataCorp, College Station, Texas) for analyses, two-sided tests, and significance-level alpha=0.05.

RESULTS

Between August 8, 2014 and November 1, 2017, we approached 1,719 patients attending the PHSKC STD Clinic about this study, of whom 332 (19%) were ineligible and 874 (51%) declined participation. The main reasons for declining were inability to attend cohort study follow-up visits (43%)

and preference not to collect urethral swabs (22%). Those who declined were similar to those who enrolled with respect to race but less often reported Hispanic/Latino ethnicity (P<0.01) and more often were screened for the cohort study only (P<0.01). Of the 513 patients who enrolled, a further 81 (16%) were excluded from this analysis because they did not meet the study NGU definition. The remaining 432 patients constituted this study sample, including 181 MSM and two TGWSM (analyzed together due to the small number of TGWSM, 118 with and 65 without NGU) and 249 MSW (126 with and 123 without NGU).

Among all participants, the mean age was 34 years (standard deviation=9.9, range=19-71), most reported white (59%) or black (18%) race, and 14% reported Hispanic/Latino ethnicity. Most participants with NGU were attending the clinic for evaluation of urethral symptoms (77%), while most participants without NGU sought STD testing (78%) (**Table 1**).

Compared to participants without NGU, those with NGU were more likely to be black (P=0.02); participants with non-CT/non-MG NGU reported a lower income-level (P=0.04). History of previous NGU, GC, and CT were each associated with any NGU and non-CT/non-MG NGU (P<0.01). CT and MG were detected in 72 (30%) and 49 (20%) participants with NGU, respectively. Sociodemographic and clinical characteristics associated with NGU were similar for MSM/TGWSM and MSW with a few exceptions. Among MSM/TGWSM but not MSW, higher education-level was associated with any NGU (P=0.01). Among MSW but not MSM/TGWSM, black race (P<0.01) and lower income-level (P=0.04) were associated with any NGU.

Participants' clinical presentation of NGU was similar, irrespective of whether CT or MG were detected (**Table 2**). Of 244 participants with any NGU, 218 (89%) reported urethral symptoms. Dysuria was the most common symptom (64%). On examination, 218 (89%) participants with any NGU had a visible urethral discharge, which was most often clear (59%); most (77%) had ≥10PMNs/HPF. The clinical presentation of MSM/TGWSM and MSW with NGU was similar with one exception. Among participants

with visible discharge, MSM/TGWSM were somewhat more likely to have clear discharge than MSW (66% versus 53%, *P*=0.054).

Sexual behavior

Among MSM/TGWSM, 49 (27%) reported IOS as their only urethral exposure at last sexual episode, 24 (13%) reported IAI only, and 84 (47%) reported both IOS and IAI (**Table 3**). The remaining 23 (13%) MSM/TGWSM reported only non-urethral exposures at last sex. In bivariate analyses, IAI only and IAI with IOS were more commonly reported at last sex among MSM/TGWSM with than without NGU (P<0.01). The sexual behaviors associated with non-CT/non-MG NGU among MSM/TGWSM were similar to those observed for any NGU (data not shown).

Nearly all MSW (95%) reported VS at last sex, irrespective of NGU status. More specifically, 10 (4%) MSW reported IOS alone, 110 (45%) reported VS alone, 120 (49%) reported IOS and VS, 2 (1%) reported IAI and VS, and 4 (2%) reported all three behaviors (IOS, IAI, VS). No MSW reported only non-urethral exposures at last sex. In bivariate analyses, none of the urethral exposures at last sex were more common among MSW with than without NGU (*P*=0.8). MSW with NGU reported more partners in their lifetime (*P*=0.01) but a similar number of partners in the past two months compared to MSW without NGU. However, MSW with NGU less often reported always using condoms with their last partner (15% versus 27%, *P*=0.03). No sexual behaviors were associated with non-CT/non-MG NGU among MSW (data not shown).

Multivariable analyses – any NGU

Compared to MSM/TGWSM reporting only non-urethral exposures at last sexual episode, MSM/TGWSM whose urethral exposures were IAI only and IAI with IOS had significantly higher odds of

NGU, adjusting for condom use with last partner and number of IOS and number of IAI partners in the past two months (adjusted odds ratio [AOR]=4.46 [95% confidence interval (CI)=1.09-18.19] and AOR=7.88 [95%CI=2.67-23.26], respectively) (**Table 4**). MSM/TGWSM whose only urethral exposure was IOS had no significant increased odds (AOR=1.67, 95%CI=0.58-4.85). Further adjustment for age, known HIV-positive status, and PrEP use did not appreciably change these estimates.

Among MSW, those whose only urethral exposure at last sex was VS had similar odds of NGU as those reporting IOS and VS only (OR=0.84, 95%CI=0.50-1.41). Adjusting for age, condom use with last partner, and number of IOS, IAI, or VS partners in the past two months did not meaningfully change these estimates.

Multivariable analyses – non-CT/non-MG NGU

The results were similar when we restricted the analysis to patients without CT and MG. Compared to MSM/TGWSM reporting only non-urethral exposures at last sex, those whose urethral exposures were IOS only, IAI only, and IOS and IAI had 2.03 (95%CI=0.58-7.18), 3.54 (95%CI=0.81-15.55), and 6.85 (95%CI=1.87-25.04) times the odds of non-CT/non-MG NGU, respectively, adjusting for number of IOS and number of IAI partners in the past two months. MSW whose urethral exposures at last sex were IOS and VS had similar odds of non-CT/non-MG NGU as those whose only urethral exposure was VS.

DISCUSSION

Among MSM and TGWSM attending an STD clinic in Seattle, Washington, IAI at last sex with or without IOS was strongly associated with any NGU and non-CT/non-MG NGU, while IOS alone was not associated with either outcome. Sociodemographic characteristics and urethral sexual exposures

associated with NGU differed somewhat for MSM/TGWSM and MSW, although the clinical presentation of NGU was similar. Among MSW, there was minimal variation in urethral exposures at last sex, and no evidence that IOS in combination with VS was associated with higher odds of NGU or non-CT/non-MG NGU than VS alone.

We hypothesized that IOS at last sex would be associated with NGU among MSM and TGWSM but not MSW based on four prior studies in Seattle. Two studies of MSM found that IOS in the past two months was associated with non-CT NGU,[16,17] while two studies of MSW found that IOS in the past two months was not associated with NGU[20] or pathogen-negative NGU.[19] In contrast, we did not observe any association between IOS at last sex and either NGU or non-CT/non-MG NGU, and this was true for MSM/TGWSM and MSW. Differences in the timing of sexual exposures, NGU definitions, comparison groups, or methodology across studies may explain the inconsistent results.

Our study suggests that IAI at last sex is associated with both NGU overall and non-CT/non-MG NGU among MSM and TGWSM. This reinforces that transmission of CT and MG can occur via IAI and suggests that rectal microorganisms may be important in the etiology of non-CT/non-MG NGU. Prior studies of IAI and NGU have yielded inconsistent results. Two studies of MSM suggested that IAI in the past two months was independently associated with urethral CT but reported conflicting results for whether IAI was associated with non-CT NGU.[16,17] In contrast, a study of Australian MSM and MSW found that condomless IAI in the past month was consistently *less* common among men with NGU, although they did not evaluate MSM and MSW separately.[21] In our study, IAI with and without IOS at the most recent sexual episode was associated with over 4-fold increased odds of NGU among MSM and TGWSM. The disparate results across studies may be due to variation in the epidemiology of NGU-associated pathogens across settings, definition of NGU across studies, time-frame of sexual exposures considered, or study methodology. Future studies of the composition of the urethral microbiota of MSM

and TGWSM with and without NGU would help determine whether known rectal bacteria cause non-CT/non-MG NGU in some cases.

Our finding that MSW had minimal variation in urethral exposures at last sex and similar odds of NGU after VS alone or in combination with IOS suggests that specific anatomic sites of urethral exposure are not key for understanding NGU risk among MSW. Instead, population-level factors associated with prevalence of pathogens or polymicrobial communities among sex partners may be more relevant for NGU risk among MSW. For example, among MSW in Seattle, *Leptotrichia/Sneathia* species were associated with NGU and detected in 15% of men with pathogen-negative NGU.[19] These bacteria are prevalent in women with bacterial vaginosis (BV)[23,24] and may have been acquired from or shared with female partners with BV during VS. Future studies of partner characteristics and urethral microbiota associated with non-CT/non-MG NGU among MSW may help define risk factors for these NGU cases.

Our analysis was strengthened by the requirement of objective evidence of urethritis (≥5 PMNs/HPF), use of CASI to minimize social desirability bias in our sexual behavior data,[25-27] and consideration of urethral exposures at last sex which may be most etiologically relevant. However, there were also limitations. First, while we collected data on condom use with participants' last partner, we did not obtain condom use separately for each urethral exposure. People may use a condom for some behaviors but not others within a sexual episode.[28] Second, the last sexual episode may not be the etiologically relevant exposure for some individuals, depending on how quickly they develop symptoms and seek care and how often they have sex. Third, IAI at last sex may be a marker of more frequent IAI, and cumulative exposure to rectal bacteria may be more important for NGU risk than the exposure to rectal bacteria at last sexual episode. We were not able to evaluate the frequency of IAI, although a proxy measure of this (number of different IAI partners) did not influence the relationship between IAI at last sex and NGU among MSM and TGWSM. Fourth, due to the small number of MSW who reported recent IAI (n=6), we could not estimate the association between IAI and NGU among MSW. Fifth, we did

not test participants for some known causes of NGU that are uncommon in our population. Nonetheless, these pathogens may have caused some cases of non-CT/non-MG NGU. Sixth, our secondary analysis evaluating the relationship between urethral sexual exposures and non-CT/non-MG NGU had limited power; larger studies would be useful to confirm our findings. Seventh, a large percentage of the patients approached about our study declined participation, many due to inability to attend parent cohort study follow-up. This may have biased our estimates if their sexual behavior and NGU etiology differ from that of the participants. Finally, our study only included two TGWSM, so our findings among MSM/TGWSM are most generalizable to MSM.

In conclusion, IAI appears to play a key role in the etiology of non-CT/non-MG NGU among MSM and TGWSM, in addition to transmission of CT and MG. In contrast, anatomic sites of urethral sexual exposure among MSW do not appear to be important for understanding risk of non-CT/non-MG NGU, likely due to minimal variation in behavior. Additional studies of the urethral microbiota, its determinants, and the role of rectal bacteria are needed to fully describe the etiologies of NGU and inform clinical management and prevention.

TABLES AND FIGURES

Table 2.1. Characteristics of men who have sex with men, transgender women who have sex with men, and men who have sex with women with and without non-gonococcal urethritis, overall and for non-*Chlamydia trachomatis*/non-*Mycoplasma genitalium* non-gonococcal urethritis

Characteristic*	**No NGU** N=188 N (%)	**Any NGU** N=244 N (%)	**P-value**†	**Non-CT/non-MG NGU**‡ N=129 N (%)	**P-value**†‡
Sociobehavioral					
Gender and sex of sex partners					
MSM	64 (34)	117 (48)	**0.006**	56 (43)	0.127
TGWSM	1 (1)	1 (<1)		1 (1)	
MSW	123 (65)	126 (52)		72 (56)	
Age, mean ±SD	34.3 ±10.4	33.8 ±9.6	0.596§	35.4 ±10.3	0.383§
Race					
White	123 (65)	130 (53)	**0.019**	72 (56)	0.064
Black	24 (13)	53 (22)		30 (23)	
Other/Multiple/Unknown	41 (22)	61 (25)		27 (21)	
Hispanic or Latino ethnicity					
Yes	28 (15)	33 (14)	0.953	21 (16)	0.428
No	154 (82)	203 (83)		102 (79)	
Unknown	6 (3)	8 (3)		6 (5)	
Education completed					
≤High School or GED	74 (40)	94 (39)	0.074	54 (42)	0.086
Some college	31 (17)	61 (25)		31 (24)	
College graduate	81 (44)	87 (36)		43 (34)	
Income					
<$10,000	36 (19)	52 (21)		24 (19)	
$10,000-49,999	72 (38)	96 (39)	0.051	60 (47)	**0.043**
≥$50,000	56 (30)	48 (20)		21 (16)	
Declined/Unknown	24 (13)	48 (20)		24 (19)	
Clinical					
Reason for visit‖					
Urethral symptoms	--	187 (77)	--	92 (71)	--
Other symptoms	32 (17)	1 (<1)	**<0.0005**	1 (1)	**<0.0005**
STD Testing	146 (78)	169 (70)	0.063	92 (72)	0.287
History of previous STD					
History of NGU	13 (7)	52 (21)	**<0.0005**	29 (23)	**<0.0005**
History of gonococcal infection	40 (21)	93 (38)	**<0.0005**	45 (35)	**0.006**
History of CT infection	52 (28)	117 (48)	**<0.0005**	57 (45)	**0.001**

Characteristic*	No NGU N=188 N (%)	Any NGU N=244 N (%)	P-value†	Non-CT/non-MG NGU‡ N=129 N (%)	P-value†‡
Known HIV-positive¶	2 (1)	12 (5)	**0.028**	4 (3)	0.237
HIV pre-exposure prophylaxis use¶Δ	10 (5)	18 (8)	0.432	7 (6)	0.801
STD pathogen detected					
Chlamydia trachomatis	0 (0)	72 (30)	**<0.0005**	--	--
Mycoplasma genitalium	6 (3)	49 (20)	**<0.0005**	--	--

Abbreviation: CT, *Chlamydia trachomatis*; GED, General Education Diploma; HIV, human immunodeficiency virus; MG, *Mycoplasma genitalium*; MSM, cisgender men who have sex with men only; MSW, cisgender men who have sex with women only; NGU, non-gonococcal urethritis; SD, standard deviation; STD, sexually transmitted disease; TGWSM, transgender women who have sex with men only.

*n=4 missing education completed, n=1 missing reason for visit is STD testing, n=4 missing history of NGU, n=2 missing history of gonococcal infection, n=3 missing history of CT.

†Fisher's exact test unless otherwise specified. Bold indicates significance at alpha=0.05 level.

‡Urine specimen tested for CT and MG via Aptima assay. Comparison group is participants without NGU whose urine samples tested negative for CT or MG (excludes n=6 participants with MG).

§Two-sample *t*-test with unequal variances.

‖Multiple reasons for visit possible. Other symptoms include genital lesions/rash, non-genital rash, anorectal symptoms, testicular symptoms, oral/pharyngeal symptoms, and other symptoms.

¶All participants known to be living with HIV or taking HIV pre-exposure prophylaxis were MSM or TGWSM.

ΔAmong participants who were not known to be HIV-positive (n=186 no NGU, n=232 any NGU, n=125 non-CT/non-MG NGU).

Table 2.2. Clinical presentation of participants with non-gonococcal urethritis and non-*Chlamydia trachomatis*/non-*Mycoplasma genitalium* non-gonococcal urethritis

Characteristic	**Any NGU** N=244 N (%)	**Non-CT/non-MG NGU*** N=129 N (%)
Self-reported urethral symptoms	218 (89)	110 (85)
Type of urethral symptoms		
Urethral discharge	124 (57)	58 (53)
Dysuria	139 (64)	65 (59)
Other urethral symptoms[†]	112 (51)	61 (55)
Visible urethral discharge on examination	218 (89)	112 (87)
Character of urethral discharge		
Clear	129 (59)	66 (59)
Cloudy	77 (35)	40 (36)
Purulent or mucoid	12 (6)	6 (5)
Amount of urethral discharge		
Small	147 (67)	72 (64)
Moderate	69 (32)	40 (36)
Large	2 (1)	0 (0)
Average PMNs/HPF on urethral Gram stain		
5-9 PMNs/HPF	55 (23)	35 (27)
≥10 PMNs/HPF	189 (77)	94 (73)

Abbreviation: CT, *Chlamydia trachomatis*; HPF, high power field; MG, *Mycoplasma genitalium*; NGU, non-gonococcal urethritis; PMN, polymorphonuclear leukocytes.

*Urine specimen tested for CT and MG via Aptima assay.

[†]Includes urethral itching, tingling, or other irritation.

Table 2.3. Sexual behavior of participants with and without non-gonococcal urethritis, stratified by sex of sex partners

	MSM/TGWSM			MSW		
Sexual Behavior*	**No NGU** N=65 N (%)	**NGU** N=118 N (%)	**P-value†**	**No NGU** N=123 N (%)	**NGU** N=126 N (%)	**P-value†**
Lifetime number of sex partners (any type of sex)						
1-4	3 (5)	3 (3)		5 (4)	1 (1)	
5-9	3 (5)	3 (3)	0.735	28 (23)	12 (10)	**0.012**
10-24	8 (13)	16 (14)		32 (26)	42 (36)	
≥25	49 (78)	91 (81)		56 (46)	62 (53)	
Number of sex partners in the past 2 months, mean ±SD						
Any type of sex	5.0 ±7.1	4.7 ±5.8	0.798‡	2.0 ±2.2	2.3 ±2.3	0.229‡
Vaginal sex	--	--	--	2.2 ±3.1	2.3 ±2.3	0.794‡
Insertive anal sex	2.2 ±5.1	3.4 ±4.2	0.125‡	0.3 ±0.7	0.2 ±0.5	0.269‡
Insertive oral sex	3.9 ±5.5	4.4 ±4.9	0.523‡	2.0 ±2.4	1.8 ±1.3	0.537‡
New sex partner in past 2 months	45 (73)	96 (83)	0.117	79 (66)	90 (73)	0.265
Urethral exposures at last sexual episode§						
None (non-urethral exposures only)	14 (22)	9 (8)		0 (0)	0 (0)	
Insertive oral sex only	25 (40)	24 (21)		4 (3)	6 (5)	
Insertive anal sex only	6 (10)	18 (15)		0 (0)	0 (0)	
Insertive oral sex and insertive anal sex only	18 (29)	66 (56)		0 (0)	0 (0)	
Vaginal sex only	--	--	**<0.0005**	51 (43)	59 (47)	0.783
Insertive anal sex and vaginal sex only	--	--		1 (1)	1 (1)	
Insertive oral sex and vaginal sex only	--	--		61 (51)	59 (47)	
Insertive oral sex, insertive anal sex, and vaginal sex	--	--		3 (3)	1 (1)	
Always used condoms with last sex partner	18 (29)	20 (18)	0.125	33 (27)	18 (15)	**0.027**

Abbreviation: MSM, cisgender men who have sex with men only; MSW, cisgender men who have sex with women only; NGU, non-gonococcal urethritis; SD, standard deviation; STD, sexually transmitted disease; TGWSM, transgender women who have sex with men only.

*n=18 missing lifetime number of sex partners, n=13 missing number of sex partners in past 2 months, n=6 missing vaginal sex partners past 2 months, n=4 missing insertive anal sex partners past 2 months, n=8 missing insertive oral sex partners past 2 months, n=13 missing new sex partner past 2 months, n=4 missing behaviors at last sexual episode, n=14 missing condom use with last sex partner.

†Fisher's exact test unless otherwise specified. Bold indicates significance at alpha=0.05 level.

‡Two-sample *t*-test with unequal variances.

§Mutually exclusive categories.

Table 2.4. Multivariable analyses of associations of specific urethral exposures at last sexual episode with non-gonococcal urethritis and with non-*Chlamydia trachomatis*/non-*Mycoplasma genitalium* non-gonococcal urethritis, stratified by sex of sex partners

Specific urethral exposures at last sexual episode	Any NGU		Non-CT/non-MG NGU[†]	
	Unadjusted OR (95%CI)	Adjusted OR (95%CI)*	Unadjusted OR (95%CI)	Adjusted OR (95%CI)[‡]
MSM/TGWSM[§]				
Non-urethral exposures only	Ref.	Ref.	Ref.	Ref.
Insertive oral sex only	1.49 (0.54-4.10)	1.67 (0.58-4.85)	1.31 (0.41-4.48)	2.03 (0.58-7.18)
Insertive anal sex only	4.67 (1.34-16.30)[‖]	4.46 (1.09-18.19)[‖]	3.50 (0.85-14.39)	3.54 (0.81-15.55)
Insertive oral sex & insertive anal sex	5.70 (2.12-15.34)[¶]	7.88 (2.67-23.26)[¶]	4.20 (1.33-13.28)[‖]	6.85 (1.87-25.04)[¶]
MSW[Δ]				
Vaginal sex only	Ref.	--	Ref.	--
Vaginal sex & insertive oral sex only	0.84 (0.50-1.41)	--	0.98 (0.53-1.82)	--

Abbreviations: CI, confidence interval; CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MSM, cisgender men who have sex with men only; MSW, cisgender men who have sex with women only; NGU, non-gonococcal urethritis; OR, odds ratio; TGWSM, transgender women who have sex with men only.

*For MSM/TGWSM, model adjusted for condom use with last partner (always/not always) and number of IAI and number of IOS partners in the past two months; adjusting for age (years), HIV status, and PrEP use did not change these estimates by >10%. For MSW, adjusting for age, condom use with last partner, and number of IAI, IOS, or VS partners in the past two months did not change these estimates by >10%.

[†]Analysis restricted to participants whose urine specimen tested negative for CT and MG via Aptima assay.

[‡]For MSM/TGWSM, model adjusted for number of IOS and number of IAI partners in the past two months; adjusting for age, HIV status, PrEP use, and condom use with last partner did not change these estimates by >10%. For MSW, adjusting for age, condom use with last partner, and number of IOS, IAI, or VS partners in the past two months did not change these estimates by >10%.

[§]Per our study inclusion criteria, no MSM/TGWSM had vaginal sex in the past year.

[‖]$P<0.05$.

[¶]$P<0.01$.

[Δ]Excludes n=16 MSW who reported other combinations of urethral exposures at last sexual episode (n=10 IOS only, n=2 IAI and VS, and n=4 IOS, IAI, and VS). No MSW reported non-urethral exposures only at last sexual episode.

CHAPTER 3. The incidence of sexual behaviors and relationship to the urethral microbiota among men who have sex with men in Seattle, Washington

ABSTRACT

Background: Studies suggest that sexual behavior influences the composition of the male urethral microbiota, but this hypothesis has not been tested.

Methods: From 12/2014-5/2018, we enrolled MSM with NGU attending an STD clinic into a cohort study. Men attended five in-clinic visits at 3-week intervals, collected weekly urine specimens at home, and reported daily antibiotics and sexual activity on weekly diaries. We applied broad-range 16S rRNA gene PCR with deep sequencing to urine. We estimated incidence of insertive oral sex (IOS) only, condomless insertive anal intercourse (CIAI) only, and IOS with CIAI (IOS+CIAI) after NGU diagnosis using Poisson regression with robust standard errors. We estimated the association between Shannon Index (diversity) and log10 number of bacterial species (richness) and urethral sexual exposures (referent group=none) in seven 3-day time windows before specimen collection using generalized estimating equations, adjusting for recent antibiotics, age, race/ethnicity, HIV status, and HIV pre-exposure prophylaxis use. For each exposure category, we tested whether all seven window coefficients were equal to zero (i.e., no overall association) using a Wald test.

Results: Among 92 MSM with NGU, median age was 31 (interquartile range [IQR]=28-40); 55% were non-Hispanic white. They contributed 1,095 person-weeks of behavioral data (median=12 diaries/man, IQR=12-13). Incidence of any sex, IOS only, CIAI only, and IOS+CIAI were 1.07 (95% confidence interval

[CI]=0.93-1.24), 0.40 (95%CI=0.32-0.49), 0.10 (95%CI=0.07-0.15), and 0.40 (95%CI=0.30-0.52) episodes per person-week, respectively. Among 894 observations (median=10 observations/man, IQR=8-12), median diversity was 1.33 (IQR=0.76-1.99), and median richness was 14 species (IQR=9-23). Overall, diversity and log10-richness were associated with CIAI only (*P*<0.01 in each model) but not IOS only or IOS+CIAI in the 21 days prior. Diversity and log10-richness were lower 1-3 days after and higher 16-18 days after CIAI only.

Conclusions: Among MSM after NGU, CIAI only in the prior 21 days was independently associated with diversity and richness of the urethral microbiota, potentially due to the acquisition of rectal micro-organisms. Additional research is needed on the relationship between the urethral microbiota and NGU.

INTRODUCTION

Although many cases of non-gonococcal urethritis (NGU) are caused by *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG),[2] up to 50% of cases are of unknown etiology.[6] Clinicians must treat NGU syndromically,[11] with treatment failure rates of up to 20%.[12] While antibiotic resistant MG[12,13] and reinfection account for some persistent and recurrent NGU cases, unidentified pathogens and polymicrobial communities are also likely culprits.

Epidemiologic studies suggest that specific sexual exposures can influence the composition of the male urethral microbiota, in addition to transmission of known pathogens. Among adolescent men, certain genera of bacteria (*Sneathia*, *Mycoplasma*, and *Ureaplasma*) were only detected in urine from men who were sexually active, suggesting colonization via urethral sexual exposures.[29] Moreover, recent insertive anal intercourse (IAI)[17,30] and insertive oral sex (IOS)[15-17] have been associated with pathogen-negative NGU, suggesting that exposure to certain anatomic sites leads to either the acquisition of unidentified pathogens or changes to the polymicrobial communities that contribute to NGU. However, studies have been inconsistent, with some detecting no association for either or both sexual exposures,[15,16,19,20,30,31] potentially due to variation in the timing of sexual exposures, NGU definitions, and study methodologies. Some studies of any NGU[4,18] but not others[21] suggest associations between specific sexual exposures and NGU, although these associations could be driven by acquisition of known pathogens such as CT and MG. Finally, a culture-based study of men found oral streptococci strains only in the urethras of men reporting IOS in the past month; however, the prevalence among these men was very low (4%), and the association was not significant.[32] Cross-sectional studies that used sensitive molecular methods have not detected an association between specific types of sex and specific bacteria/taxa,[19,31] although these studies compared relatively broad measures of sexual activity.

Importantly, prior studies evaluating the relationship between sexual activity and the male urethral microbiota that used broad-range 16S rRNA gene PCR and sequencing analyses have been

relatively small or cross-sectional.[29,31] Additionally, the hypothesis that urethral sexual exposures influence the male urethral microbiota has not been tested using longitudinal data. To address this knowledge gap, we conducted a cohort study of MSM with NGU and, in an exploratory analysis, estimated the association between specific urethral sexual exposures and the ecological diversity and richness of species in the urethral microbiota, among men enrolled through May 16, 2018. We also sought to define the relevant time-frame for the urethral sexual exposures.

METHODS

Study design and procedures

We recruited Public Health – Seattle and King County (PHSKC) Sexually Transmitted Disease (STD) Clinic patients age ≥16 years with NGU who were assigned male sex at birth and only had sex with people assigned male sex at birth in the past year. Patients reporting no sexual activity in the past 60 days, antibiotic use in the past 30 days, or urethral contact to *Neisseria gonorrhoeae* (GC) were not eligible. Additionally, patients were required to have valid contact information and a freezer at home in which they could store urine specimens.

This prospective cohort study included in-clinic visits every three weeks for three months (five visits total). Participants collected weekly urine specimens at home and completed a weekly symptom and sex diary.

Enrollment Visit. One of two study clinicians offered enrollment to interested and eligible patients. The clinician conducted a standard clinical examination, including collection of a urethral swab specimen for Gram staining and 30-45 mL of first-void urine. The clinician examined the Gram-stained slide of urethral exudates to quantitate polymorphonuclear leukocytes (PMNs) and check for Gram-negative intracellular diplococci indicative of GC infection.[11] We defined NGU as urethral symptoms or

visible urethral discharge on examination, in combination with an average of ≥5 PMNs per high power field (HPF). Participants received presumptive treatment for NGU according to clinic standard of care, which specifies either azithromycin (1g, single-dose) or doxycycline (100mg, twice daily, 7 days).[11] Participants also provided sociobehavioral data on a computer-assisted self-interview (CASI) developed with Research Electronic Data Capture (REDCap).[22]

Follow-Up Visits. Each follow-up visit included the same study procedures as the enrollment visit (i.e., clinical examination, collection of a urethral swab specimen and urine, and completion of a CASI). Participants with urethral symptoms between scheduled visits were asked to return for an interim visit with these same study procedures.

Home Collection of Urine. Throughout study follow-up, participants collected 30-45 mL of first-void urine at home each week. They were instructed to collect the specimen the first time that they urinated every Monday morning, store the specimens in their freezer, and bring them to their next study visit. We provided men with specimen collection materials, including specimen tubes, biohazard bags, labels, an opaque container in which to store the specimens privately in their freezer, and an insulated bag with ice-packs to prevent the specimens from melting during transport to the clinic.

Web-Based Diaries. Throughout study follow-up, participants completed weekly diaries, reporting daily instances of sexual activity, antibiotic therapy, and urethral symptoms. Participants reported the specific types of sex at each sexual episode, as well as condom use for IAI and receptive anal intercourse (RAI). We programmed and automated the diaries using REDCap and timed-out incomplete diaries after 7 days. Participants had the option to complete paper diaries, but none chose this method.

Laboratory testing

We tested clinic-collected urine specimens for GC and CT using the Aptima Combo 2 assay and MG using the Aptima assay with analyte-specific reagents (Hologic, Inc., Marlborough, Massachusetts). The first 166 specimens (37%) were tested retrospectively for MG in batches, while the subsequent 277 specimens (63%) were tested for MG within ten days of the visit. The clinicians treated participants with a positive test result according to clinic standard of care.

We applied broad-range 16S rRNA gene PCR and deep sequencing to all clinic- and home-collected urine specimens. Laboratory staff was blinded to clinical findings. We used PCR primers targeting the V3-V4 region and 2 X 300 base-pair chemistry on the Illumina MiSeq for sequencing.[33] For each specimen, we excluded sequence variants of which <25 copies were present within that specimen. Additionally, specimens with <1,000 sequence reads were excluded from statistical analyses.

Statistical analysis

Using the daily diary data, we estimated the incidence rate of specific sexual behaviors in the three months after NGU diagnosis (i.e., beginning on the day of enrollment) using Poisson regression with robust standard errors. Using daily diary data and broad-range 16S rRNA gene PCR and sequencing data for clinic- and home-collected urine specimens, we estimated the association between specific urethral sexual exposures and ecological diversity (Shannon Index[34]) and richness (i.e., number of bacterial species represented; similar to the Chao index[35]) using generalized estimating equations (GEEs). We considered urethral sexual exposures in the following mutually exclusive categories: (1) IOS (presumed to be condomless) but not condomless IAI, (2) condomless IAI but not IOS, (3) both IOS and condomless IAI, (4) missing urethral sexual exposure data, and (5) neither IOS nor condomless IAI

(referent category). The distribution of richness was highly skewed, so we conducted statistical analyses on log10 richness.

Compared to known bacterial pathogens with estimated incubation periods, there were minimal prior data with which to hypothesize the relevant time-frame for the sexual exposures to influence the microbial community. Thus, we estimated this empirically. We considered urethral sexual exposures within seven 3-day time windows: 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, and 19-21 days prior to specimen collection and microbiota characterization. We developed a single model for diversity and another for richness, specifying GEEs with a Gaussian model, an identity link function, an exchangeable covariance matrix, and robust standard errors. We included variables for the mutually exclusive urethral sexual exposure categories within each 3-day time window and adjusted for recent antibiotic therapy (yes, no, or missing), as reported on diaries, by including variables for antibiotic exposure within each 3-day time window. We also adjusted for hypothesized baseline confounders: age (continuous), race/ethnicity (non-Hispanic black; non-Hispanic white; Hispanic [any race]; and non-Hispanic other, multiple, or unknown race), HIV status, and HIV pre-exposure prophylaxis (PrEP) use. We excluded specimens collected within the first 21 days after enrollment because participants had not yet provided sufficient daily diary data to inform all seven exposure time windows. Finally, for each urethral sexual exposure category in each model, we tested whether all seven coefficients were equal to zero (indicating no association during the 21-day time-period) using a Wald test.

This study was approved by the University of Washington Human Subjects Division. Participants provided written, informed consent. We compensated participants $25-60 per visit and $5-20 per diary, with the amount increasing throughout follow-up, for a total of $360. We conducted analyses using RStudio version 1.1.463 (RStudio, Inc., Boston, Massachusetts) and Stata 13 (StataCorp, College Station, Texas). We used two-sided statistical tests and a significance-level of alpha=0.05.

RESULTS

Between December 16, 2014 and May 16, 2018, we approached 471 patients attending the PHSKC STD Clinic about this study, of whom 186 (39%) were ineligible, 179 (38%) declined, and 106 (23%) enrolled. The main reasons for declining were inability to stay to enroll (21%) or attend follow-up visits (41%). We excluded one participant (1%) who self-identified as a transgender woman due to insufficient sample size to stratify our analyses by gender. The remaining 105 cisgender MSM comprised the analysis sample.

Baseline characteristics

Among the 105 MSM with NGU, median age was 30 years (range=20-60), and about half (53%) of men were non-Hispanic white (**Table 1**). Thirteen men (12%) were known to be living with HIV and, of the remaining HIV-negative men, 17 (18%) were taking PrEP at study enrollment. Over half of men reported a history of GC infection (55%) and CT infection (59%). Most men had urethral symptoms (93%), visible urethral discharge on examination (91%), and ≥10 PMNs/HPF on a urethral Gram stain (81%). Thirty-seven men (35%) had urethral CT infection, while 25 (24%) had urethral MG infection. Three men (3%) had CT/MG co-infection.

At baseline, 66 men (65%) reported ≥50 sex partners in their lifetime (**Table 2**). Men reported a median of three sex partners in the past two months (interquartile range [IQR]=2-6). Most men (81%) reported a new sex partner in the past two months.

Longitudinal analyses

Ninety-two (88%) of these 105 men were included in the longitudinal analyses. Of the 13 men excluded from the longitudinal analyses, 11 (85%) were lost to follow-up or withdrew after the enrollment visit, and 2 (15%) did not have specimens with sufficient bacterial DNA for broad-range 16S rRNA gene PCR and sequencing from >21 days after enrollment. Men included and excluded from the longitudinal analyses had similar baseline sociobehavioral characteristics, except excluded men were younger (*P*=0.004), were more likely to be non-Hispanic black (*P*=0.04), and had lower education (*P*=0.04). The remaining 92 men in the longitudinal analyses provided 1,095 person-weeks of behavioral data (median=87 days/man, IQR=82.5-92.5, range=31-108). Overall, they completed 1,170 (93%) of their 1,253 diaries.

In the three months after NGU diagnosis, the incidence rate of any sexual activity was 1.07 episodes per person-week (95% confidence interval [CI]=0.93-1.24) (**Table 3**). Urethral sexual exposures were common, especially IOS (0.79 episodes/person-week, 95%CI=0.66-0.95), which was the most common specific behavior. Most IAI (85%) and RAI (82%) episodes were condomless. The incidence rate of condomless IAI and condomless RAI were 0.50 (95%CI=0.40-0.63) and 0.19 (95%CI=0.14-0.25) episodes per person-week, respectively. The incidence rate of IOS in the absence of condomless IAI (0.40, 95%CI=0.32-0.49) was similar to that of IOS with condomless IAI (0.40, 95%CI=0.30-0.52) and much higher than condomless IAI in the absence of IOS (0.10, 95%CI=0.07-0.15).

These 92 men collected 1,104 urine specimens during the analysis period (n=346 [31%] clinic-collected, n=758 [69%] home-collected). Of these 1,104 specimens, 208 (19%) had insufficient bacterial DNA for the broad-range 16S rRNA gene PCR and sequencing analyses; two additional specimens (0.2%) had <1,000 sequence reads and were excluded from statistical analyses. Insufficient bacterial DNA was associated with clinic-collection (versus home-collection) of urine (*P*=0.001). Among the clinic-collected specimens, insufficient bacterial DNA was not associated with NGU (*P*=0.9). Among the 894 specimens

with sufficient bacterial DNA (median=10 specimens/man, IQR=8-12, range=1-15), median Shannon diversity index was 1.33 (IQR=0.76-1.99, range=0-3.48), and median richness was 14 species (IQR=9-23, range=1-101).

Overall, 141 specimens (16%) were missing sexual exposure and/or antibiotic exposure data for ≥1 time window. These specimens were retained in the statistical analysis by including categories for missing exposure data within each window. Within all 3-day time windows in the 21 days before specimen collection, the most common urethral sexual exposures reported were IOS and condomless IAI together (13-20%), followed by IOS only (9-14%) and condomless IAI only (2-3%) (**Table 4**). In bivariate analyses, HIV infection was associated with higher diversity (*P*=0.05) and higher log-10 richness (*P*=0.01) over follow-up. Age, race/ethnicity, and PrEP use were not associated with diversity or log10-richness.

Overall, diversity was associated with condomless IAI in the absence of IOS over the entire 21-day period (*P*<0.001) but not with IOS in the absence of condomless IAI (*P*=0.07) or both IOS and condomless IAI (*P*=0.10) during this same time-period, adjusting for urethral sexual exposures in the other time windows, antibiotic exposure in all time windows, missing exposure data in the other time windows, age, race/ethnicity, HIV status, and PrEP use (**Figure 1**). Examination of the individual time windows revealed that specimens collected within 1-3 days of condomless IAI only had lower diversity than those with no urethral sexual exposures in the 1-3 days prior (*P*=0.02), while higher diversity was associated with condomless IAI only 16-18 days prior (*P*<0.01). Although diversity was not associated with IOS only in the prior 21 days overall, higher diversity was associated with IOS only 13-15 days prior (*P*=0.01).

The results were similar for log10-richness (**Figure 2**). Log10-richness was associated with condomless IAI in the absence of IOS over the entire 21-day period (*P*<0.001) but not with IOS without condomless IAI (P=0.14) or both IOS and condomless IAI (*P*=0.14) during this time-period, adjusting for confounders. Within the individual time windows, lower log-10 richness was associated with

condomless IAI only 1-3 days prior (P=0.03), and higher log-10 richness was associated with condomless IAI only 16-18 days prior (*P*<0.01). As with diversity, higher log10-richness was associated with IOS only 13-15 days prior (P=0.02), despite the absence of an overall association between IOS only and log10-richness during the 21 day period. Additionally, higher log10-richness was associated with both IOS and condomless IAI 13-15 days prior (*P*=0.05), despite no overall association during the 21 day period.

DISCUSSION

In a cohort of MSM attending an STD clinic in Seattle, we found that men engaged in sex just over once per week in the three months after NGU diagnosis. The most common specific sexual behavior was IOS. Condom use during IAI and RAI was uncommon (<20% of episodes). Overall, in an exploratory analysis, condomless IAI without IOS was independently associated with the ecological diversity and richness of bacterial species. Diversity and log10-richness were lower 1-3 days after condomless IAI only and higher 16-18 days after condomless IAI only. There was minimal evidence of an association between IOS and diversity or richness, and the influence of IOS appeared to counteract the strong association with IAI when both behaviors were practiced together in the same time window.

Our study suggests that urethral sexual exposures influence the composition of the male urethral microbiota, even after accounting for the influence of antibiotic exposure and potential baseline sociodemographic and clinical confounders. This finding is consistent with prior observations that men[29] and women[36,37] acquire new urogenital bacteria after becoming sexually active, despite the presence of urogenital bacteria since infancy.[38] Additionally, studies have detected bacterial vaginosis (BV) associated bacteria in the male urethra and coronal sulcus,[19,29,39] which may suggest these bacteria can be acquired from or shared with their female sex partners. However, one of these studies detected many BV-associated bacteria in men who were not yet sexually active, so urethral colonization with

these bacteria may also result from vertical transmission from the mother, other anatomic sites of the individual, or environmental exposures.[29]

Our finding that condomless IAI but not IOS was associated with the diversity and richness of the urethral microbiota is consistent with our prior finding that IAI but not IOS at last sexual episode was associated with non-CT/non-MG NGU.[30] We found that very recent (1-3 days prior) condomless IAI was associated with the lower diversity and richness, while more distal (16-18 days prior) condomless IAI was associated with higher diversity and richness. Although speculative, this may suggest that, in the short-term after condomless IAI, the urethral microbiota is inundated with a large quantity of a few rectal micro-organisms. Subsequently, as the new organisms grow and compete for resources with the pre-existing organisms, the community composition may slowly shift and become more diverse.

It is estimated that the usual incubation period of NGU is roughly 14-21 days,[2] which might suggest that the higher diversity 16-18 days after IAI could contribute to some NGU cases. However, our preliminary cross-sectional data suggest that decreased diversity is associated with NGU among MSM,[40] which is inconsistent with this hypothesis. Alternatively, the median time since last sexual episode among MSM with non-CT/non-MG NGU in our cross-sectional study[30] was 5 days (IQR=3-8), and IAI at last sex was associated with non-CT/non-MG NGU, which might suggest that more recent IAI contributed to decreased diversity which contributed to NGU (consistent with our preliminary cross-sectional data). To further complicate things, it is plausible that the delay between a sexual exposure and a shift in the microbial community would differ from the delay between sexual exposure and the development of NGU symptoms. Additional analyses of non-CT/non-MG NGU, especially with longitudinal data, are needed to determine whether specific changes in the urethral microbiota may cause some of these NGU cases. We have ongoing analyses that are exploring this.

In the three months after NGU diagnosis, the MSM in our study engaged in just over one sexual episode per week. If their behavior were similar in the subsequent nine months, this would suggest

roughly 55 sexual episodes per year (range=48-64). Wall and colleagues previously estimated the frequency of sexual activity among predominantly heterosexual men and women of median age 41 years (70-80 sex acts/year) and among MSM of median age 22 years (~81 annualized acts/year with their last male partner) using data from retrospective surveys.[41] While advantageous to have data from large population surveys, questionnaires with long recall periods may over-estimate the frequency of common behaviors.[42] Our diary data from sexually active MSM attending an STD clinic in Seattle suggest that the incidence rate of sex may be lower than previously thought, although the three months after NGU diagnosis observed in our study may under-estimate the usual incidence of sex among these men.

Our study was strengthened by its longitudinal study design, which clearly documented the temporal sequence of exposures and outcomes allowing for better causal inference. Moreover, we used weekly web- and mobile-phone enabled electronic diaries to collect sexual behavior data, which helps minimize recall and social desirability bias.[43,44] Additionally, our bioinformatics pipeline allowed classification of bacteria to the species-level in most cases,[24] allowing for accurate estimates of diversity and richness and reducing bias in the associations of interest.

However, this study was also subject to important limitations. First, 19% of urine specimens had insufficient bacterial DNA for sequencing analyses. The exclusion of these specimens from our statistical analyses may have biased our results if having insufficient bacterial DNA was not random given our measured covariates. Home-collected urine specimens less often had insufficient bacterial DNA, perhaps because early morning first-void urine has a greater quantity of bacteria than first-void urine collected later in the day or because home storage conditions contributed to bacterial growth. However, adjusting for clinic- vs. home-collection did not meaningfully change our results. Although 16% of the remaining specimens had missing exposure data for ≥1 time window, we were able to retain them in our statistical analyses by including a missing data category for each exposure in each time window. Additionally, younger men, non-Hispanic black men, and men with lower education were under-represented in our

longitudinal analysis. Their behavior or microbiota may differ from that of men who attended follow-up visits, although neither age nor race/ethnicity was associated with diversity or log-10 richness among the remaining men. Second, we did many statistical tests, which increases the likelihood of detecting false associations. Due to the exploratory study objectives, we did not correct for multiple comparisons. Instead, we gave the greatest weight to the global tests for an association across the entire 21-day period, and then used the analysis of the time windows to understand the general pattern over the period. Third, we only evaluated two measures of the composition of the urethral microbiota. Sexual exposures may have influenced the microbial composition in other unmeasured ways. Fourth, we adjusted for recent urethral sexual exposures, recent antibiotic exposure, and baseline characteristics thought to be associated with sexual behavior and the urethral microbiota; however, our estimates may be subject to residual confounding. In particular, although we adjusted for antibiotic therapy in the 21 days before specimen collection, we did not have day-level data on the antibiotic type, and antibiotics may influence the microbiota for an extended period of time.

In conclusion, men's urethral sexual exposures likely influence certain dimensions of the composition of the urethral microbiota. As we learn more about the association between the urethral microbiota and NGU, this highlights the potential for prevention targets, particularly for men who experience persistent and recurrent episodes.

TABLES AND FIGURES

Table 3.1. Characteristics of men who have sex with men with non-gonococcal urethritis attending an STD clinic at enrollment

Characteristic*	N=105 n (%)
Sociodemographic	
Age, median (IQR)	30 (27-39)
Race and ethnicity	
Non-Hispanic black	7 (7)
Non-Hispanic white	56 (53)
Hispanic (any race)	19 (18)
Non-Hispanic other, multiple, or unknown race	23 (22)
Education completed	
≤High school or GED	27 (26)
Some college	28 (27)
Bachelor's degree	37 (36)
Graduate or professional degree	12 (12)
Clinical history	
Known HIV-positive	13 (12)
HIV pre-exposure prophylaxis use prior to enrollment†	17 (18)
History of NGU	37 (36)
History of GC infection	57 (55)
History of CT infection	61 (59)
Clinical presentation	
Urethral symptoms	98 (93)
Visible urethral discharge	96 (91)
PMNs/HPF	
5-9	20 (19)
≥10	85 (81)
Urethral CT infection (Aptima)‡	37 (35)
Urethral MG infection (Aptima)‡	25 (24)

Abbreviation: CT, *Chlamydia trachomatis*; GED, General Education Diploma; HIV, human immunodeficiency virus; IQR, interquartile range; MG, *Mycoplasma genitalium*; MSM, men who have sex with men only; NGU, non-gonococcal urethritis.

*n=1 missing education completed; n=1 missing history of NGU, GC, and CT.

†Among n=92 not known to be HIV-positive.

‡n=3 co-infected with CT and MG.

Table 3.2. Sexual behavior reported by men who have sex with men with non-gonococcal urethritis at enrollment

Behavior*	**Overall** N=105 n (%)
Lifetime number of sex partners	
1-9	5 (5)
10-49	30 (30)
50-99	25 (25)
≥100	41 (40)
Number of sex partners in past 2 months, median (IQR)	
Any type of sex	3 (2-6)
Receptive anal intercourse	1 (0-2)
Insertive anal intercourse	2 (1-4)
Receptive oral sex (his mouth on partner's penis)	2 (1-4)
Insertive oral sex (partner's mouth on his penis)	3 (2-5)
He rimmed partner	1 (0-2)
Partner rimmed him	1 (0-2)
New sex partner in past 2 months	83 (81)
Sexual exposure(s) at last sexual episode	
Receptive anal intercourse	29 (28)
Insertive anal intercourse	76 (73)
Insertive oral sex	79 (76)
Partner rimmed him	26 (25)
Partner's hands/fingers on his penis	69 (66)
Always uses condoms with most recent sex partner	17 (17)

Abbreviation: IQR, interquartile range; MSM, men who have sex with men only; NGU, non-gonococcal urethritis.

*n=4 missing lifetime number of sex partners; n=4 missing number sex partners past 2 months; n=1 missing number receptive anal sex partners past 2 months; n=3 missing number insertive anal sex partners past 2 months; n1= missing number of receptive oral sex partners past 2 months; n=1 missing number insertive oral sex partners past 2 months; n=1 missing number of partners who rimmed him; n=2 missing new sex partner past 2 months; n=1 missing sexual exposures at last sexual episode; n=3 missing always condom use with most recent sex partner.

Table 3.3. Incidence rates of specific sexual exposures reported by men who have sex with men in the three months after non-gonococcal urethritis diagnosis

Sexual exposures	All men* 1,095 person-weeks	
	Number of episodes	IR per person-week (95%CI)
Overall		
Any sex	1,173	1.07 (0.93, 1.24)
Any RAI	253	0.23 (0.18, 0.30)
Any condomless RAI	206	0.19 (0.14, 0.25)
Any IAI	656	0.60 (0.49, 0.73)
Any condomless IAI	548	0.50 (0.40, 0.63)
Any IOS	870	0.79 (0.66, 0.95)
Any receptive rimming†	228	0.21 (0.15, 0.29)
Any receptive hand-penile contact‡	804	0.73 (0.60, 0.90)
Combinations of urethral sexual exposures		
IOS without condomless IAI	435	0.40 (0.32, 0.49)
Condomless IAI without IOS	114	0.10 (0.07, 0.15)
IOS and condomless IAI	434	0.40 (0.30, 0.52)

Abbreviation: IAI, insertive anal intercourse; IOS, insertive oral sex; IR, incidence rate; MSM, men who have sex with men only; NGU, non-gonococcal urethritis; RAI, receptive anal intercourse.

*Among 92 in the longitudinal analysis.

†Partner rimmed him.

‡Partner's fingers/hands on his penis.

Table 3.4. Association between recent urethral sexual exposures and composition of the urethral microbiota in the three months after non-gonococcal urethritis diagnosis*

Urethral sexual exposures by time window before specimen collection	Observations	Diversity†		Log10-richness‡	
	N=894* n (%)	Coefficient (95%CI)§	P¶	Coefficient (95%CI)§	P ¶
Prior 1-3 days					
None	545 (65)	Ref.		Ref.	
IOS only	103 (12)	-0.08 (-0.22, 0.06)	0.28	0.003 (-0.04, 0.05)	0.91
Condomless IAI only	26 (3)	-0.29 (-0.54, -0.04)	**0.02**	-0.11 (-0.21, -0.01)	**0.03**
Both IOS and condomless IAI	171 (20)	-0.07 (-0.19, 0.05)	0.28	0.001 (-0.05, 0.05)	0.98
Prior 4-6 days					
None	593 (69)	Ref.		Ref.	
IOS only	96 (11)	0.07 (-0.05, 0.18)	0.26	0.04 (-0.01, 0.08)	0.09
Condomless IAI only	27 (3)	0.05 (-0.19, 0.29)	0.68	-0.003 (-0.09, 0.09)	0.95
Both IOS and condomless IAI	138 (16)	-0.06 (-0.17, 0.05)	0.31	-0.03 (-0.06, 0.01)	0.16
Prior 7-9 days					
None	545 (65)	Ref.		Ref.	
IOS only	110 (13)	-0.01 (-0.15, 0.14)	0.93	0.01 (-0.03, 0.05)	0.66
Condomless IAI only	25 (3)	-0.04 (-0.28, 0.20)	0.77	-0.04 (-0.11, 0.03)	0.31
Both IOS and condomless IAI	165 (20)	0.03 (-0.09, 0.16)	0.60	0.01 (-0.03, 0.04)	0.69
Prior 10-12 days					
None	563 (67)	Ref.		Ref.	
IOS only	114 (14)	-0.02 (-0.15, 0.11)	0.81	0.002 (-0.04, 0.04)	0.93
Condomless IAI only	28 (3)	0.06 (-0.15, 0.28)	0.58	0.05 (-0.02, 0.12)	0.17
Both IOS and condomless IAI	137 (16)	-0.03 (-0.14, 0.08)	0.56	0.002 (-0.04, 0.05)	0.94
Prior 13-15 days					
None	598 (72)	Ref.		Ref.	
IOS only	93 (11)	0.18 (0.04, 0.31)	**0.01**	0.04 (0.01, 0.08)	**0.02**
Condomless IAI only	20 (2)	0.19 (-0.04, 0.43)	0.10	0.05 (-0.04, 0.14)	0.30
Both IOS and condomless IAI	122 (15)	0.06 (-0.06, 0.17)	0.33	0.04 (-0.001, 0.08)	**0.05**
Prior 16-18 days					
None	563 (67)	Ref.		Ref.	
IOS only	106 (13)	0.01 (-0.13, 0.14)	0.94	0.01 (-0.03, 0.05)	0.59
Condomless IAI only	23 (3)	0.36 (0.13, 0.59)	**<0.01**	0.14 (0.06, 0.21)	**<0.01**
Both IOS and condomless IAI	143 (17)	0.01 (-0.10, 0.13)	0.83	-0.03 (-0.07, 0.02)	0.23
Prior 19-21 days					
None	645 (76)	Ref.		Ref.	
IOS only	73 (9)	-0.004 (-0.14, 0.13)	0.96	-0.02 (-0.07, 0.04)	0.56
Condomless IAI only	20 (2)	-0.003 (-0.22, 0.22)	0.98	0.03 (-0.05, 0.11)	0.43
Both IOS and condomless IAI	113 (13)	-0.10 (-0.21, 0.02)	0.09	-0.03 (-0.07, 0.001)	0.06

Abbreviation: CI, confidence interval; IAI, insertive anal intercourse; IOS, insertive oral sex; P, p-value; Ref., referent group.

*894 time-points with urethral microbiota assessment among 92 men (1-15 observations per man). Sum of all n within each time window may not sum to column total (N) because some time-points were missing sexual exposure data for some windows.

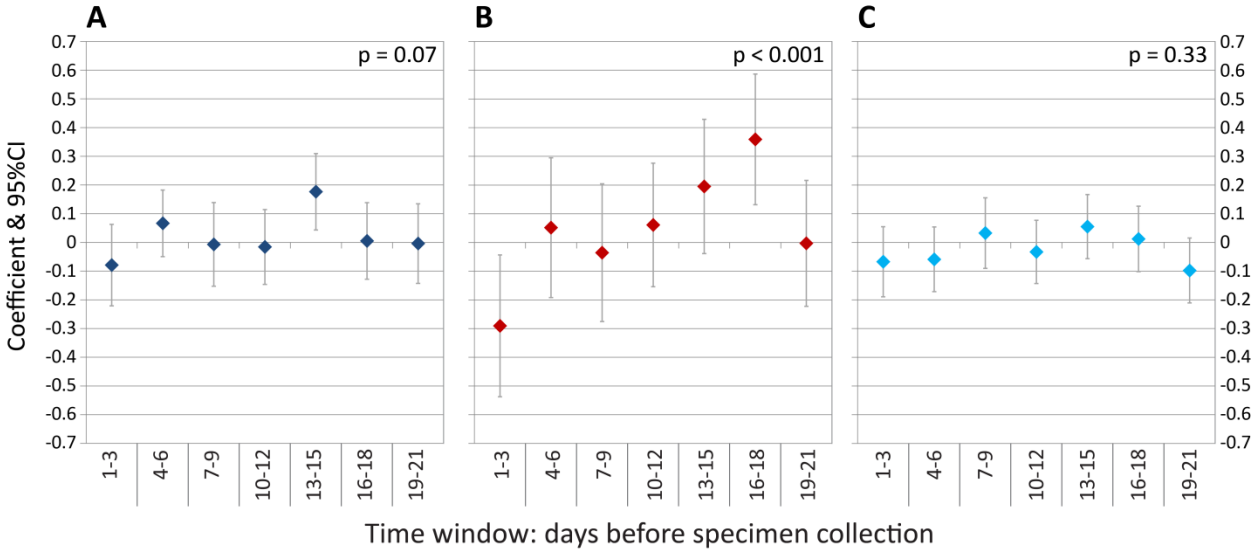
†Diversity measured by Shannon index.

‡Richness defined as number of bacterial species.

§Adjusted for recent antibiotics (in each of the 7 time windows), urethral sexual exposures (in each of the other time windows), missing antibiotic and urethral sexual exposure data (in each of the other time windows), and baseline age, race/ethnicity, HIV status, and PrEP use.

¶Bold indicates $P \leq 0.05$.

Figure 3.1. Association between recent urethral sexual exposures and the diversity* of the urethral microbiota in the three months after non-gonococcal urethritis diagnosis (insertive oral sex only [A], condomless insertive anal intercourse only [B], insertive oral sex and condomless insertive anal intercourse [C])[†§¶]



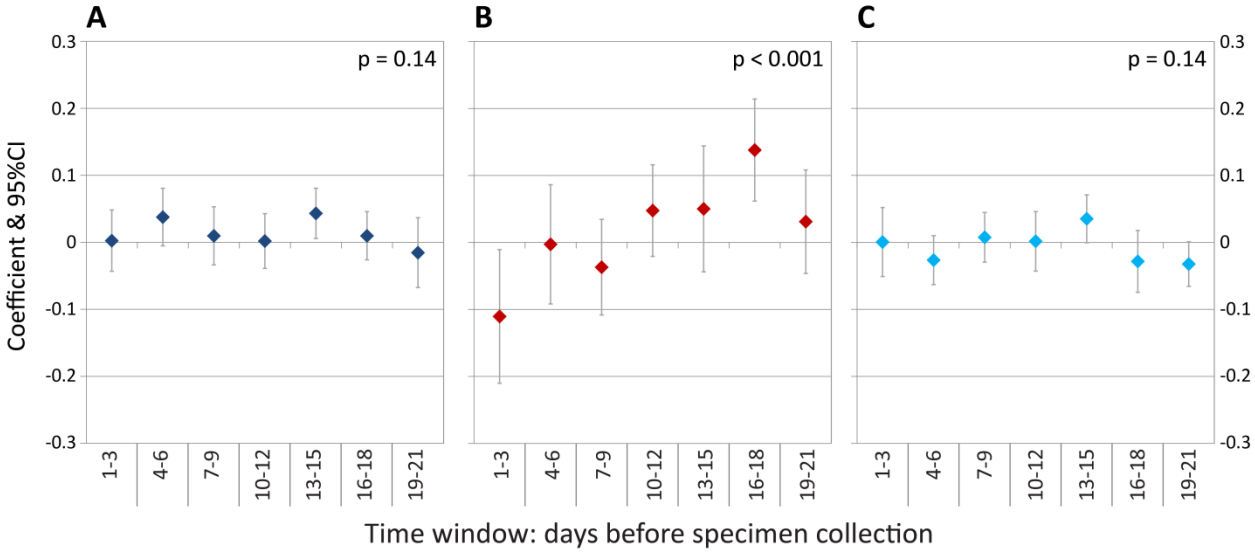
Abbreviation: CI, confidence interval; IAI, insertive anal intercourse; IOS, insertive oral sex.

*Diversity measured by Shannon index.

†894 time-points with urethral microbiota assessment among 92 men (1-15 observations per man).

‡Estimates adjusted for recent antibiotics (in each of the 7 time windows), urethral sexual exposures (in each of the other time windows), missing antibiotic and urethral sexual exposure data (in each of the other time windows), and baseline age, race/ethnicity, HIV status, and PrEP use.

Figure 3.2. Association between recent urethral sexual exposures and log10-richness* of the urethral microbiota in the three months after non-gonococcal urethritis diagnosis (insertive oral sex only [A], condomless insertive anal intercourse only [B], insertive oral sex and condomless insertive anal intercourse [C])[†§¶]



Abbreviation: CI, confidence interval; IAI, insertive anal intercourse; IOS, insertive oral sex.
*Richness defined as number of bacterial species.
†894 time-points with urethral microbiota assessment among 92 men (1-15 observations per man).
‡Estimates adjusted for recent antibiotics (in each of the 7 time windows), urethral sexual exposures (in each of the other time windows), missing antibiotic and urethral sexual exposure data (in each of the other time windows), and baseline age, race/ethnicity, HIV status, and PrEP use.

CHAPTER 4. Resolution of symptoms and resumption of sex after diagnosis of non-gonococcal urethritis among men who have sex with men

ABSTRACT

Background: Standard counseling at non-gonococcal urethritis (NGU) diagnosis includes advice to abstain from sex for seven days and until symptoms resolve.

Methods: We enrolled men who have sex with men and received azithromycin (1g) for NGU (12/16/2014-07/27/2018). Over 12 weeks, participants reported daily symptoms and sexual behavior on diaries. NGU was defined as symptoms or discharge plus ≥5 polymorphonuclear leukocytes per high-power field.

Results: Of 103 men with NGU, three (3%) were excluded due to *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) co-infection. Of the remaining 100 men, 36 (36%), 22 (22%), and 42 (42%) had CT-NGU, MG-NGU, and non-CT/non-MG NGU, respectively. Among men with MG-NGU, 94% had a macrolide resistance mutation. For all etiologies, median time to symptom resolution after azithromycin therapy was seven days (95% confidence interval [CI]=5-9); 37% had symptoms lasting >7 days. For men with CT-NGU, MG-NGU, and non-CT/non-MG NGU, median time to symptom resolution was four (95%CI=2-6, 16% >7 days), undefined (95%CI=7-undefined, 60% >7 days), and seven (95%CI=5-11, 46% >7 days) days, respectively. Median time to urethral sexual exposure after treatment was 16 days (95%CI=12-18); 27% did not avoid exposure for the recommended period.

Conclusions: Counseling at NGU diagnosis should educate patients that symptoms may persist >7 days and should emphasize the rationale for behavioral recommendations, as over a quarter of men did not follow counseling advice.

INTRODUCTION

Non-gonococcal urethritis (NGU), the most common male genital tract syndrome, is characterized by urethral symptoms and elevated polymorphonuclear leukocytes (PMNs).[2] In the United States, patients diagnosed with NGU are generally treated presumptively with antibiotics active against *Chlamydia trachomatis* (CT), the most common bacterial cause of NGU, and advised to (1) abstain from sex for at least seven days and until their symptoms resolve and (2) avoid sex with exposed sex partners until seven days after those partners have been treated.[11] The behavioral recommendations are intended to prevent transmission of pathogens to partners and to prevent reinfection from infected partners.

Time to symptom resolution after treatment is generally assumed to be roughly seven days based on studies of objective evidence of clinical cure rather than patient-reported symptoms.[2] Objective clinical and microbiologic cure are critical measures for defining treatment efficacy; however, patients' experience of symptom resolution is also a key part of clinical care and counseling at NGU diagnosis. Moreover, the timing of symptom resolution may vary by NGU etiology and efficacy of therapy. In particular, *Mycoplasma genitalium* (MG), the second leading cause of NGU, has rapidly developed resistance to the common first-line presumptive NGU therapy in the US, the macrolide azithromycin.[45] In addition, the efficacy of the recommended etiology-specific therapy for MG, the fluoroquinolone moxifloxacin, is declining.[46] Finally, the frequency with which patients follow counseling advice to avoid sexual exposure while the infection clears is unknown.

An improved understanding of time to symptom resolution and adherence to behavioral recommendations would inform counseling messages, help patients know what to expect following treatment for NGU, and further our understanding of time to clearance of NGU and the associated pathogens. To address these knowledge gaps, we used daily diary data to (1) estimate time to symptom resolution and time to recurrent urethral sexual exposure after presumptive azithromycin therapy for

NGU, overall and by etiology, and (2) estimate time to symptom resolution after initiation of moxifloxacin therapy for MG-NGU, among men who have sex with men (MSM).

METHODS

Study design and procedures

We enrolled Public Health – Seattle and King County (PHSKC) Sexually Transmitted Disease (STD) Clinic patients age ≥16 years with NGU who were assigned male sex at birth and only had sex with people assigned male sex at birth in the past year. Patients who had no sex in the past 60 days, antibiotics in the past 30 days, known urethral contact to *Neisseria gonorrhoeae* (GC), no valid contact information, or no freezer at home in which they could store urine specimens (not included in this analysis) were not eligible.

This prospective cohort study included in-clinic study visits every three weeks for three months (five visits total). Data from follow-up visits were not included in this analysis, so they are not described. Throughout study follow-up, participants completed weekly symptom and sex diaries.

Enrollment Visit. Potentially eligible patients saw one of two study clinicians who confirmed eligibility and offered enrollment. The clinician conducted a standard clinical examination, including collection of a urethral swab specimen for Gram staining and 30-45 mL of first-void urine, and examined the Gram-stained slide of urethral exudates to quantitate PMNs and diagnose urethral GC based on the presence of Gram-negative intracellular diplococci.[11] We defined NGU as urethral symptoms (urethral discharge, dysuria, or other urethral symptoms) or visible urethral discharge on examination, in combination with an average of ≥5 PMNs per high power field (HPF) assessed over the three most populated oil immersion fields. Participants received presumptive treatment for NGU according to PHSKC STD Clinic standard of care during this period, which was to provide either azithromycin 1g orally

as a single-dose or doxycycline 100mg orally twice daily for seven days.[11] Participants also provided sociobehavioral data on a computer-assisted self-interview developed with Research Electronic Data Capture (REDCap).[22]

The urine specimen was tested for GC and CT using the Aptima Combo 2 assay and MG using the Aptima assay with analyte-specific reagents (Hologic, Inc., Marlborough, Massachusetts). Prior to April 21, 2016, before MG testing was implemented as a routine part of this study, we conducted retrospective MG testing after participants completed study follow-up. During this time, participants with persistent or recurrent NGU were treated with moxifloxacin (400mg orally daily for 10-14 days[11]). We asked participants who were MG-positive at their final study visit to return for another test and provided moxifloxacin to those who still tested MG-positive. On April 21, 2016, we implemented real-time MG testing (i.e., conducted with GC and CT testing), and participants testing MG-positive were recalled to the clinic and provided moxifloxacin.

We retrospectively tested specimens that were MG-positive by Aptima assay for macrolide resistance mediating mutations (MRMM)[47] and fluoroquinolone resistance-associated mutations in the *parC* gene.[45] We performed DNA extraction from specimens in Aptima transport medium using a MagNA Pure 96 Instrument (Roche, Pleasanton, California) with large volume (1mL) universal pathogen extraction protocol and elution in 50µL. We used polymerase-chain-reaction and PyroMark Q96 sequencing to detect MRMM.[48,49] We amplified and sequenced the *parC* gene by conventional Sanger sequencing.[50] Finally, we used quantitative polymerase-chain-reaction to estimate the MG organism load.[49]

Web-Based Diaries. Throughout study follow-up, participants completed weekly diaries, reporting daily instances of urethral symptoms (discharge, dysuria, pruritus, or any other unusual feeling in/on their penis), sexual behavior (including types of sex and condom use), and antibiotic therapy. Questions referred to specific dates to minimize recall error. We programmed and automated the

diaries using REDCap, which is web-based and mobile phone enabled. REDCap e-mailed participants a unique URL to access their diary every Monday and sent reminders for incomplete diaries on Thursdays and Sundays. We timed out incomplete diaries every Sunday night to ensure compliance with the recall interval. Although we gave participants the option to complete their diaries on paper, none chose this method.

Statistical analysis

We excluded men with CT/MG co-infection from etiology-specific analyses. We compared baseline characteristics of participants by NGU etiology (CT-NGU, MG-NGU, and non-CT/non-MG NGU) using Fisher's exact tests and Kruskal-Wallis tests. We used the Kaplan-Meier method to estimate median time until symptom resolution after presumptive azithromycin therapy for NGU; men who received additional treatment (e.g., moxifloxacin) were censored when they started the second treatment. Time of symptom resolution was defined as the first of five consecutive days without urethral symptoms. For participants with missing symptom diary data before symptom resolution (n=11, 12%), their symptom resolution event time was the midpoint between the first day with missing data and the first day their symptoms were known to be resolved. In bivariate analyses, we tested for between-group differences by NGU etiology and type of urethral symptoms at enrollment using log-rank tests. We fit a Cox proportional hazards model with robust standard errors to estimate the association between NGU etiology and time to symptom resolution, adjusting for confounders. We considered potential confounding by age (years), race/ethnicity (non-Hispanic black; non-Hispanic white; Hispanic [any race]; and other, multiple, or unknown), known HIV-positive status, HIV pre-exposure prophylaxis (PrEP) use at enrollment, duration of urethral symptoms at enrollment (days), and type of urethral symptoms at enrollment (discharge only, dysuria only, other urethral symptoms only, or >1 symptom

type). These confounders were included in the final model if they changed an exposure effect estimate by >10%. Due to the use of retrospective MG testing early in our study, we also evaluated potential effect modification of the exposure-outcome associations by MG testing-period (retrospective vs. real-time). In a separate analysis, for participants with MG-NGU during the real-time testing period and persistent urethral symptoms when they started moxifloxacin, we estimated median time until symptom resolution after initiation of moxifloxacin using the Kaplan-Meier method; men who received additional treatment were censored when they started the third treatment. Due to uncertainty in the ideal definition of time of symptom resolution when using daily data, we conducted sensitivity analyses for time to symptom resolution after azithromycin and after moxifloxacin varying our definition from the first of 4-6 consecutive asymptomatic days.

Finally, we used the Kaplan-Meier method to estimate median time until first reported urethral sexual exposure after NGU diagnosis and treatment. We defined urethral sexual exposure as insertive oral sex or condomless insertive anal sex. We tested for between-group differences by NGU etiology using a log-rank test. For participants with ≥1 day with missing sexual exposure diary data before their first reported urethral exposure (n=10, 11%), we assumed they had no urethral exposure on missing days. We identified baseline characteristics independently associated with not following behavioral recommendations using logistic regression with robust standard errors.

This study was approved by the University of Washington Human Subjects Division. Participants provided written, informed consent. We compensated participants a total of $360 for their time. We used Stata 15 (StataCorp, College Station, Texas) for analyses, two-sided tests, and significance-level alpha=0.05.

RESULTS

Between December 16, 2014 and July 27, 2018, we approached 502 patients attending the PHSKC STD Clinic about this study, of whom 204 (41%) were ineligible, 187 (37%) declined, and 111 (22%) enrolled. The main reasons for declining were inability to stay to enroll (21%) or attend follow-up visits (41%). Of 111 people enrolled, we excluded eight (7%) from this analysis because they received presumptive doxycycline (n=7) or moxifloxacin (n=1, reported contact to MG) therapy for their NGU and our sample size was insufficient to stratify analyses by initial therapy. The remaining 103 participants were included in baseline analyses.

Baseline characteristics

All 103 participants with NGU self-identified as cisgender men. The median age was 30 years (range=20-60), and 55 men (53%) were non-Hispanic white (**Table 1**). Twelve men (12%) were known to be living with HIV. Urine specimens from 36 (35%), 22 (21%), and three (3%) men tested positive for CT only, MG only, and both CT and MG, respectively. The remaining 42 men (41%) tested negative for both pathogens and were considered to have non-CT/non-MG NGU. At enrollment, 97 men (94%) reported urethral symptoms, and 93 (90%) had visible urethral discharge (**Table 2**). Most (81%) had ≥10 PMNs/HPF on a urethral Gram stain. The baseline characteristics of men with CT-NGU, MG-NGU, and non-CT/non-MG NGU were similar. Men with MG-NGU were somewhat more likely to be taking PrEP than those with CT-NGU and non-CT/non-MG NGU (37% vs. 17% and 13%, respectively; *P*=0.09).

Of 22 men with MG-NGU, four (18%) enrolled during the retrospective MG testing period (none of whom received moxifloxacin for persistent or recurrent NGU), and 18 (82%) enrolled during the real-time MG testing period. Among the 17 men with MG-NGU with resistance test results, 16 (94%) had

MRMM and two (12%) had the S83I fluoroquinolone resistance-associated ParC mutation in their pre-treatment specimen.

Longitudinal analyses

Ten men (10%) were excluded from the longitudinal analyses because they did not report urethral symptoms at enrollment (n=5) or did not have any diary data (n=5). Men excluded from the longitudinal analyses were younger (*P*=0.009) and reported lower education (*P*=0.02) compared to men included in the longitudinal analyses. The remaining 93 men included in longitudinal analyses reported symptom and sexual behavior data over a median of 86 days (interquartile range [IQR]=79-92, range=7-108). Overall, 89% of diaries were completed. Thirty-five men (38%) missed ≥1 diary.

Symptom resolution. Overall, 21 men (23%) had intermittent days (≥1) without urethral symptoms before meeting our definition of symptom resolution. Across all etiologies, median time to symptom resolution after presumptive azithromycin therapy alone for NGU was seven days (95% confidence interval [CI]=5-9) (**Table 3**). Among 72 men with an observed event time (i.e., not censored due to additional treatment or the end of their follow-up time), time to symptom resolution ranged from one to 39 days. Among 83 men not censored within the first eight days, 31 (37%) had symptoms lasting >7 days following treatment. For men with CT-NGU, MG-NGU, and non-CT/non-MG NGU, median time to symptom resolution after azithromycin therapy alone was four (95%CI=2-6), undefined (95%CI=7-undefined), and seven (95%CI=5-11) days, respectively (*P*=0.001) (**Figure 1**). Among men who were not censored within the first eight days, five (16%), nine (60%), and 16 (46%) had symptoms lasting >7 days for CT-NGU, MG-NGU, and non-CT/non-MG NGU, respectively. The undefined median time to symptom resolution after azithromycin alone among men with MG-NGU was due to the majority of men

starting moxifloxacin before experiencing symptom resolution. Median time to symptom resolution did not vary by type of urethral symptoms at enrollment (*P*=1.0).

Men with MG-NGU (hazard ratio [HR]=0.29, 95%CI=0.13-0.68) and non-CT/non-MG NGU (HR=0.56, 95%CI=0.36-0.89) had a decreased "hazard" for symptom resolution (i.e., longer time to symptom resolution) after azithromycin therapy compared to those with CT-NGU (**Table 4**). Adjustment for age, race/ethnicity, HIV status, PrEP use at enrollment, duration and type of urethral symptoms at enrollment, and MG testing period did not meaningfully change these estimates.

There was evidence that the association between MG-NGU and time to symptom resolution after azithromycin therapy differed for men enrolled during the retrospective versus real-time MG testing period (*P*=0.002). During the retrospective testing period, time to symptom resolution was shorter for men with MG-NGU compared to CT-NGU. In contrast, during the real-time testing period, time to symptom resolution was longer for men with MG-NGU than CT-NGU. However, only 4 men had MG-NGU during the retrospective testing period, and the pre-treatment MG organism load was substantially lower for those enrolled during the retrospective versus real-time testing period (median eight vs. 1,371 genome equivalents per 5µL template, *P*=0.01). When a covariate for organism load was added to the model, there was no longer evidence of effect modification by MG testing period (*P*=0.12).

Importantly, of 12 men with MG-NGU during the real-time MG testing period known to have persistent urethral symptoms at the time they started moxifloxacin, eight of nine (89%) with resistance test results had MRMM in their pre-azithromycin specimen. None had the S83I fluoroquinolone-resistance associated ParC mutation. Among these 12 men, median time to symptom resolution after initiation of moxifloxacin was four days (95%CI=1-8). Among the 11 men who were not censored within the first eight days, two (18%) had symptoms lasting >7 days.

In the sensitivity analysis varying the definition of time of symptom resolution from the first of 4-6 asymptomatic days, median time to symptom resolution after presumptive azithromycin therapy

alone ranged from 6-7 days overall (34-40% >7 days) and 7-10 days for non-CT/non-MG NGU (43-47% >7 days); it remained stable for CT-NGU (four days, 13-19% >7 days) and MG-NGU (undefined, 53-67% >7 days). Median time to symptom resolution after initiation of moxifloxacin for MG-NGU remained stable (four days, 20-25% >7 days).

Resumption of sex. Among the 93 men with urethral symptoms at enrollment and some diary data, median time to first reported urethral sexual exposure after diagnosis and treatment of NGU was 16 days (95%CI=12-18) (**Table 5**). Among the 89 men with an observed event time, time to resumption of urethral sexual exposure ranged from three to 85 days. Median time to first reported urethral exposure did not differ by NGU etiology (*P*=0.8). Twenty-five men (27%) did not follow counseling advice to abstain from urethral sexual exposure for ≥7 days and until symptoms resolve. Seventeen men (18%) reported urethral exposure within 1-7 days, although 71% of these men experienced symptom resolution before their first exposure. Additionally, 13 men (14%) reported a urethral exposure before their symptoms resolved. Not following counseling advice was positively associated with report of a new sex partner in the past two months (adjusted odds ratio [aOR]=8.57, 95%CI=1.22-60.21, *P*=0.03) and inversely associated with ≥10 versus 5-9 PMNs/HPF (aOR=0.26, 95%CI=0.08-0.85, *P*=0.03) at enrollment.

DISCUSSION

Among MSM in Seattle, over 35% had urethral symptoms lasting >7 days after presumptive azithromycin therapy alone for NGU. The time to symptom resolution differed by NGU etiology. Urethral symptoms lasted at least three days longer for men with MG-NGU and non-CT/non-MG NGU compared to CT-NGU. Among MSM with MG-NGU, longer time to symptom resolution after azithromycin was likely due to near universal MRMM in MG. Although symptoms persisted longer for MG-NGU than CT-NGU after azithromycin, time to symptom resolution after starting effective therapy (i.e., moxifloxacin

for MG, azithromycin for CT) was similar for MG-NGU and CT-NGU (four days). Over 25% of men did not follow counseling advice to abstain from sex for ≥7 days and until symptoms resolve.

We were surprised that urethral symptoms lasting >7 days after azithromycin therapy were so common, given the high efficacy of azithromycin for NGU cases not caused by MG and prior evidence that most patients with NGU experience some or complete clinical improvement by the end of treatment.[2] Studies of tetracycline hydrochloride,[51] oxytetracycline and erythromycin stearate,[52] minocycline,[6] and azithromycin and doxycycline[12,53-55] therapy suggest that 64-98% of patients experience clinical cure by 6-35 days after treatment initiation based on objective criteria (generally visible discharge and/or elevated PMNs). The studies with the shortest follow-up intervals (~6-10 days) found that 92% and 64% experienced clinical cure after minocycline[6] and tetracycline hydrochloride,[51] respectively. Studies of azithromycin reported high clinical cure rates (87-94%) but after longer follow-up intervals (14-35 days), and two of them were conducted at a time when MG with MRMM was rare.[12,53-55] Importantly, none of these studies reported the percentage of patients whose urethral symptoms resolved or the time until symptom resolution.

Our finding that time until symptom resolution after azithromycin therapy varied by NGU etiology is consistent with prior evidence that the efficacy of azithromycin for achieving clinical cure is higher for CT-NGU than for MG-NGU and non-CT/non-MG NGU. Among men with NGU in Seattle, three weeks after presumptive azithromycin therapy, the clinical cure rates for men with CT, MG, and neither pathogen were 87%, 63%, and roughly 81%, respectively.[12] Moreover, MRMM in MG have been associated consistently with azithromycin treatment failure.[45] Thus, the 94% prevalence of MRMM in MG among men with MG-NGU in our study likely contributed to their long duration of symptoms after azithromycin (60% with symptoms >7 days). In contrast, symptom resolution occurred more rapidly after initiation of moxifloxacin for MG (18% with symptoms >7 days). However, we were surprised that, among men with MG-NGU, median time to symptom resolution was shorter for the four men enrolled

during the retrospective MG testing period (none treated with moxifloxacin) compared to the 14 enrolled during the real-time testing period (all treated with moxifloxacin). The very low MG organism load among the four men enrolled during the retrospective testing period likely led to their prompt symptom resolution, as there was no difference between the two time-periods after adjusting for organism load. The difference in symptom resolution timing across the two time-periods may also be related to a difference in the duration of infection before enrollment (although adjusting for duration of urethral symptoms at enrollment did not change our estimates), a difference in the pathogenicity of circulating MG strains, the small number of men with MG-NGU during the retrospective testing period (i.e., an unstable estimate), and/or a difference in perception of symptoms when patients anticipate receiving MG test results.

To our knowledge, adherence to counseling advice on sexual abstinence immediately following NGU diagnosis has not been evaluated previously. We found that a substantial percentage of men (18%) resumed sexual activity during the recommended 7-day period of abstinence, although many of these men (71%) waited for their symptoms to resolve first. The implications of this finding for transmission of pathogens to partners depend on how long it takes men to clear NGU-associated pathogens after treatment and how well this correlates with symptom resolution. A study of CT clearance in women found that three, seven, 10, and 14 days after azithromycin 1g treatment 88%, 54%, 34%, and 21% of women, respectively, still had detectable CT ribosomal ribonucleic acid,[56] although the extent to which these nucleic acids represent viable organisms is uncertain. Evidence suggests that MG clears more quickly when effective treatment is provided. Among men and women with genital MG infection, 38% and 4% had detectable MG nucleic acids three and eight days, respectively, after azithromycin 1.5g treatment, and none had detectable nucleic acids three days after starting moxifloxacin.[57] However, we expect MG clearance to depend on the prevalence of MRMM and fluoroquinolone resistance-associated mutations.[45] Nonetheless, the anti-inflammatory properties of azithromycin[58,59] may lead to symptom

resolution prior to pathogen clearance, suggesting that a recommended period of abstinence is critical irrespective of symptom resolution. Currently, US, Australian, and European NGU management guidelines emphasize a 7-day period of abstinence.[11,60,61]

Our analysis has limitations. First, there was no precedent for defining time of symptom resolution with daily data, and 23% of our participants reported intermittent symptoms. Our definition (the first of five consecutive asymptomatic days) was intended to capture the subjective threshold at which intermittent symptoms would be considered persistent rather than recurrent clinically. Varying this definition from the first of 4-6 consecutive asymptomatic days did not meaningfully change our findings. Nonetheless, our definition likely differs from that of patients, who may consider it "safe" to resume sex after one symptomless day. Second, we did not consider objective evidence of clinical or microbiologic cure in this analysis. Some men with prompt symptom resolution may have had persistent NGU and/or pathogen infection. Similarly, some men with a long duration of symptoms may have experienced rapid NGU resolution and/or pathogen clearance. Nevertheless, understanding the patient experience of symptom resolution is an important aspect of clinical care. Third, this analysis did not consider recurrent symptoms after initial resolution, and recurrent NGU is common.[2] Fourth, we did not have information on sex partner treatment with which to evaluate that aspect of clinical counseling. Fifth, younger men were slightly under-represented in the longitudinal analyses. Symptoms may resolve slower for younger patients who have had no or fewer prior cases of NGU. Finally, the true event times for some men (11-12% for each outcome) are uncertain due to missing diary data. For our time to symptom resolution analysis, we also fit a proportional hazards model with interval censoring (versus use of the midpoint), and our estimates remained stable. For our time to resumption of sex analysis, we assumed men had no urethral sexual exposure on missing days, so we likely over-estimated the true time to first exposure.

Among MSM in Seattle, NGU symptoms often persist for >7 days following presumptive azithromycin therapy for NGU, particularly in those with MG-NGU or non-CT/non-MG NGU. Using current treatment guidelines,[11] only 40% of men with MG-NGU would experience symptom resolution within seven days, and only 54% of men with non-CT/non-MG NGU would have resolution within seven days. The very high prevalence of MRMM among men with MG-NGU likely led to their long duration of symptoms after azithromycin; their symptoms resolved relatively rapidly after initiation of moxifloxacin. Inadequate treatment of unidentified pathogens or polymicrobial communities may have contributed to the long duration of symptoms for men with non-CT/non-MG NGU. Over 1-in-4 men did not follow counseling advice to abstain from sex for ≥7 days and until symptoms resolve. Standard counseling at NGU diagnosis should educate patients that symptoms may persist for >7 days and emphasize the rationale for the 7-day abstinence period.

TABLES AND FIGURES

Table 4.1. Baseline characteristics of men who have sex with men with non-gonococcal urethritis, overall and by etiology

Characteristic*	Overall N=103 n (%)	CT-NGU† n=36 n (%)	MG-NGU† n=22 n (%)	Non-CT/non-MG NGU n=42 n (%)	P-value‡
Age, median (IQR)	30 (27-39)	31 (27-37)	31 (28-40)	30 (27-40)	0.782§
Race/ethnicity					
Non-Hispanic black	7 (7)	4 (11)	0 (0)	3 (7)	
Non-Hispanic white	55 (53)	18 (50)	12 (55)	23 (55)	0.157
Hispanic (any race)	19 (19)	5 (14)	2 (9)	11 (26)	
Other, multiple, or unknown	22 (21)	9 (25)	8 (36)	5 (12)	
Education completed					
≤High School or GED	25 (25)	12 (34)	5 (23)	7 (17)	0.306
Some college	28 (27)	6 (17)	6 (27)	15 (36)	
College graduate	49 (48)	17 (49)	11 (50)	20 (47)	
Known HIV-positive	12 (12)	6 (17)	3 (14)	3 (7)	0.383
HIV pre-exposure prophylaxis use‖	18 (20)	5 (17)	7 (37)	5 (13)	0.092
History of previous STD					
History of NGU	37 (36)	12 (33)	9 (41)	15 (36)	0.831
History of GC infection	58 (56)	20 (56)	15 (68)	21 (50)	0.391
History of CT infection	60 (58)	22 (61)	14 (64)	22 (53)	0.633
Lifetime number of sex partners					
1-9	5 (5)	1 (3)	1 (5)	3 (8)	
10-49	29 (29)	12 (34)	5 (23)	12 (31)	0.573
50-99	24 (24)	11 (31)	6 (27)	6 (15)	
≥100	41 (42)	11 (31)	10 (45)	18 (46)	
Number of sex partners in past 2 months, median (IQR)					
Any type of sex	3 (2-6)	3 (2-5)	4 (3-7)	3 (2-6)	0.253§
Insertive anal sex	2 (1-4)	2 (1-4)	2 (2-5)	2 (1-4)	0.814§
Receptive anal sex	1 (0-2)	1 (0-2)	1.5 (1-2)	1 (0-2)	0.101§
Insertive oral sex	3 (2-5)	2.5 (2-5)	4 (3-6)	3 (2-5)	0.387§
New sex partner in past 2 months	83 (81)	28 (78)	19 (86)	34 (81)	0.719

Abbreviation: CT, *Chlamydia trachomatis*; GC, *Neisseria gonorrhoeae*; GED, General Education Diploma; HIV, human immunodeficiency virus; IQR, interquartile range; MG, *Mycoplasma genitalium*; MSM, men who have sex with men only; NGU, non-gonococcal urethritis; STD, sexually transmitted disease.

*n=1 missing education completed, n=4 missing lifetime number of sex partners, n=3 missing number sex partners past 2 months, n=3 missing number insertive anal sex partners past 2 months, n=1 missing number of receptive anal sex partners past 2 months, and n=1 missing number of insertive oral sex partners past 2 months.

†Excludes men co-infected with CT and MG (n=3).

‡Fisher's exact test unless otherwise specified.

[§]Kruskal-Wallis test.
[‖]Among men not known to be HIV-positive (n=91 overall, n=30 CT-NGU, n=19 MG-NGU, n=39 non-CT/non-MG NGU).

Table 4.2. Baseline clinical presentation of non-gonococcal urethritis among men who have sex with men, overall and by etiology

Characteristic	Overall N=103 n (%)	CT-NGU* n=36 n (%)	MG-NGU* n=22 n (%)	Non-CT/non-MG NGU n=42 n (%)	P-value[†]
Urethral symptoms	97 (94)	35 (97)	21 (95)	39 (93)	0.841
Type(s)					
Urethral discharge	51 (53)	18 (51)	12 (57)	20 (51)	0.930
Dysuria	62 (64)	22 (63)	16 (76)	23 (59)	0.410
Other urethral symptoms	54 (56)	21 (60)	8 (38)	24 (62)	0.206
Duration (days), median (IQR)[§]	4 (2-9)	4.5 (2-9)	2.5 (2-10)	4.5 (2.5-7)	0.744[‖]
Visible urethral discharge	93 (90)	34 (94)	20 (91)	36 (86)	0.429
Character					
Clear	61 (66)	25 (74)	12 (60)	22 (61)	0.308
Cloudy	25 (27)	9 (26)	6 (30)	10 (28)	
Mucoid or purulent	7 (7)	0 (0)	2 (10)	4 (11)	
Amount					
Small	57 (61)	22 (65)	12 (60)	20 (56)	0.916
Moderate	34 (37)	11 (32)	8 (40)	15 (42)	
Large	2 (2)	1 (3)	0 (0)	1 (3)	
PMNs/HPF on a urethral Gram stain					
5-9	20 (19)	4 (11)	7 (32)	8 (19)	0.163
≥10	83 (81)	32 (89)	15 (68)	34 (81)	
Macrolide resistance mediating mutation[¶]	-	-	16 (94)	-	-
S83I fluoroquinolone resistance-associated ParC mutation[¶]	-	-	2 (12)	-	-
Organism load (genome equivalents / 5 µL template), median (IQR)[¶]	-	-	403 (11-2,230)	-	-

Abbreviation: CT, *Chlamydia trachomatis*; HPF, high power field; IQR, interquartile range; MG, *Mycoplasma genitalium*; MSM, men who have sex with men only; NGU, non-gonococcal urethritis; PMN, polymorphonuclear leukocytes.

*Excludes men co-infected with CT and MG (n=3).

[†]Fisher's exact test unless otherwise indicated.

[§]Missing for n=2 who reported unknown or sporadic duration of urethral symptoms.

[‖]Kruskal-Wallis test.

[¶]n=5 with MG-NGU missing data on resistance-associated mutations and MG organism load.

Table 4.3. Time to symptom resolution by treatment type among men who have sex with men with non-gonococcal urethritis, overall and by etiology*[†]

	Etiology	**Median** days (95%CI)	**P-value**[‡]	**>7 days, among men not censored within the first 8 days**[§] n (%)
After presumptive azithromycin therapy for NGU	Overall (n=93)	7 (5-9)	-	31 (37)
	By etiology			
	CT-NGU (n=32)[‖]	4 (2-6)		5 (16)
	MG-NGU (n=22)[‖]	undefined (7-undefined)	0.001	9 (60)
	Non-CT/non-MG (n=37)	7 (5-11)		16 (46)
After initiation of moxifloxacin therapy for MG	MG-NGU (n=12)[¶]	4 (1-8)	-	2 (18)

Abbreviation: CI, confidence interval; CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MSM, men who have sex with men only; NGU, non-gonococcal urethritis.

*Among men with urethral symptoms at enrollment (n=5 excluded) and some diary data (n=5 additional excluded).

[†]Time of symptom resolution defined as the first of 5 consecutive asymptomatic days.

[‡]Log-rank test.

[§]Among men not censored within the first 8 days for the azithromycin analysis (excludes n=10 overall, n=7 MG-NGU, n=2 non-CT/non-MG NGU) and moxifloxacin analysis (excludes n=1).

[‖]Excludes men co-infected with CT and MG (n=2).

[¶]Among men with MG-NGU during the real-time testing period (n=4 excluded) known to have persistent urethral symptoms when they started moxifloxacin (n=6 additional excluded).

Table 4.4. Cox proportional hazards model to estimate the association between non-gonococcal urethritis etiology and time to symptom resolution after presumptive azithromycin therapy alone for non-gonococcal urethritis among men who have sex with men*[†]

Etiology	HR (95%CI)[‡]	P-value
CT-NGU (n=32)	Ref.	
MG-NGU (n=22)	0.29 (0.13-0.68)	0.004
Non-CT/non-MG (n=37)	0.56 (0.36-0.89)	0.013

Abbreviation: CI, confidence interval; CT, *Chlamydia trachomatis*; HR, hazard ratio; MG, *Mycoplasma genitalium*; MSM, men who have sex with men only; NGU, non-gonococcal urethritis.

*Among men with urethral symptoms at enrollment (n=5 excluded), with some diary data (n=5 additional excluded), and not co-infected with CT and MG (n=2 additional excluded).

[†]Time of symptom resolution defined as the first of 5 consecutive asymptomatic days.

[‡]Adjustment for age (years), race/ethnicity (non-Hispanic black, non-Hispanic white, Hispanic [any race], other/multiple/unknown), known HIV-positive status, PrEP use at enrollment, duration of urethral symptoms prior to enrollment (days), type of urethral symptoms at enrollment (discharge only, dysuria only, other urethral symptoms only, >1 symptom), and MG testing period (retrospective vs. real-time) did not change these estimates by >10%.

Table 4.5. Time to first known urethral sexual exposure after presumptive azithromycin therapy for non-gonococcal urethritis among men who have sex with men, overall and by etiology*[†]

Etiology	**Median** days (95%CI)	**P-value**[‡]	**>7 days, among men not censored within the first 7 days**[§] n (%)
Overall (n=93)	16 (12-18)		75 (82)
By etiology			
CT-NGU (n=32)[‖]	14 (10-23)		28 (88)
MG-NGU (n=22)[‖]	14 (8-22)	0.753	17 (77)
Non-CT/non-MG NGU (n=37)	17 (10-22)		28 (78)

Abbreviation: CI, confidence interval; CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MSM, men who have sex with men only; NGU, non-gonococcal urethritis.

*Among men with urethral symptoms at enrollment (n=5 excluded) and some diary data (n=5 additional excluded).

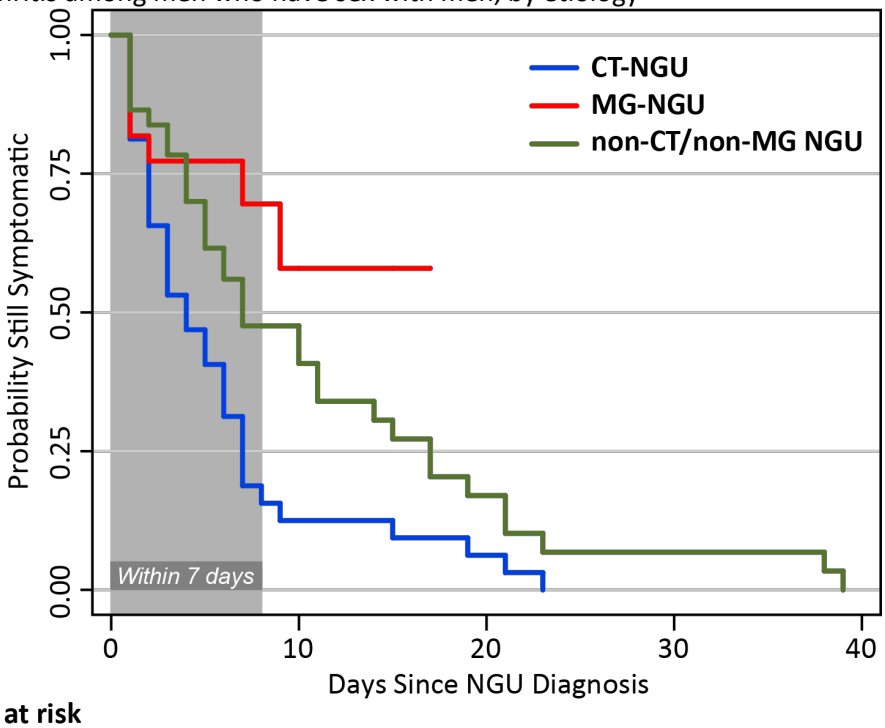
[†]Urethral sexual exposure defined as any insertive oral sex or condomless insertive anal sex.

[‡]Log-rank test.

[§]Among men not censored within the first 7 days (excludes n=1 overall, n=1 non-CT/non-MG NGU).

[‖]Excludes men co-infected with CT and MG (n=2).

Figure 4.1. Time to symptom resolution after presumptive azithromycin therapy alone for non-gonococcal urethritis among men who have sex with men, by etiology*†



Numbers at risk

CT-NGU	32	15	4	4	2	0	0	0	0
MG-NGU	22	17	4	2	0	0	0	0	0
non-CT/non-MG NGU	37	25	14	9	5	2	2	2	0

Abbreviation: CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MSM, men who have sex with men only; NGU, non-gonococcal urethritis.

*Among men with urethral symptoms at enrollment (n=5 excluded), with some diary data (n=5 additional excluded), and who were not co-infected with CT and MG (n=2 additional excluded).

†Time of symptom resolution defined as the first of 5 consecutive asymptomatic days.

CHAPTER 5. Conclusion

In this dissertation, we conducted three analyses from two studies of patients attending the Public Health – Seattle and King County STD Clinic. In the cross-sectional study of patients with and without non-gonococcal urethritis (NGU) described in Chapter 2, we found that, among men who have sex with men (MSM) and transgender women who have sex with men, insertive anal intercourse (IAI) with and without insertive oral sex (IOS) at last sexual episode was associated with higher odds of NGU and *Chlamydia trachomatis* (CT)- and *Mycoplasma genitalium* (MG)-negative NGU (i.e., non-CT/non-MG NGU) compared to neither urethral exposure, but IOS alone was not. In contrast, among men who have sex with women (MSW), there was minimal variation in urethral sexual exposures at last sexual episode and no evidence that vaginal sex with IOS at last sexual episode was associated with higher odds of NGU compared to vaginal sex alone. In our cohort study of MSM with NGU at baseline described in Chapter 3, we found that condomless IAI alone in the prior 21 days was independently associated ecological diversity and richness of bacterial species of the urethral microbiota. More specifically, diversity and richness were lower 1-3 days after condomless IAI and higher 16-18 days after condomless IAI. There was minimal evidence of an association between recent IOS and diversity or richness. Finally, in Chapter 4, we reported that NGU symptoms often persisted for more than seven days following presumptive azithromycin, particularly for those with MG-NGU and non-CT/non-MG NGU. The very high prevalence (94%) of macrolide resistance mediating mutations among men with MG-NGU likely led to their long duration of symptoms after azithromycin. Over 1-in-4 MSM with NGU did not follow counseling guidance to abstain from sex for at least seven days and until symptoms resolved.

The findings from these studies have several important implications. First, based on our findings among MSM, it is likely that IAI leads to the transmission of yet-unidentified rectal micro-organisms that cause some non-CT/non-MG NGU, in addition to transmission of known pathogens. This is further supported by our finding that condomless IAI influences the diversity and richness of the urethral

microbiota among MSM, both in the short-term (1-3 days) and subsequent 2-3 weeks. This may also be true for MSW; however, minimal variation in urethral sexual exposures at last sexual episode prohibited us from estimating the association between IAI and NGU among MSW in the cross-sectional study. We have ongoing analyses of a cohort of MSW, which are considering the influence of sexual exposures on the urethral microbiota and NGU and may provide insight on the role of IAI in this population. Finally, our analysis of symptom resolution and recurrent urethral sexual exposure among MSM after syndromic therapy for NGU suggest that counseling at NGU diagnosis should educate patients that symptoms may persist for more than seven days and should emphasize the rationale for the 7-day abstinence period.

Our studies also highlight several gaps in the field of NGU research which may inform future studies. First, additional research is needed to define the role of the urethral microbiota in NGU and non-CT/non-MG among MSM and MSW, particularly with longitudinal data. It would also be useful to determine the role of rectal micro-organisms in non-CT/non-MG NGU. More generally, little is known about the determinants of the composition of the urethral microbiota. Our analysis suggests that urethral sexual exposures influence the microbiota, but it may also be influenced by one's microbial communities at other body sites, the microbial communities of one's mother, and environmental exposures, among other things. In addition, the relative importance of cumulative sexual exposures, very recent sexual exposures, and sexual exposure to new partners in determining the composition of the urethral microbiota remains unknown. Furthermore, among MSW, it would be useful to better characterize partner characteristics that are associated with non-CT/non-MG, as specific anatomic sites of urethral sexual exposure varied little among the MSW in our study. Longitudinal studies and/or larger cross-sectional studies of MSW are needed to define the role of IAI in the etiology of NGU among MSW, as very recent IAI was relatively uncommon among MSW in our study. Finally, this dissertation focused on risk factors for and clinical management of initial NGU episodes. Additional research is needed to determine the extent to which persistent and recurrent NGU episodes are similar to initial episodes.

In conclusion, among MSM, IAI may lead to the acquisition of rectal micro-organisms which may cause some non-CT/non-MG NGU, in addition to transmission of known pathogens such as CT and MG. As we learn more about the association between rectal micro-organisms and the urethral microbiota and NGU, this highlights the potential for prevention targets.

ACKNOWLEDGEMENTS

I owe deepest gratitude to my dissertation chair and mentor, Lisa Manhart, for her guidance and support and for facilitating countless professional opportunities for me in the field of STD epidemiology. I would also like to thank the other members of my supervisory committee, David Fredricks, Matthew Golden, James Hughes, Christine Khosropour, and Richard Darveau, for being incredibly generous with their time and for their invaluable guidance and perspective.

I would like to recognize many others who contributed to the work in this dissertation. These studies would not have been possible without the study participants attending the Public Health – Seattle and King County STD Clinic; our dedicated study clinicians, Jennifer Morgan and Sylvan Lowens; study data manager, Sean Proll; and our research coordinators, Tashina Robinson, Sarah Romano, and Gina Leipertz. Thank you to the staff at the STD Clinic for referring patients to the study and facilitating study appointments. Thank you also to Sujatha Srinivasan, Noah Hoffman, Ken Tapia, Krista Yuhas, Anna Wald, Jeanne Marrazzo, and the rest of the Sexually Transmitted Infections Cooperative Research Centers leadership and staff for their support, scientific expertise, and regular feedback. Many thanks to the Fredricks Laboratory staff and to Olusegun Soge, Patricia Totten, Jørgen Jensen, and their laboratory staff for laboratory expertise. Thank you to Sara Glick for guidance on working with diary data. I gratefully acknowledge Hologic, Inc. for donation of test kits and reagents.

This work would not have been possible without financial support from the National Institutes of Health (NIH). The research in Chapter 2 was supported by NIH U19 AI113173091 (Center PI: Anna Wald, Project 3 PIs: Lisa Manhart and Sujatha Srinivasan) and NIH R01 AI110666-01 (PIs: Lisa Manhart and David Fredricks), while the research in Chapters 3 and 4 was supported by the aforementioned U19. I was supported by the U19 (2014-2019) and the TL1 Translational Research Training Grant NIH TL1 TR002319 (PIs: Joie Whitney and Linda LeResche) (2017-2018). Finally, our study data were collected

using REDCap tools hosted at the University of Washington Institute of Translational Health Sciences, which is supported by NIH UL1 TR002319.

Finally, I would like to thank my partner, family, and friends for their unwavering support and encouragement.

REFERENCES

1. Centers for Disease Control and Prevention. *Sexually Transmitted Disease Surveillance 2000.* Atlanta, GA; 2001.
2. Martin DH. Urethritis in Males. In: Holmes KK, Sparling PF, Stamm WE, et al., eds. *Sexually Transmitted Diseases*. 4th ed. New York: McGraw Hill; 2008: 1107-1126.
3. Deguchi T, Yoshida T, Miyazawa T, et al. Association of *Ureaplasma urealyticum* (biovar 2) with nongonococcal urethritis. *Sex Transm Dis.* 2004; 31(3):192-195.
4. Wetmore CM, Manhart LE, Lowens MS, et al. *Ureaplasma urealyticum* is associated with nongonococcal urethritis among men with fewer lifetime sexual partners: a case-control study. *J Infect Dis.* 2011; 204(8):1274-1282.
5. Bradshaw CS, Denham IM, Fairley CK. Characteristics of adenovirus associated urethritis. *Sex Transm Infect.* 2002; 78(6):445-447.
6. Bowie WR, Alexander ER, Stimson JB, Floyd JF, Holmes KK. Therapy for Nongonococcal UrethritisDouble-Blind Randomized Comparison of Two Doses and Two Durations of Minocycline. *Ann Intern Med.* 1981; 95(3):306-311.
7. Cohen MS, Hoffman IF, Royce RA, et al. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV- 1. AIDSCAP Malawi Research Group. *Lancet.* 1997; 349(9069):1868-1873.
8. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect.* 1999; 75(1):3-17.
9. Santucci RA, Joyce GF, Wise M. Male urethral stricture disease. *J Urol.* 2007; 177(5):1667-1674.

10. Holmes KK, Stamm WE, Sobel JD. Lower Genital Tract Infection Syndromes in Women. In: Holmes KK, Sparling PF, Stamm WE, et al., eds. *Sexually Transmitted Diseases*. 4th ed. New York City: McGraw Hill; 2008: 987-1016.

11. Centers for Disease Control and Prevention. *Sexually Transmitted Diseases Treatment Guidelines 2015.* Atlanta, GA; 2015.

12. Manhart LE, Gillespie CW, Lowens MS, et al. Standard Treatment Regimens for Nongonococcal Urethritis Have Similar but Declining Cure Rates: A Randomized Controlled Trial. *Clin Infect Dis.* 2013; 56(7):934-42.

13. Schwebke JR, Rompalo A, Taylor S, et al. Re-evaluating the treatment of nongonococcal urethritis: emphasizing emerging pathogens--a randomized clinical trial. *Clin Infect Dis.* 2011; 52(2):163-170.

14. Rane VS, Fairley CK, Weerakoon A, et al. Characteristics of acute nongonococcal urethritis in men differ by sexual preference. *J Clin Microbiol.* 2014; 52(8):2971-2976.

15. Bradshaw CS, Tabrizi SN, Read TRH, et al. Etiologies of Nongonococcal Urethritis: Bacteria, Viruses, and the Association with Orogenital Exposure. *J Infect Dis.* 2006; 193(3):336-345.

16. Lafferty WE, Hughes JP, Handsfield HH. Sexually transmitted diseases in men who have sex with men. Acquisition of gonorrhea and nongonococcal urethritis by fellatio and implications for STD/HIV prevention. *Sex Transm Dis.* 1997; 24(5):272-278.

17. Barbee LA, Khosropour CM, Dombrowski JC, Manhart LE, Golden MR. An estimate of the proportion of symptomatic gonococcal, chlamydial and non-gonococcal non-chlamydial urethritis attributable to oral sex among men who have sex with men: a case-control study. *Sex Transm Infect.* 2016; 92(2):155-160.

18. Iser P, Read TH, Tabrizi S, et al. Symptoms of non-gonococcal urethritis in heterosexual men: a case control study. *Sex Transm Infect.* 2005; 81(2):163-165.

19. Manhart LE, Khosropour CM, Liu C, et al. Bacterial vaginosis-associated bacteria in men: association of Leptotrichia/Sneathia spp. with nongonococcal urethritis. *Sex Transm Dis.* 2013; 40(12):944-949.

20. Schwartz MA, Lafferty WE, Hughes JP, Handsfield HH. Risk Factors for Urethritis in Heterosexual Men: The Role of Fellatio and Other Sexual Practices. *Sex Transm Dis.* 1997; 24(8):449-455.

21. Couldwell DL, Gidding HF, Freedman EV, et al. Ureaplasma urealyticum is significantly associated with non-gonococcal urethritis in heterosexual Sydney men. *Int J STD AIDS.* 2010; 21(5):337-341.

22. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* 2009;42(2):377-381.

23. Haggerty CL, Totten PA, Ferris M, et al. Clinical characteristics of bacterial vaginosis among women testing positive for fastidious bacteria. *Sex Transm Infect.* 2009; 85(4):242-248.

24. Srinivasan S, Hoffman NG, Morgan MT, et al. Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One.* 2012; 7(6):e37818.

25. Kurth AE, Martin DP, Golden MR, et al. A Comparison Between Audio Computer-Assisted Self-Interviews and Clinician Interviews for Obtaining the Sexual History. *Sex Transm Dis.* 2004; 31(12):719-726.

26. Rogers SM, Willis G, Al-Tayyib A, et al. Audio computer assisted interviewing to measure HIV risk behaviours in a clinic population. *Sex Transm Infect.* 2005; 81(6):501-507.

27. Richens J, Copas A, Sadiq ST, et al. A randomised controlled trial of computer-assisted interviewing in sexual health clinics. *Sex Transm Infect.* 2010; 86(4):310-314.

28. Tieu HV, Li X, Donnell D, et al. Anal sex role segregation and versatility among men who have sex with men: EXPLORE Study. *J Acquir Immune Defic Syndr.* 2013; 64(1):121-125.

29. Nelson DE, Dong Q, Van der Pol B, et al. Bacterial communities of the coronal sulcus and distal urethra of adolescent males. *PLoS One.* 2012; 7(5):e36298.

30. Chambers LC, Morgan JL, Lowens MS, et al. Cross-sectional study of urethral exposures at last sexual episode associated with non-gonococcal urethritis among STD clinic patients. *Sex Transm Infect.* 2018. Epub ahead of print.

31. Frolund M, Wikstrom A, Lidbrink P, et al. The bacterial microbiota in first-void urine from men with and without idiopathic urethritis. *PLoS One.* 2018; 13(7):e0201380.

32. Forsum U, Hjelm E, Holmberg K, Nord CE, Wallin J. Genital occurrence of oral microbiota. *Acta Derm Venereol.* 1978; 58(4):353-356.

33. Golob JL, Pergam SA, Srinivasan S, et al. Stool Microbiota at Neutrophil Recovery Is Predictive for Severe Acute Graft vs Host Disease After Hematopoietic Cell Transplantation. *Clin Infect Dis.* 2017;65(12):1984-1991.

34. Shannon CE. A mathematical theory of communication. *Bell Syst Tech J.* 1948; 27:379-423.

35. Chao A. Estimating the population size for capture-recapture data with unequal catchability. *Biometrics.* 1987; 43(4):783-791.

36. Mitchell CM, Fredricks DN, Winer RL, Koutsky L. Effect of sexual debut on vaginal microbiota in a cohort of young women. *Obstet Gynecol.* 2012; 120(6):1306-1313.

37. Jespers V, Hardy L, Buyze J, Loos J, Buve A, Crucitti T. Association of Sexual Debut in Adolescents With Microbiota and Inflammatory Markers. *Obstet Gynecol.* 2016; 128(1):22-31.

38. Lee JW, Shim YH, Lee SJ. Lactobacillus colonization status in infants with urinary tract infection. *Pediatr Nephrol.* 2009; 24(1):135-139.

39. Liu CM, Hungate BA, Tobian AA, et al. Male circumcision significantly reduces prevalence and load of genital anaerobic bacteria. *mBio.* 2013; 4(2): e00076-13.

40. Srinivasan S, Chambers LC, Hoffman NG, et al. The urethral microbiota in nongonococcal urethritis. Paper presented at: 2017 STI and HIV World Congress; July 9-12, 2017, 2017; Rio de Janeiro, Brazil.

41. Wall KM, Stephenson R, Sullivan PS. Frequency of sexual activity with most recent male partner among young, Internet-using men who have sex with men in the United States. *J Homosex.* 2013; 60(10):1520-1538.

42. Garry M, Sharman SJ, Feldman J, Marlatt GA, Loftus EF. Examining memory for heterosexual college students' sexual experiences using an electronic mail diary. *Health Psychol.* 2002; 21(6):629-634.

43. Glick SN, Winer RL, Golden MR. Web-based sex diaries and young adult men who have sex with men: assessing feasibility, reactivity, and data agreement. *Arch Sex Behav.* 2013; 42(7):1327-1335.

44. Hensel DJ, Fortenberry JD, Harezlak J, Craig D. The feasibility of cell phone based electronic diaries for STI/HIV research. *BMC Med Res Methodol.* 2012; 12:75.

45. Jensen JS, Bradshaw C. Management of Mycoplasma genitalium infections – can we hit a moving target? *BMC Infect Dis.* 2015; 15:343.

46. Li Y, Le WJ, Li S, Cao YP, Su XH. Meta-analysis of the efficacy of moxifloxacin in treating Mycoplasma genitalium infection. *Int J STD AIDS.* 2017; 28(11):1106-1114.

47. Jensen JS, Bradshaw CS, Tabrizi SN, Fairley CK, Hamasuna R. Azithromycin treatment failure in Mycoplasma genitalium-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. *Clin Infect Dis.* 2008; 47(12):1546-1553.

48. Salado-Rasmussen K, Jensen JS. Mycoplasma genitalium testing pattern and macrolide resistance: a Danish nationwide retrospective survey. *Clin Infect Dis.* 2014; 59(1):24-30.

49. Jensen JS. Protocol for the detection of Mycoplasma genitalium by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. *Methods Mol Biol.* 2012; 903:129-139.

50. Deguchi T, Maeda S, Tamaki M, et al. Analysis of the gyrA and parC genes of Mycoplasma genitalium detected in first-pass urine of men with non-gonococcal urethritis before and after fluoroquinolone treatment. *J Antimicrob Chemother.* 2001; 48(5):742-744.

51. Handsfield HH, Alexander ER, Pin Wang S, Pedersen AH, Holmes KK. Differences in the therapeutic response of chlamydia-positive and chlamydia-negative forms of nongonococcal urethritis. *J Am Vener Dis Assoc.* 1976; 2(3):5-9.

52. Oriel JD, Ridgway GL, Tchamouroff S. Comparison of erythromycin stearate and oxytetracycline in the treatment of non-gonococcal urethritis: their efficacy against Chlamydia trachomatis. *Scott Med J.* 1977; 22(5):375-379.

53. Martin DH, Mroczkowski TF, Dalu ZA, et al. A controlled trial of a single dose of azithromycin for the treatment of chlamydial urethritis and cervicitis. The Azithromycin for Chlamydial Infections Study Group. *N Engl J Med.* 1992; 327(13):921-925.

54. Stamm WE, Hicks CB, Martin DH, et al. Azithromycin for empirical treatment of the nongonococcal urethritis syndrome in men. A randomized double-blind study. *JAMA.* 1995; 274(7):545-549.

55. Schwebke JR, Rompalo A, Taylor S, et al. Re-Evaluating the Treatment of Nongonococcal Urethritis: Emphasizing Emerging Pathogens–A Randomized Clinical Trial. *Clin Infect Dis.* 2011; 52(2):163-170.

56. Renault CA, Israelski DM, Levy V, Fujikawa BK, Kellogg TA, Klausner JD. Time to clearance of Chlamydia trachomatis ribosomal RNA in women treated for chlamydial infection. *Sex Health.* 2011; 8(1):69-73.

57. Falk L, Enger M, Jensen JS. Time to eradication of Mycoplasma genitalium after antibiotic treatment in men and women. *J Antimicrob Chemother.* 2015; 70(11):3134-3140.

58. Banjanac M, Munic Kos V, Nujic K, et al. Anti-inflammatory mechanism of action of azithromycin in LPS-stimulated J774A.1 cells. *Pharmacol Res.* 2012; 66(4):357-362.

59. Bosnar M, Kelneric Z, Munic V, Erakovic V, Parnham MJ. Cellular uptake and efflux of azithromycin, erythromycin, clarithromycin, telithromycin, and cethromycin. *Animicrob Agents Chemother.* 2005;49(6):2372-2377.

60. Australasian Sexual Health Alliance. Sexually Transmitted Infection Management Guidelines: Urethritis - male. Available at: http://www.sti.guidelines.org.au/syndromes/urethritis-male. Accessed November 29, 2018.

61. Horner PJ, Blee K, Falk L, van der Meijden W, Moi H. 2016 European guideline on the management of non-gonococcal urethritis. *Int J STD AIDS.* 2016; 27(11):928-937.