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MAJOR ARTICLE

Neisseria gonorrhoeae Infection in Women Increases With Rising Gonococcal Burdens in Partners: Chlamydia Coinfection in Women Increases Gonococcal Burden

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Background. Likelihood of *Neisseria gonorrhoeae* infection in women exposed to male sex partners with increasing *N. gonorrhoeae* burdens and enhancement by *Chlamydia trachomatis* is not defined.

Methods. We identified men with urethritis and their regular female sex partners. Exposure to *N. gonorrhoeae* burdens in men was compared in *N. gonorrhoeae*-infected versus -uninfected partners. Association of *N. gonorrhoeae* infection in women with burdens in male partners was estimated using logistic regression. Association of *C. trachomatis* coinfection and *N. gonorrhoeae* burdens in women adjusted for burdens in male partners was estimated by linear regression.

Results. In total, 1816 men were enrolled; 202 had ≥2 partners, 91 who confirmed monogamy and were enrolled; 77% were married. Seventy were partners of *N. gonorrhoeae*-infected men; 58 (83%) were *N. gonorrhoeae* infected, 26 (45%) *C. trachomatis* coinfected. Infected women had partners with 9.3-fold higher *N. gonorrhoeae* burdens than partners of uninfected women ($P = .0041$). Association of *N. gonorrhoeae* infection in women with upper quartiles of *N. gonorrhoeae* burdens in partners increased (odds ratios ≥ 2.97)compared to the first quartile ($P = .032$). *N. gonorrhoeae* burdens in *C. trachomatis*-coinfected women were 2.82-fold higher than in *C. trachomatis*-uninfected women ($P = .036$).

Conclusions. *N. gonorrhoeae* infections increased in women whose partners were infected with higher *N. gonorrhoeae* burdens. *C. trachomatis* coinfection was associated with increased *N. gonorrhoeae* burdens in women.

Keywords. *Chlamydia trachomatis* infection in women; *Neisseria gonorrhoeae*.

The impact of *Neisseria gonorrhoeae* burdens in infected men with urethritis upon the likelihood of female sex partners becoming infected with *N. gonorrhoeae* is not defined. An increase in the infectious dose of pathogenic bacteria is often associated with a corresponding increase in the likelihood of infection and generally is considered axiomatic [1]. However, individual strains of *N. gonorrhoeae* vary in their ability to escape innate host defense mechanisms [2]; therefore, *N. gonorrhoeae* burdens required to infect may also vary [3]. We examined exposure of women to large burdens of *N. gonorrhoeae* present in gonococcal urethritis in men to circumvent potential differences in infectious potential of clinical isolates to override natural immunity and thereby identify dose as a major variable.

N. gonorrhoeae is second to *Chlamydia trachomatis* as the most common cause of bacterial sexually transmitted infection (STI); the 2 often coinfect [4–6]. In certain geographic locales, *N. gonorrhoeae* may be expanding into the *C. trachomatis* niche because of increasing *N. gonorrhoeae* antimicrobial resistance [7]. Frequent coexistence of dual infection suggests that a biological variable(s) may increase *N. gonorrhoeae* burdens present during infection when *C. trachomatis* is present [8, 9]. Coinfection is reported in 10%–40% of persons with *N. gonorrhoeae* infection in the United States and United Kingdom [10]. Coinfection may preclude spontaneous clearance of *N. gonorrhoeae* infection [11]; persons repeatedly infected with *N. gonorrhoeae* are more likely coinfected [12]. Consequently, coinfection may prolong the burden of *N. gonorrhoeae* in the clinical and epidemiological reservoir. We examined the effect of *C. trachomatis* coinfection in women on their *N. gonorrhoeae* burden to better understand increased *N. gonorrhoeae* infection in *C. trachomatis*-infected women. Abnormal vaginal flora, that include diminished lactobacilli and increases in anaerobic

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bacteria, is a risk factor for the development of *N. gonorrhoeae/C. trachomatis* infection [13–15]. We examined relative abundance of lactobacilli and bacterial vaginosis (BV)-like flora in women infected with *N. gonorrhoeae* and or *C. trachomatis* in the context of exposure to and transmission of *N. gonorrhoeae*.

METHODS

Participants

Men

Our study was conducted from April 2011 to August 2015, at the Institute of Dermatology, Chinese Academy of Medical Sciences, STD Clinic, Nanjing, China. In total, 1816 Chinese speaking men, ≥18 years old, were evaluated for symptomatic urethritis, defined by urethral discharge and/or dysuria. The Institutional Review Boards of the Institute of Dermatology, (approval No. 2009-62), the University of Massachusetts Chan Medical School (No. 13448) and Boston University School of Public Health (No. H28858) approved the study; all participants provided written informed consent. Men who indicated they had unprotected sex with 2 or more women were asked to identify their regular partner(s) with whom they had 1 or more episodes of unprotected vaginal intercourse in the 30 days before to 30 days after symptomatic onset of urethritis (the spread period). Two groups were identified: (1) men diagnosed with gonorrhea microscopically by Gram's stain of urethral specimens with polymorphonuclear neutrophils (PMNs) plus gram-negative intracellular diplococci (GNIDs); and (2) men with presumptive nongonococcal urethritis (NGU; PMNs without GNIDs).

Women

Chinese speaking women, identified as regular sex partners by men, were eligible if they were ≥18 years of age, stated they were not human immunodeficiency virus (HIV) infected, and indicated that they had been monogamous in the recent 30 days with the man who identified them (exclusion criteria for women are in Supplementary Data). Baseline characteristics included: demographic information; contraception use; a history of STIs and reports of genital symptoms. A vaginal speculum examination was performed and specimens collected for laboratory testing. Cervicovaginal lavage (CVL) was also performed [16]; fluid was collected and stored at −80°C for additional microbial testing. A bimanual pelvic examination was also performed.

Laboratory Testing

Microbiology and Molecular Methods: Men

First-voided urine specimens and urethral swabs were collected. Gram stains were performed and swabs streaked onto modified Thayer–Martin medium (Zhuhai DL Biotech Co. Ltd). Gonococci (*N. gonorrhoeae*) were identified by colonial morphology, Gram's stain and oxidase testing.

Urethral exudates were inoculated into liquid culture media (Mycoplasma IST2; bioMerieux) to identify *Ureaplasma* species and *Mycoplasma hominis*. Urine specimens were examined for *C. trachomatis* by polymerase chain reaction (PCR; DAAN Gene Co. Ltd.). *Mycoplasma genitalium* and *Trichomonas vaginalis* PCRs were performed as previously described [17]. *N. gonorrhoeae* colony forming unit (CFU) equivalents/mL were quantitated in male urine specimens using a modification of asymmetric quantitative polymerase chain reaction (qPCR) [18] (also described in Supplementary Data). Urine specimens from men identified as having NGU, who had monogamous partners (see below), were confirmed negative for *N. gonorrhoeae* by PCR (DAAN Gene Co. Ltd.).

Microbiology and Molecular Methods: Women

Vaginal specimens were examined for yeast forms, *T. vaginalis,* and clue cells by microscopy. Initial endocervical swab specimens were gram stained to enumerate PMNs/high-powered field and identify GNIDs, and cultured for *N. gonorrhoeae*. *C. trachomatis* PCR was performed on a second endocervical swab; a third swab was cultured for *Ureaplasma* species and *M. hominis;* and a fourth tested for *M. genitalium* and *T. vaginalis* by PCR [17]. A final swab was frozen (−80°C) and, in *N. gonorrhoeae*-culture–negative women, was tested for *N. gonorrhoeae* by PCR (DAAN Gene Co. Ltd.). *N. gonorrhoeae* CFU equivalents were quantitated in CVL specimens [16] by qPCR [18] as indicated above for male urine specimens. To characterize and differentiate vaginal microbiome signatures, CVL specimens from women with: (1) *N. gonorrhoeae* negative *C. trachomatis* positive, (2) *N. gonorrhoeae* positive *C. trachomatis* negative, or (3) *N. gonorrhoeae* positive *C. trachomatis* positive infections, and (4) uninfected with *N. gonorrhoeae* and *C. trachomatis* (*N. gonorrhoeae* negative *C. trachomatis* negative), were tested for bacterial 16s rRNA V3–V4 hypervariable regions, which were amplified, sequenced, and assigned genera. Total genomic DNA was extracted from CVLs using QuickExtract DNA Extraction Solution (Lucigen). PCR amplification, library construction, sequencing, and analysis were carried out by Novogene Co. In brief, the V3–V4 hypervariable regions of bacterial 16S rRNA genes were amplified using primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). Barcoded PCR products were sequenced on an Illumina paired-end platform to generate 250-bp paired-end raw reads. Sequences were processed through the Qiime pipeline [19] and operational taxonomic units (OTUs) quantitated against the SILVA 16s database (https://www.arb-silva.de/) [20] clustered at 97%. Alpha diversity scores were calculated for each sample using the Shannon and Simpson indices (https://digitalinsights.qiagen.com/plugins/clc-microbial-genomics-module/) and were reported at the group level. Based solely on the predominant species identified within the samples of each group, community state type (CST) equivalents

were also identified. Serologic testing of blood for syphilis (rapid plasma reagin and treponema pallidum particle agglutination) and *Herpes simplex* (HSV-1 IgM/IgG and HSV-2 IgM/IgG) were also performed.

Data Management and Statistical Analyses

Demographic, clinical, and laboratory data were recorded in a centralized relational structured query language data base with an ASP.NET web front end using standardized data collection and sample tracking forms, which were fax scanned and sent electronically to the Biostatistics and Epidemiology Data Analytics Center at the Boston University School of Public Health (additional methodological details are described in Supplementary Data). Baseline characteristics were compared among the 3 infection groups in women: (1) *N. gonorrhoeae* positive *C. trachomatis* positive, (2) *N. gonorrhoeae* positive *C. trachomatis negative*, (3) *N. gonorrhoeae* negative *C. trachomatis* positive, and the *N. gonorrhoeae/C. trachomatis* uninfected group (*N. gonorrhoeae* negative *C. trachomatis negative*) using ANOVA for testing means and Fisher exact test for testing categorical distributions. Abundance of microbial genera in each of the infection groups was compared by *t* test with the *N. gonorrhoeae/C. trachomatis* uninfected group. Geometric mean of *N. gonorrhoeae* burdens (CFU equivalents) in *N. gonorrhoeae*-infected men who were partners of *N. gonorrhoeae*-infected women (including PCR-only *N. gonorrhoeae*-positive women), was compared by *t* test with the geometric mean in *N. gonorrhoeae*-infected men who were partners of *N. gonorrhoeae*-uninfected women. Total number of coital exposures associated with *N. gonorrhoeae* infection in women was tested using a nonparametric test [21, 22] for trend. *N. gonorrhoeae* DNA sequences from dually infected partners were assessed for concordance by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST; https://pubmlst.org) [23, 24]. The association of *N. gonorrhoeae* infection status in women with increasing *N. gonorrhoeae* burdens (qPCR, divided into quartiles) present in male partners and *C. trachomatis* status in women was estimated using a logistic regression model. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each quartile of male burdens compared to the first quartile. The association of *N. gonorrhoeae* burdens ($\log_{10}$) present in women with their *C. trachomatis* status and *N. gonorrhoeae* burdens ($\log_{10}$) in men was estimated by linear regression. The interaction of *C. trachomatis* coinfection and *N. gonorrhoeae* burdens in men was tested using a likelihood ratio test.

RESULTS

Enrollment of Study Participants

In total, 1816 men were enrolled (Figure 1); 373 had 2 or more sex partners with whom they had unprotected vaginal sex during the spread period; 202 had regular female partners who they identified. Ninety-eight identified matched regular female partners were successfully contacted; all indicated monogamy in the recent 30 days with 96 male partners (2 men each identified 2 regular female partners). Two women were ineligible because

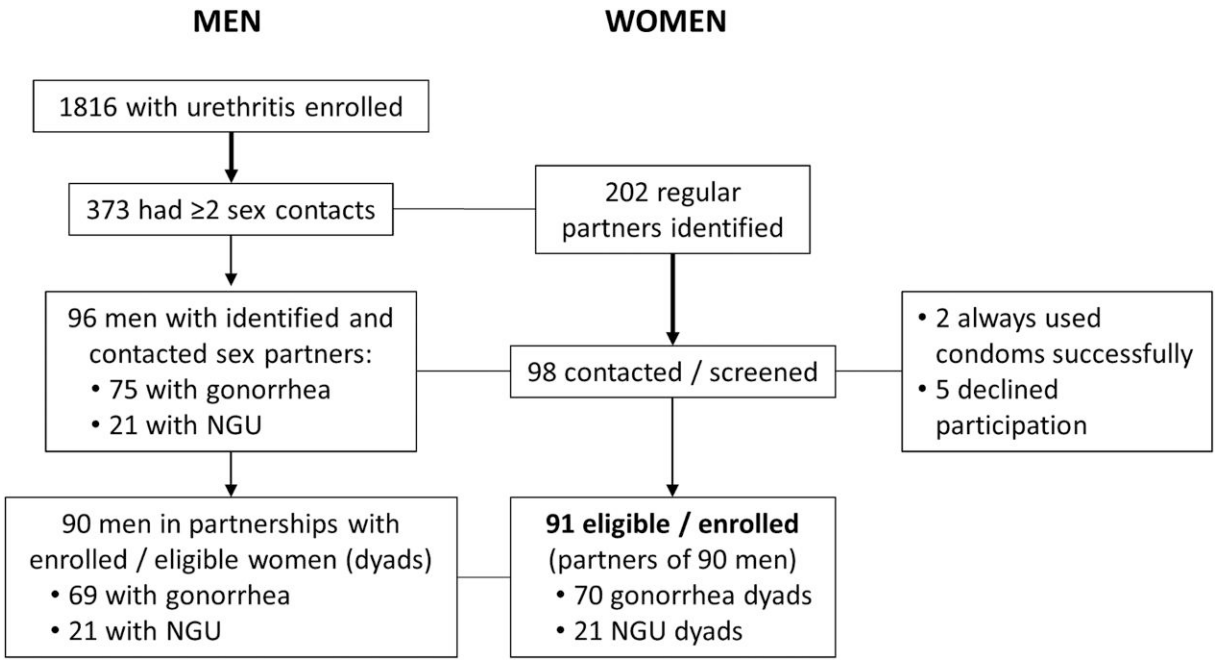

Figure 1. Men: enrollment (overall) of men with urethritis (having ≥2 sex contacts) and enrolled into this study. Women: identification of regular female sex partners, number contacted and screened, and number eligible and enrolled into this study. Abbreviation: NGU, nongonococcal urethritis.

their partners always used condoms successfully; 5 declined participation; 91 women were enrolled.

Microbiology in Men

Microbiologic characterization of urethral specimens from 1816 men is described in Supplementary Data and Supplementary Table 1. In total, 1816 were tested for *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium,* and *T. vaginalis*; a subset of 1529 were also tested for *Ureaplasma* species and *M. hominis*. The prevalence of gonorrhea (and the distribution of other organisms) was maintained in the 96 fully eligible men whose partners had been identified and screened, compared to 373 men who also had 2 or more female partners. Gonorrhea was diagnosed presumptively in 75/96 (78.1%) men by Gram's stain of urethral specimens; all were culture positive for *N. gonorrhoeae*. Sixty-nine men with gonorrhea matched with 70 partners. *N. gonorrhoeae* burdens measured by qPCR (Supplementary Data and Supplementary Figure 2) in male urine are shown in Supplementary Table 2. NGU was diagnosed in 21/96 (21.9%) men and confirmed by absence of *N. gonorrhoeae* by culture and a negative *N. gonorrhoeae* PCR; 21 men with NGU matched with 21 partners.

Microbiology in Women

Microbiologic characterization of genital specimens from 91 enrolled women is described in Supplementary Data and Supplementary Table 3. In total, 91 were tested for *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium,* and *T. vaginalis*; a subset of 64 were also tested for *Ureaplasma* species and *M. hominis*. Fifty-eight partners of 69 *N. gonorrhoeae*-infected men (82.9%; 1 infected man had 2 partners) were infected with *N. gonorrhoeae*; 26 (45%) were coinfected with *C. trachomatis*. *N. gonorrhoeae* burdens measured in CVLs by qPCR are shown in Supplementary Table 2. Twelve partners of 21 men with NGU (57.1%) were infected with *C. trachomatis*; all were negative for *N. gonorrhoeae* by culture and PCR. A flowchart (Supplementary Figure 2) shows *N. gonorrhoeae* and *C. trachomatis* infections in women after contact with infected male sex partners.

Baseline Characteristics in 91 Women

Demographic and Clinical Histories

Baseline characteristics were compared across 3 groups with cervical *N. gonorrhoeae* and or *C. trachomatis* infections: (1) *N. gonorrhoeae* negative *C. trachomatis* positive; (2) *N. gonorrhoeae* positive *C. trachomatis* negative; (3) *N. gonorrhoeae* positive *C. trachomatis* positive; and the *N. gonorrhoeae*/*C. trachomatis* uninfected group (*N. gonorrhoeae* negative *C. trachomatis* negative) (Table 1). Age, race, and college completion were similar across the 4 groups. Overall, 77% (70/91) of women were married (1 cohabited) to their sex partner. Number of days since the last menstrual period did not vary among the groups. Forty-three percent (39/91) used no regular contraception; 3 indicated they had used a condom (with spermicide) as the only means of contraception; 5 others used condoms together with other methods; condom use in 8 women in the 30 days before/after the male partner developed signs/symptoms of urethritis was infrequent (Supplementary Figure 3). Four women reported prior gonorrhea, chlamydial infection, or syphilis; 2 reported genital warts. Fifty-three percent (48/91) reported a prior negative HIV test; the rest had not been tested. Painful intercourse (4 subjects) and abdominal pain (7 subjects) were reported infrequently. Thirty-six percent (33/91) reported prior vaginal infections; there were no differences among the groups. The prevalence of vaginal discharge reported by subjects at the time of the encounter was significantly different among the groups ($P = .029$); the highest rate was in the group coinfected with *N. gonorrhoeae* and *C. trachomatis* (50%) and the lowest rate in the *N. gonorrhoeae* negative *C. trachomatis* negative group (10.5%).

Speculum and Physical Examinations

Ninety-six percent (87/91) of women had vaginal discharge on speculum examination: the quality or amount did not vary across the 4 groups (Supplementary Table 4 and Table 1). Ninety-seven percent (88/91) of women had a cervical discharge: the quality (purulent or mucoid) or amount did not vary across the groups. No genital lesions were identified. Bimanual pelvic examinations were normal (all findings negative) in greater than 91% of subjects; uterine tenderness was elicited in 8 subjects (8.7%).

Vaginal and Cervical Analyses

Results of microscopic vaginal smears (yeast, *T. vaginalis,* and clue cells) are summarized in Table 1 and Supplementary Table 5. There was no significant difference ($P = .353$) in the prevalence of clue cells among groups with abnormal wet preps. The numbers of PMNs seen by microscopy on a cervical Gram stain was significantly different ($P = .019$) among the groups: 25/58 (43%) of *N. gonorrhoeae* or *N. gonorrhoeae*/*C. trachomatis* coinfected women had ≥10 PMNs/high-powered field versus 3/19 (7%) of *N. gonorrhoeae*-negative *C. trachomatis*-negative women. Only 28% (16/58) of women infected either with *N. gonorrhoeae* or coinfected with *N. gonorrhoeae* and *C. trachomatis* had GNIDs seen on a Gram stain (Table 1).

Cervicovaginal Microbiomes

Detailed composition of vaginal microbiomes is presented in Supplementary Table 6. OTUs were normalized using a standard of sequence number corresponding to the sample with the least sequences. Normalized OTU data from the (1) *N. gonorrhoeae* negative *C. trachomatis* positive, (2) *N. gonorrhoeae* positive *C. trachomatis* negative, and (3) *N. gonorrhoeae* positive *C. trachomatis* positive infected groups were each compared with data from group (4), the *N. gonorrhoeae* negative *C. trachomatis* negative set of samples in which the abundance

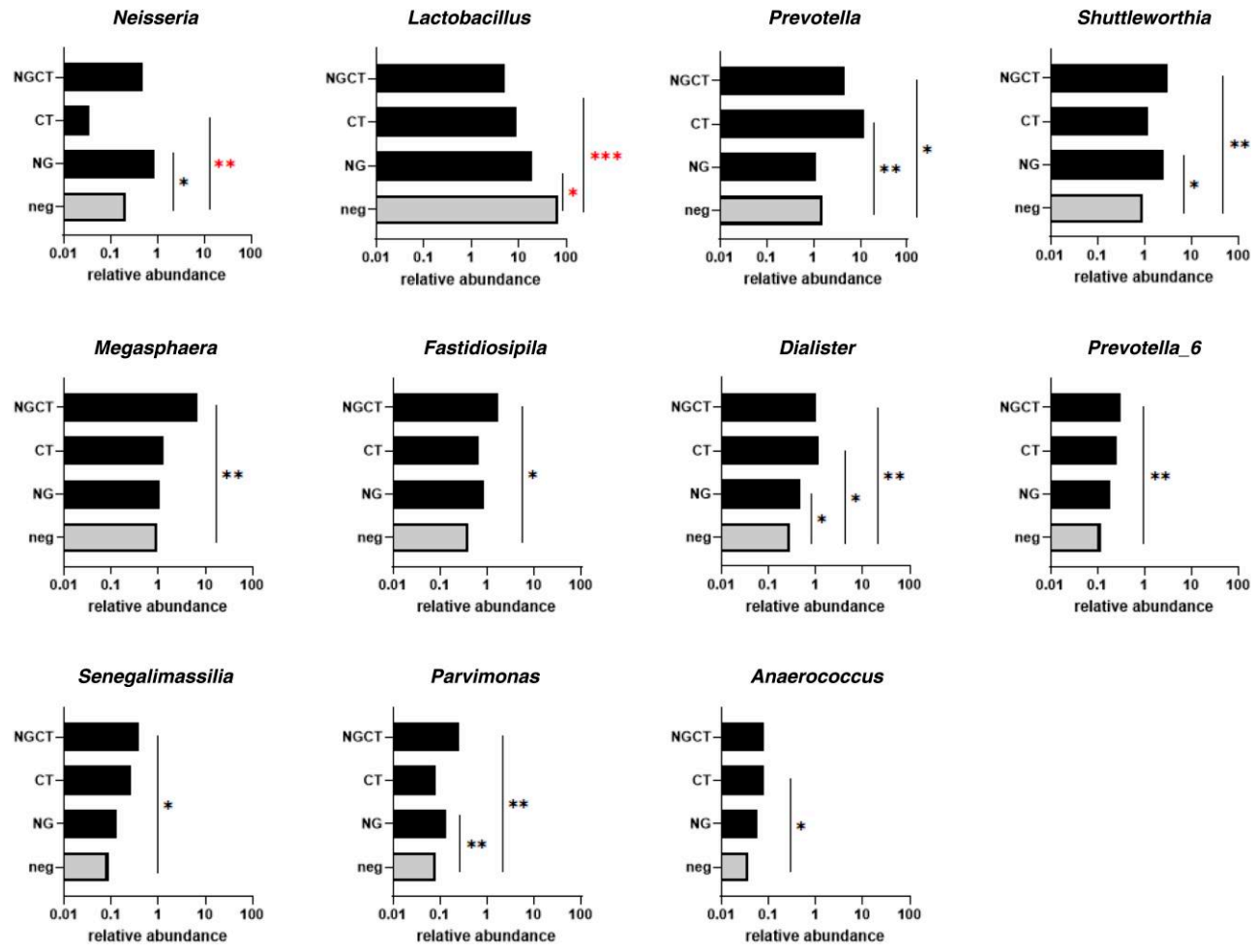

Figure 2. The relative abundance of 11 (of the top 25) bacterial genera measured in *Neisseria gonorrhoeae* (NG)-infected and/or *Chlamydia trachomatis* (CT)-infected cervicovaginal lavage specimens that had values significantly different from specimens with neither organism (neg), calculated by *t* test. Normalized read values of operational taxonomic units (OTUs), quantitated against the SILVA 16s database (https://www.arb-silva.de/) [20] that clustered at 97% OTUs were measured in specimens infected with both *N. gonorrhoeae* and *C. trachomatis* (NGCT), *C. trachomatis*, or *N. gonorrhoeae* and compared with values in specimens with neither organism (neg). $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ denote abundance values that are significantly greater than neither organism (neg). Asterisks denote values significantly greater or less than neg.

of *Lactobacillus* (using median values: 66% of 1107 total genera and 80% of the top 25 genera) was normal [25]. The top 25 genera (based on median levels of normalized OTU data) comprised greater than 95% mean total abundance in all 4 groups (Supplementary Table 5). Predominance of several major genera in each infection group was increased compared to *N. gonorrhoeae*-negative *C. trachomatis*-negative women, mostly in women with *N. gonorrhoeae*/*C. trachomatis* coinfection (Figure 2 and Supplementary Table 5). Alpha diversity indicated significant differences between the *N. gonorrhoeae*-negative *C. trachomatis*-negative group and the *N. gonorrhoeae*, *C. trachomatis*, and *N. gonorrhoeae*/*C. trachomatis* groups (Supplementary Table 7). CST 4 equivalent was most prevalent overall (55/91): *N. gonorrhoeae* (19/32); *N. gonorrhoeae*/*C. trachomatis* (22/26); *C. trachomatis* (8/14); and *N. gonorrhoeae*-negative *C. trachomatis*-negative group (6/19). The *N. gonorrhoeae*-negative *C. trachomatis*-negative group was predominantly CST 3 equivalent (11/19; *Lactobacillus iners*). The distribution of CSTs by category are shown in Supplementary Table 8.

Syphilis and HSV Serologies

Four women had positive syphilis serologies (3 rapid plasma reagin and 4 treponema pallidum particle agglutination). HSV antibody results are shown in Table 1; 27/91 (30%) women had measurable HSV-2 IgG antibody; there were no differences among the groups.

Likelihood of Infection in Women Exposed to *N. gonorrhoeae*-Infected Partners

Overall, *N. gonorrhoeae*-infected women were partners of men with 9.3-fold higher mean *N. gonorrhoeae* burden ($\log_{10} = 3.26$)

Table 1. Characteristics of Women Infected With *Neisseria gonorrhoeae, Chlamydia trachomatis*, Both Organisms, or Neither Organism

Variable	*N. gonorrhoeae* negative and *C. trachomatis* negative (n = 19)	*N. gonorrhoeae* negative and *C. trachomatis* positive (n = 14)	*N. gonorrhoeae* positive and *C. trachomatis* negative (n = 32)	*N. gonorrhoeae* positive and *C. trachomatis* positive (n = 26)	*P* Value
Baseline					
Age, y, mean ± SD	34.58 ± 7.49	33.93 ± 7.18	31.63 ± 8.22	31.46 ± 9.43	.504
Time since last MP, d, mean ± SD	16.63 ± 8.47	18.79 ± 9.41	84.47 ± 270.3	26.6 ± 36.25	.381
Age <40 y					.728
No	6 (31.6)	3 (21.4)	6 (18.8)	5 (19.2)	
Yes	13 (68.4)	11 (78.6)	26 (81.3)	21 (80.8)	
Han race					.205
No	2 (10.5)	1 (7.1)	0 (0.0)	1 (3.8)	
Yes	17 (89.5)	13 (92.9)	32 (100.0)	25 (96.2)	
College or beyond					.226
Less than college	13 (68.4)	11 (78.6)	29 (90.6)	22 (84.6)	
College or beyond	6 (31.6)	3 (21.4)	3 (9.4)	4 (15.4)	
Married or cohabiting					.281
No	3 (15.8)	1 (7.1)	9 (28.1)	8 (30.8)	
Yes	16 (84.2)	13 (92.9)	23 (71.9)	18 (69.2)	
Time since last MP, group					.895
≤7 d	3 (15.8)	3 (21.4)	3 (9.4)	5 (20.0)	
8–14 d	5 (26.3)	2 (14.3)	12 (37.5)	5 (20.0)	
15–21 d	4 (21.1)	4 (28.6)	5 (15.6)	4 (16.0)	
22–28 d	6 (31.6)	4 (28.6)	8 (25.0)	7 (28.0)	
>28 d	1 (5.3)	1 (7.1)	4 (12.5)	4 (16.0)	
Main contraception					.737
None	8 (42.1)	8 (57.1)	14 (43.8)	9 (34.6)	
Pill	1 (5.3)	0 (0.0)	2 (6.3)	0 (0.0)	
IUD	6 (31.6)	5 (35.7)	7 (21.9)	10 (38.5)	
Tubal ligation	0 (0.0)	0 (0.0)	2 (6.3)	1 (3.8)	
Condom only	3 (15.8)	0 (0.0)	6 (18.8)	4 (15.4)	
Condom with spermicide	0 (0.0)	1 (7.1)	1 (3.1)	0 (0.0)	
Emergency contraception	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.8)	
Other	1 (5.3)	0 (0.0)	0 (0.0)	1 (3.8)	
Ever tested ± for gonorrhea					.373
Never tested	2 (10.5)	2 (14.3)	8 (25.0)	9 (34.6)	
No	17 (89.5)	12 (85.7)	23 (71.9)	16 (61.5)	
Yes	0 (0.0)	0 (0.0)	1 (3.1)	1 (3.8)	
Ever tested ± for *Chlamydia*					.677
Never tested	5 (26.3)	3 (21.4)	10 (31.3)	9 (34.6)	
No	14 (73.7)	11 (78.6)	20 (62.5)	14 (53.8)	
Yes	0 (0.0)	0 (0.0)	1 (3.1)	0 (0.0)	
DK	0 (0.0)	0 (0.0)	1 (3.1)	3 (11.5)	
Ever tested ± for syphilis					.718
Never tested	10 (52.6)	4 (28.6)	13 (40.6)	10 (38.5)	
No	9 (47.4)	10 (71.4)	18 (56.3)	16 (61.5)	
Yes	0 (0.0)	0 (0.0)	1 (3.1)	0 (0.0)	
Ever had genital warts					.022
No	19 (100.0)	12 (85.7)	32 (100.0)	26 (100.0)	
Yes	0 (0.0)	2 (14.3)	0 (0.0)	0 (0.0)	
Ever had positive HIV test					.627
Never tested	10 (52.6)	5 (35.7)	17 (53.1)	10 (38.5)	
No	9 (47.4)	9 (64.3)	14 (43.8)	16 (61.5)	
DK	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Painful intercourse					.259
No	19 (100.0)	12 (85.7)	31 (96.9)	25 (96.2)	
Yes	0 (0.0)	2 (14.3)	1 (3.1)	1 (3.8)	
Abdominal pain					.662
No	18 (94.7)	14 (100.0)	28 (87.5)	24 (92.3)	

Table 1. Continued

Variable	*N. gonorrhoeae* negative and *C. trachomatis* negative (n = 19)	*N. gonorrhoeae* negative and *C. trachomatis* positive (n = 14)	*N. gonorrhoeae* positive and *C. trachomatis* negative (n = 32)	*N. gonorrhoeae* positive and *C. trachomatis* positive (n = 26)	*P* Value
Yes	1 (5.3)	0 (0.0)	4 (12.5)	2 (7.7)	
Ever had vaginal infections					.845
No	12 (63.2)	9 (64.3)	17 (53.1)	14 (53.8)	
Yes	6 (31.6)	5 (35.7)	11 (34.4)	11 (42.3)	
DK	1 (5.3)	0 (0.0)	4 (12.5)	1 (3.8)	
Vaginal discharge patient report					**.029**
No	17 (89.5)	8 (57.1)	23 (71.9)	13 (50.0)	
Yes	2 (10.5)	6 (42.9)	9 (28.1)	13 (50.0)	
Speculum examinations					
Vaginal discharge examiner					.516
No	1 (5.3)	1 (7.1)	2 (6.3)	0 (0.0)	
Yes	18 (94.7)	13 (92.9)	30 (93.8)	26 (100.0)	
Inflammation of the cervix					.254
Absent	12 (63.2)	5 (35.7)	14 (43.8)	8 (30.8)	
Minimal	6 (31.6)	5 (35.7)	12 (37.5)	9 (34.6)	
Moderate	1 (5.3)	4 (28.6)	6 (18.8)	9 (34.6)	
Cervical discharge examiner					.505
No	1 (5.3)	1 (7.1)	1 (3.1)	0 (0.0)	
Yes	18 (94.7)	13 (92.9)	31 (96.9)	26 (100.0)	
Cervical discharge [purulent]: amount					.818
Scant	2 (50.0)	4 (66.7)	9 (56.3)	5 (33.3)	
Moderate	2 (50.0)	2 (33.3)	6 (37.5)	9 (60.0)	
Large	0 (0.0)	0 (0.0)	1 (6.3)	1 (6.7)	
Vaginal/cervical microscopy/serologies					
Vaginal wet prep (WP)					.121
Abnormal	4 (21.1)	8 (57.1)	10 (31.3)	12 (46.2)	
Normal	15 (78.9)	6 (42.9)	22 (68.8)	14 (53.8)	
WP abnormal: trichomonas					.208
Positive	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.7)	
Negative	19 (100.0)	14 (100.0)	32 (100.0)	24 (92.3)	
WP abnormal: yeast					.154
Positive	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	
Negative	19 (100.0)	13 (92.9)	32 (100.0)	26 (100.0)	
WP abnormal clue cell					.222
Positive	4 (21.1)	7 (50.0)	10 (31.3)	12 (46.2)	
Negative	15 (78.9)	7 (50.0)	22 (68.8)	14 (53.8)	
Cervical Gram's stain for PMNs					**.019**
0 PMNs	0 (0.0)	0 (0.0)	3 (9.4)	0 (0.0)	
1–4 PMNs	11 (57.9)	6 (42.9)	11 (34.4)	4 (15.4)	
5–9 PMNs	5 (26.3)	6 (42.9)	8 (25.0)	7 (26.9)	
≥10 PMNs	3 (15.8)	2 (14.3)	10 (31.3)	15 (57.7)	
Cervical Gram's stain for GNIDs					**.003**
Positive	0 (0.0)	0 (0.0)	7 (21.9)	9 (34.6)	
Negative	19 (100.0)	14 (100.0)	25 (78.1)	17 (65.4)	
RPR					.826
Negative	18 (94.7)	14 (100.0)	31 (96.9)	24 (92.3)	
Positive	1 (5.3)	0 (0.0)	1 (3.1)	2 (7.7)	
TPPA					.683
Negative	18 (100.0)	14 (100.0)	30 (93.8)	24 (92.3)	
Positive	0 (0.0)	0 (0.0)	2 (6.3)	2 (7.7)	
HSV-1 IgG					.414
Negative	1 (5.6)	0 (0.0)	3 (9.4)	0 (0.0)	
Positive	17 (94.4)	14 (100.0)	29 (90.6)	26 (100.0)	
HSV-1 IgM					.084
Negative	18 (100.0)	14 (100.0)	32 (100.0)	23 (88.5)	

Table 1. Continued

Variable	*N. gonorrhoeae* negative and *C. trachomatis* negative (n = 19)	*N. gonorrhoeae* negative and *C. trachomatis* positive (n = 14)	*N. gonorrhoeae* positive and *C. trachomatis* negative (n = 32)	*N. gonorrhoeae* positive and *C. trachomatis* positive (n = 26)	*P* Value
Positive	0 (0.0)	0 (0.0)	0 (0.0)	3 (11.5)	
HSV-2 IgG					.883
Negative	14 (77.8)	10 (71.4)	21 (65.6)	18 (69.2)	
Positive	4 (22.2)	4 (28.6)	11 (34.4)	8 (30.8)	
HSV-2 IgM					.873
Negative	18 (100.0)	14 (100.0)	30 (93.8)	25 (96.2)	
Positive	0 (0.0)	0 (0.0)	2 (6.3)	1 (3.8)	

Data are No. (%) except where indicated.

Abbreviations: DK, don't know; GNID, gram-negative intracellular diplococci; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IgG, immunoglobulin G; IUD, intrauterine device; MP, menstrual period; PMN, polymorphonuclear neutrophil; RPR, rapid plasma reagin; TPPA, *Treponema pallidum* particle agglutination; WP, wet prep.

[a]Baseline characteristics were compared among the 3 infection groups in women *N. gonorrhoeae* negative *C. trachomatis* positive, *N. gonorrhoeae* positive *C. trachomatis* negative, *N. gonorrhoeae* positive *C. trachomatis* positive, and the uninfected group *N. gonorrhoeae* negative *C. trachomatis* negative using ANOVA for testing means and Fisher exact test for testing categorical distributions.

versus mean *N. gonorrhoeae* burden ($\log_{10} = 2.29$) in partners of uninfected women (Figure 3) ($P = .0041$).

Coital Exposures of Women to Gonorrhea-Infected Male Sex Partners

There was a relationship, overall, between increased numbers of coital exposures and likelihood of *N. gonorrhoeae* infection in women (Figure 4 and Supplementary Figure 3) ($P = .021$). Exposures where a condom was used were excluded from analysis.

Genotypes in Gonococcal (*N. gonorrhoeae*) Partnerships

Forty-six NG-MAST ST types were seen across partnerships where both members were *N. gonorrhoeae* infected (Supplementary Table 9); 57/58 (98%) *N. gonorrhoeae* dually infected couples shared an identical NG-MAST ST.

Gonococcal (*N. gonorrhoeae*) Burden in Men and Likelihood (or Risk) of *N. gonorrhoeae* Infection in Women

The unadjusted estimated association of *N. gonorrhoeae* infection in women and quartile of *N. gonorrhoeae* burdens in men (Table 2)was significantly different, overall ($P = .032$). The highest risk was exposure to the third and fourth quartiles versus the first quartile (OR = 10.82; 95% CI, 1.17–100.44 and OR = 10.18; 95% CI, 1.09–94.83, respectively). When adjusted for *C. trachomatis* infection in women, the adjusted estimated association (aORs) of women acquiring *N. gonorrhoeae* infection remained similar with exposure to each successive quartile (Table 2; overall $P = .052$); the highest risk was again exposure to the third and fourth quartiles versus the first quartile (aOR = 8.66; 95% CI, .90–83.26 and aOR = 10.17; 95% CI, 1.06–97.08, respectively). The unadjusted estimated association of *N. gonorrhoeae* and *C. trachomatis* coinfection versus *C. trachomatis*-negative status in *N. gonorrhoeae*-infected women showed a positive trend (OR = 4.06; 95% CI, .82–20.20), which persisted when adjusted for *N. gonorrhoeae* burdens in male sex partners (OR = 3.47; 95% CI, .65–18.59; Table 2).

Chlamydia (*C. trachomatis*) Infection in Women and the Burden of Gonococcal (*N. gonorrhoeae*) Infection

N. gonorrhoeae burdens in female sex partners of infected men was associated with burdens in their infected male partners (β coefficient [regression slope] = 0.527, $P < .001$), adjusted for *C. trachomatis* status in women (Table 3). The estimated association within *C. trachomatis*-positive women was slightly greater (β = 0.65, $P < .001$) than within *C. trachomatis*-negative women (β = 0.45, $P < .001$) but the difference (interaction) was not significant ($P = .306$) (Supplementary Figure 4). The number of organisms in *N. gonorrhoeae*-infected women was also associated with female *C. trachomatis* status; 2.82-fold higher in *C. trachomatis*-coinfected versus *C. trachomatis*-uninfected women (β = 0.451, $P = .036$), adjusted for *N. gonorrhoeae* burdens in male partners (Table 3).

DISCUSSION

Our study showed that the prevalence of *N. gonorrhoeae* infection in men with urethritis increased by a third in men who indicated they had 2 or more sex partners, and mirrors studies that have shown an increased risk of gonorrhea in men with multiple female sex partners [26–28]. Even so, transmission of *N. gonorrhoeae* differs markedly, depending on directionality; it was approximately 4-fold lower from women to men (19%–22% [29, 30]), compared to 83% transmission from men to women in our study involving predominantly Chinese married couples. Men with symptomatic *N. gonorrhoeae* urethritis possess 3.7×10^6 DNA copies per urethral swab by qPCR [31] compared to lower *N. gonorrhoeae* burdens reported in infected women: 2.0×10^4 copies per mL (vaginal swabs) [32] and 1.45×10^5 CFU/mL (CVLs) [33].

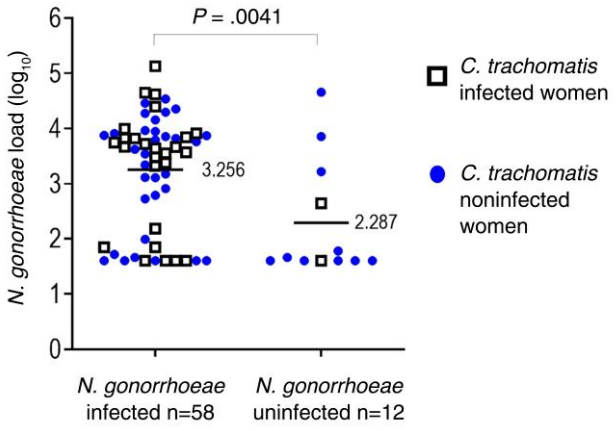

Figure 3. The load (log_{10} of colony forming unit equivalents) of *Neisseria gonorrhoeae* in men and *N. gonorrhoeae* infection status in 70 exposed monogamous female sex partners. Comparison of the means of *N. gonorrhoeae* burdens in men whose partners were *N. gonorrhoeae*-infected versus *N. gonorrhoeae*-uninfected partners was calculated by *t* test.

The risk of *N. gonorrhoeae* infection in women was driven primarily by exposure to increasing burdens in male partners. Nonetheless, we also demonstrated a trend in the likelihood of *N. gonorrhoeae* infection in women when *C. trachomatis* infection was present; the odds ratio was decreased by 15% after adjustment for *N. gonorrhoeae* burdens in male sex partners. Earlier, we reported 73% transmission of *N. gonorrhoeae* from men to women in the US; coinfection with *C. trachomatis* (42%) [34] was similar to 45% in our current study. Transmission studies often use concordant infection (both members of the dyad infected) as the measure of transmission (in either direction). In a US study that examined *C. trachomatis* transmission across dyads, which also included subjects with *N. gonorrhoeae* infection, the percent of *C. trachomatis* concordant dyads when women were *N. gonorrhoeae* infected was significantly higher than in *C. trachomatis*-concordant dyads with *N. gonorrhoeae*-uninfected women [35]. In a second US study, 100% of dyads with *N. gonorrhoeae*-infected women were *C. trachomatis* concordant [36].

N. gonorrhoeae burdens in infected women range widely [33, 37] but have not been shown to increase during the period of infection and are borderline higher when women are coinfected with *C. trachomatis* [37]. When adjusted for *N. gonorrhoeae* burdens in male partners, we found that coinfected women had almost a 3-fold higher *N. gonorrhoeae* burden compared to women infected with *N. gonorrhoeae* alone. This suggests that *N. gonorrhoeae* may flourish when *C. trachomatis* is present in women and that an increase in the *N. gonorrhoeae* burden takes place after infection. Killing of gonococci by macrophages and neutrophils in vitro is impaired by type I

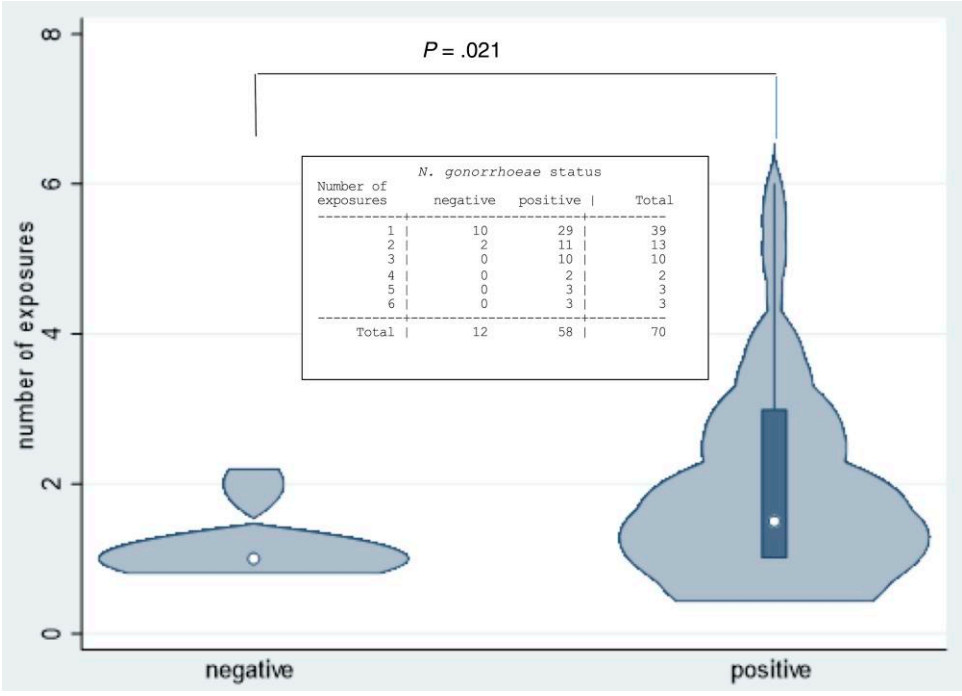

Figure 4. The number of coital exposures between *Neisseria gonorrhoeae*-infected men and their female sex partners and the resultant number of infections in women. The total number of exposures associated with *N. gonorrhoeae* infection in women was tested using a nonparametric test of trend that examined if the rate of *N. gonorrhoeae* increased with increasing number of exposures.

Table 2. Logistic Regression Model That Examined Factors Associated With *Neisseria gonorrhoeae* Infection in Women

Association With *N. gonorrhoeae* Infection in Women	Unadjusted Odds Ratios (95% CI)	Adjusted[a] Odds Ratios (95% CI)
N. gonorrhoeae burden, quartiles in male sex partner		
1st quartile (ref)	1	1
2nd quartile	2.97 (0.62–14.22)	2.70[b] (.54–13.46)
3rd quartile	10.82 (1.17–100.44)	8.66[b] (.90–83.26)
4th quartile	10.18 (1.09–94.83) *P* = .032	10.17[b] (1.06–97.08) *P* = .052
Chlamydia trachomatis status, positive vs negative, in women	4.06 (.82–20.20) *P* = .087	3.47[c] (.65–18.59) *P* = .146

Abbreviations: CI, confidence interval; ref, reference.

[a]Model estimates below remained similar after additional adjustment for nonsignificant factors: male *C. trachomatis* status; male *Mycoplasma genitalium* status and male age.

[b]Adjusted for *C. trachomatis* infection in women.

[c]Adjusted for *N. gonorrhoeae* burdens in male sex partners.

Table 3. Linear Regression Model That Examined Factors Associated With *Neisseria gonorrhoeae* Burdens in Women

Association of *N. gonorrhoeae* Burdens (log_{10}) in Women	Unadjusted β Coefficient (95% CI)	Adjusted[a] β Coefficient (95% CI)[b]
N. gonorrhoeae burdens, log_{10}, in male sex partners	0.549 (.354–.743) *P* < .001	0.527[b] (.336–.718) *P* < .001
Chlamydia trachomatis status, positive vs negative, in women	0.575 (.076–1.075) *P* = .25	0.451[c,d] (.031–.871) *P* = .036

Abbreviation: CI, confidence interval.

[a]Model estimates below are similar after additional adjustment for nonsignificant factors: male *C. trachomatis* status, male *Mycoplasma genitalium* status, and male age.

[b]Adjusted for *C. trachomatis* status in women.

[c]Adjusted for burdens of *N. gonorrhoeae* in male sex partners.

[d]Fold increase: $10^{0.451} = 2.82$.

interferons [38]. We speculate that induction of type I interferons by *C. trachomatis* [39, 40] may contribute to impaired clearance of *N. gonorrhoeae* in the setting of coinfection.

Molecular analysis of vaginal microbiome indicated that *N. gonorrhoeae*/*C. trachomatis*-negative specimens in our study contained a normal abundance of lactobacilli [25], principally *L. iners* (CST 3 equivalent), similar to normal microbiomes otherwise reported in women of Asian ethnicity [41]. Diversity indices indicated significant differences between the *N. gonorrhoeae*, *C. trachomatis,* and *N. gonorrhoeae*/*C. trachomatis* groups compared to the *N. gonorrhoeae*-negative/*C. trachomatis*-negative group, which aligns with the traditional diagnosis of BV [42, 43] and a meta-analysis of vaginal microbiota studies [44] that included reports that diagnosed BV exclusively by sequence analysis [45–48]. Several longitudinal studies have established that a high prevalence of BV elevates the risk for incident (subsequent) *N. gonorrhoeae* and/or *C. trachomatis* genital infection [13–15].

We identified 21% of *N. gonorrhoeae*-infected women who had underlying *C. trachomatis* infection (their partners were free of *C. trachomatis*; Supplementary Figure 2), similar to the 22% in our earlier study [34]. We speculate that these women had been exposed earlier to previously infected regular partners whose infections may have been asymptomatic and had cleared spontaneously [49] or had undergone treatment. Prior infection in these women with *C. trachomatis* may not have been apparent until they became infected with *N. gonorrhoeae* [34, 50].

Our study has limitations. We used qPCR to quantitate *N. gonorrhoeae* in male urine; *N. gonorrhoeae* burdens measured in urines collected from men with symptomatic urethritis can be as much as 3 log_{10} lower compared to those measured using urethral swabs [31], potentially diminishing accuracy. We showed that *N. gonorrhoeae* burdens measured in male urine and cervicovaginal swabs were not different, similar to a study that showed that men and women have similar *N. gonorrhoeae* burdens measured in vaginal swabs, anorectal swabs from men and women, and male first voided urines [32]. An additional limitation was the comparison of multiple factors and characteristics using statistical hypothesis testing. There was the potential of false-positive findings based merely on statistical significance. The estimates of association (odds ratios or regression coefficients) provided a best assessment of the strength of the associations while the CIs provided an assessment of variability of these measures.

In summary, our study showed that women who are exposed to large *N. gonorrhoeae* burdens such as occur in men with symptomatic urethritis are more likely to become infected; coinfection with *Chlamydia* increases their burden of *N. gonorrhoeae*.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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