Men and Women Have Similar Neisseria gonorrhoeae Bacterial Loads

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Men and Women Have Similar *Neisseria gonorrhoeae* Bacterial Loads: a Comparison of Three Anatomical Sites

Brian M. J. W. van der Veer,[a] Christian J. P. A. Hoebe,[a,b] Nicole H. T. M. Dukers-Muijrers,[b,c] Lieke B. van Alphen,[a] Petra F. G. Wolffs[a]

[a]Department of Medical Microbiology, Care and Public Health Research Institute (CAPHRI), Maastricht University Medical Centre (MUMC+), Maastricht, the Netherlands
[b]Department of Sexual Health, Infectious Diseases and Environmental Health, South Limburg Public Health Service, Heerlen, the Netherlands
[c]Department of Health Promotion, Care and Public Health Research Institute (CAPHRI), Maastricht University Medical Centre (MUMC+), Maastricht, the Netherlands

ABSTRACT *Neisseria gonorrhoeae* is a common bacterial sexually transmitted infection (STI). Currently, there are limited data on the bacterial load in both men and women and on both genital and extragenital sites. Therefore, we quantified *N. gonorrhoeae* bacterial loads in a large population of women, heterosexual men, and men who have sex with men (MSM) at three different anatomical sites. *N. gonorrhoeae*-positive samples ($n = 1265$) of STI clinic consultations ($n = 944$) were tested for *N. gonorrhoeae* with the Roche Cobas 4800 system, and quantification cycle (*Cq*) values were used as an inversely proportional measure for *N. gonorrhoeae* bacterial load after interpolation from a standard curve. Bacterial loads were compared between sample materials and sexes using *t* tests. The following mean *N. gonorrhoeae* loads were observed: urine, 4.5 ± 1.0 $\log_{10}$ CFU/ml; vaginal swabs, 4.3 ± 1.1 $\log_{10}$ CFU/ml; anorectal swabs (women), 4.0 ± 1.2 $\log_{10}$ CFU/ml; anorectal swabs (men), 4.5 ± 1.3 $\log_{10}$ CFU/ml; oropharyngeal swabs (women), 2.8 ± 0.9 $\log_{10}$ CFU/ml; and oropharyngeal swabs (men), 3.2 ± 1.0 $\log_{10}$ CFU/ml. Oropharyngeal swabs had a significantly lower *N. gonorrhoeae* load ($P < 0.001$) than genital and anorectal samples. Loads did not differ between men and women. This is the first study that determined *N. gonorrhoeae* load in both women and men at three anatomical sites. The substantial *N. gonorrhoeae* load at all sample sites suggest that all sites may have transmission potential. However, the oropharyngeal site presents the lowest bacterial load. Men and women have a similar *N. gonorrhoeae* loads on separate anatomical sites, arguing for similar transmission potential and similar clinical relevance.

KEYWORDS *Neisseria gonorrhoeae*, bacterial load, symptoms, extragenital, transmission

Neisseria gonorrhoeae accounts for 87 million of the estimated 376 million new curable sexually transmitted infection (STI) cases worldwide every year (1). Limiting the transmission of *N. gonorrhoeae* is a major public health challenge. Many infections remain hidden to care, especially in women, because of stigma and their asymptomatic nature (2–5). Untreated *N. gonorrhoeae* infections can affect the vagina, cervix, urethra, the rectum, or the throat and could lead to severe sequelae. In women, *N. gonorrhoeae* can cause pelvic inflammatory disease, infertility, ectopic pregnancy, and maternal death (2). In men, urethral infections are frequently symptomatic and can cause urethritis with pus-like discharge, dysuria, and pain or swelling in one testicle (6). Current guidelines recommend urogenital testing for *N. gonorrhoeae* in both men and women (7–9). The need for extragenital testing is recognized for men who have sex with men (MSM) but not for women and heterosexual men. Aside from MSM, extragenital testing is only recommended for patients with an indication, such as a history

Citation van der Veer BMJW, Hoebe CJPA, Dukers-Muijrers NHTM, van Alphen LB, Wolffs PFG. 2020. Men and women have similar *Neisseria gonorrhoeae* bacterial loads: a comparison of three anatomical sites. J Clin Microbiol 58:e01171-20. https://doi.org/10.1128/JCM.01171-20.

Editor Erik Munson, Marquette University

Address correspondence to Petra F. G. Wolffs, p.wolffs@mumc.nl.

Received 18 May 2020
Returned for modification 21 June 2020
Accepted 8 August 2020
Accepted manuscript posted online 12 August 2020
Published 21 October 2020

of direct exposure by anal or oral sex or symptoms. The prevalence of extragenital *N. gonorrhoeae* is 3% to 21% in MSM but substantially lower in heterosexual men and women (0% to 3%) (10, 11). However, as current health care is mainly focused on genital testing, many *N. gonorrhoeae* infections remain hidden to care. Thus, 29% to 30% of *N. gonorrhoeae* infections in women and 14% of *N. gonorrhoeae* infections in heterosexual men will be missed if extragenital testing is not performed (11, 12). Hidden *N. gonorrhoeae* infections may facilitate ongoing transmission, although the extent of underdiagnosis and undertreatment of extragenital *N. gonorrhoeae* infections remains unknown (6, 10–13). In addition, oropharyngeal infections are thought to have a key role in antimicrobial resistance (AMR) development in *N. gonorrhoeae* because of the common presence of commensal *Neisseria* species that readily exchange DNA (14).

Aside from poor access to health care and high-risk behavior, the transmissibility of *N. gonorrhoeae* can also be linked to bacterial load, as a higher inoculum dose in an experimental infection study resulted in higher infection probability (2, 15). The site of infection and symptoms could also be associated with *N. gonorrhoeae* bacterial load. Previous studies have demonstrated a higher *N. gonorrhoeae* load in urethral infections of symptomatic men and anorectal infections of symptomatic MSM (16, 17). Lower *N. gonorrhoeae* load was observed in oropharyngeal swabs of MSM than in urethral and anorectal swabs (16, 17). However, these studies were relatively small, and no study has assessed the *N. gonorrhoeae* bacterial load in women in relation to symptoms.

Therefore, the aim of this study was to assess the bacterial load of *N. gonorrhoeae* at three different anatomical sites (urogenital, anorectal, and oropharyngeal) in a large population of women, heterosexual men, and MSM visiting a large STI clinic. Bacterial loads were compared between sample materials and associations with symptoms were assessed. Results might provide insights in the transmission potential of different anatomical sites in women and men and might also inform testing guidelines for clinical practice.

MATERIALS AND METHODS

Study population and procedures. The South Limburg public health service STI clinic offers STI testing for patients with high-risk of acquiring an STI free of charge. Between January 2012 and July 2017, *N. gonorrhoeae* was diagnosed in 923 individuals, yielding data from 1,095 consultations for retrospective analysis. The *N. gonorrhoeae* positivity rates in this time frame were 1.7% in women, 1.7% in heterosexual men, and 12.0% in MSM. Patients were tested urogenitally for *N. gonorrhoeae* using first-void urine (men) or a self-collected vaginal swab (women). According to regular STI clinic guidelines, MSM were routinely and systematically tested at both anorectal and oropharyngeal sites (18). However, heterosexual men and women were tested extragenitally when reporting symptoms or receptive anal sex (women) or oral sex in the last 6 months (18). Patients were requested to collect a self-taken anorectal swab or a nurse-taken oropharyngeal swab for extragenital testing. A standardized medical and sexual history was routinely taken by nurses for all patients, including self-reported symptoms and sexual behavior in the last 6 months. Urogenital symptoms were defined as one or more of the following: genital discharge, bleeding, itching, swelling, pain, burning sensation, and more frequent urination. Anal symptoms were defined as rectal discharge, bleeding, pain, and itching. Any oropharyngeal symptoms were defined as symptomatic. We excluded samples ($n = 215$) from consultations that had missing data on the Cobas 4800 CT/NG test (Roche Diagnostics, Basel, Switzerland) quantification cycle (*Cq*) value, sexual risk group, symptoms, and number of sex partners in the previous 6 months, leaving 944 consultations (800 individuals) and 1,265 samples for analysis (see Fig. S1 in the supplemental material).

***N. gonorrhoeae* diagnosis and bacterial load.** All samples were tested for *N. gonorrhoeae* with the Cobas 4800 CT/NG test. This assay targets a direct repeat region called DR-9 that is specific for *N. gonorrhoeae* (19). The Cobas test utilizes amplification using two sets of primers and probes to detect two highly conserved variations within the DR-9 region. Little variation is expected between *N. gonorrhoeae* strains, because this assay is not prone to copy number variation and the targets are highly conserved. Therefore, bacterial load can be quantified by interpolation of *Cq* values to a standard curve with known concentrations of *N. gonorrhoeae*. For quantification, a standard curve was made for each specimen type (e.g., swab or urine) as the Cobas 4800 CT/NG test requires different volumes of input material (20). *N. gonorrhoeae* suspension from log-phase culture was spiked (100 μl) and serially diluted 10-fold. A part of the suspension was plated to determine the amount of *N. gonorrhoeae* by counting the colonies resulting in *n* CFU per ml. To resemble urine samples, 5 ml of an *N. gonorrhoeae*-negative male urine pool was added and stabilized before *N. gonorrhoeae* was spiked.

Data analysis. We performed a retrospective data analysis of the bacterial loads of 1,265 *N. gonorrhoeae*-positive samples from 944 routine diagnostic consultations stratified by sample material and sexual risk group (data available in Data Set S1). We binned the number of sex partners in the previous 6 months in tertiles (0 to 2, 3 to 7, and ≥8). Descriptive statistics were used to describe the

TABLE 1 Characteristics of the study population by sexual risk group

Characteristic	Women (*n* = 197)	Heterosexual men (*n* = 144)	MSM[a] (*n* = 603)	Total (*n* = 944)
Age (yr) (median [interquartile range])	31 (22–44)	30 (23–47)	32 (24–45)	31 (23–45)
No. of *N. gonorrhoeae*-positive samples within one patient (% [*n*])[b]				
1	73.6 (145)	98.6 (142)	64.8 (391)	71.8 (678)
2	20.3 (40)	1.4 (2)	28.0 (169)	22.4 (211)
3	6.1 (12)	0.0 (0)	7.1 (43)	5.8 (55)
N. gonorrhoeae diagnosis (% [*n*])				
Genital only	43.7 (86)	75.7 (109)	6.8 (41)	25.0 (236)
Anorectal only	5.1 (10)	0.0 (0)	32.8 (198)	22.0 (208)
Oropharyngeal only	24.9 (49)	22.9 (33)	25.2 (152)	24.8 (234)
Genital-anorectal	14.2 (28)	0.0 (0)	10.8 (65)	9.9 (93)
Genital-oropharyngeal	5.1 (10)	1.4 (2)	1.5 (9)	2.2 (21)
Anorectal-oropharyngeal	1.0 (2)	0.0 (0)	15.8 (95)	10.3 (97)
All three sites	6.1 (12)	0.0 (0)	7.1 (43)	5.8 (55)
Symptoms (any site) (% [*n*])				
Asymptomatic	40.1 (79)	38.9 (56)	46.9 (283)	44.3 (418)
Symptomatic	59.9 (118)	61.1 (88)	53.1 (320)	55.7 (526)
Number of sex partners (% [*n*])				
0–2	31.5 (62)	34.7 (50)	18.2 (110)	23.5 (222)
3–7	34.0 (67)	47.9 (69)	43.6 (263)	42.3 (399)
≥8	34.5 (68)	17.4 (25)	38.1 (230)	34.2 (323)

[a]MSM, men who have sex with men.
[b]*n*, number of consultations.

proportion of concurrent *N. gonorrhoeae* infections at separate anatomical sites, symptoms, and number of sex partners per sexual risk group and sample material. Bacterial load values were $\log_{10}$ transformed for analyses and compared between sample materials using Student's *t* tests (all combinations of two sample materials). Using multivariable linear regression, the bacterial load comparison between sample materials was adjusted for age, sexual risk group, symptoms, and number of sex partners. Bacterial loads were also compared per sample material between sexes with a Student's *t* test. Using multivariable linear regression, the bacterial load comparison between sample materials and sexes was adjusted for age, sexual risk group, symptoms, and number of sex partners. Next, associations between symptoms (i.e., the dependent variable with the asymptomatic group as the reference group) and bacterial load (i.e., the outcome) were assessed. Therefore, univariable and multivariable linear regression analyses were performed on data stratified by sample material and sexual risk group. These models were adjusted for age and number of sex partners in multivariable linear regression. A *P* value of less than 0.05 was considered statistically significant. All analyses were performed using R statistical software, version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria). Figures were made using the R package ggplot2 version 3.1.0.

Ethics. The Medical Ethical Committee of Maastricht University Medical Centre (MUMC+; number METC 2017-2-0251) approved the study protocol, as only deidentified retrospective data from standard care were used. Consultations were part of routine STI clinic procedures where individuals did not object to the use of their samples and data for research. For this study, we used two retrospective databases from the STI clinic and laboratory, with data collected within standard care.

RESULTS

Proportion of extragenital positivity. *N. gonorrhoeae* was detected in 944 routine diagnostic consultations with 1,265 *N. gonorrhoeae*-positive samples, among which, 603 belonged to MSM, 144 to heterosexual men, and 197 to women (Table 1). In most patients, only one sample was *N. gonorrhoeae* positive, irrespective of sexual risk group (73.6% of women, 98.6% of heterosexual men, and 64.8% of MSM) (Table 1). Some patients were *N. gonorrhoeae* positive at all three tested anatomical sites (55/944 [5.8%]). Extragenital-only, anorectal, or oropharyngeal *N. gonorrhoeae*-positive samples were observed in 31.0% (61/197) of women, 22.9% (33/144) of heterosexual men, and 73.8% (445/603) of MSM who were diagnosed (Table 1). Of these, most of the women (49/61 [80.3%]) and all heterosexual men were *N. gonorrhoeae*-positive only at the oropharyngeal site.

***N. gonorrhoeae* bacterial loads compared between sample materials and sexes.** In total, 269 urine samples (genital site, men), 136 vaginal swabs (genital site, women),

TABLE 2 Characteristics of the study population by sample material

Category	% (no.) of samples				
	Urine (n = 269)	Vaginal (n = 136)	Anorectal (n = 453)	Oropharyngeal (n = 407)	Total (n = 1,265)
Sexual risk group					
Women	NA[a]	100 (136)	11.5 (52)	17.9 (73)	20.6 (261)
Heterosexual men	41.3 (111)	NA	0.0 (0)	8.6 (35)	11.5 (146)
MSM[b]	58.7 (158)	NA	88.5 (401)	73.5 (299)	67.8 (858)
Symptoms					
Asymptomatic	23.0 (62)	31.6 (43)	75.1 (340)	88.9 (362)	63.8 (807)
Symptomatic	77.0 (207)	68.4 (93)	24.9 (113)	11.1 (45)	36.2 (458)

[a]NA, not applicable, as no samples were collected as part of routine diagnostic procedures.
[b]MSM, men who have sex with men.

453 anorectal swabs, and 407 oropharyngeal swabs were available for analysis (Table 2). The mean bacterial loads per sample material were as follows: urine samples, 4.5 ± 1.0 $\log_{10}$ CFU/ml; vaginal swabs, 4.3 ± 1.1 $\log_{10}$ CFU/ml; anorectal swabs, 4.4 ± 1.3 $\log_{10}$ CFU/ml; and oropharyngeal swabs, 3.1 ± 1.0 $\log_{10}$ CFU/ml. Oropharyngeal swabs had a lower mean load (16- to 25-fold lower) than all other sample materials ($P < 0.001$, t tests), while no statistically significant difference was observed between the other sample materials. The observation of lower load in oropharyngeal swabs persisted even when corrected for age, sexual risk group, symptoms, and number of sex partners in a linear regression model ($P < 0.001$).

Stratified by sex, the mean bacterial loads were as follows: urine samples, 4.5 ± 1.0 $\log_{10}$ CFU/ml; vaginal swabs, 4.3 ± 1.1 $\log_{10}$ CFU/ml; anorectal swabs of women, 4.0 ± 1.2 $\log_{10}$ CFU/ml; anorectal swabs of men, 4.5 ± 1.3 $\log_{10}$ CFU/ml; oropharyngeal swabs of women, 2.8 ± 0.9 $\log_{10}$ CFU/ml; and oropharyngeal swabs of men, 3.2 ± 1.0 $\log_{10}$ CFU/ml (Fig. 1). Anorectal and oropharyngeal swabs from women had a significantly lower load than those from men, 2.8-fold ($P = 0.02$) and 2.3-fold ($P = 0.002$),

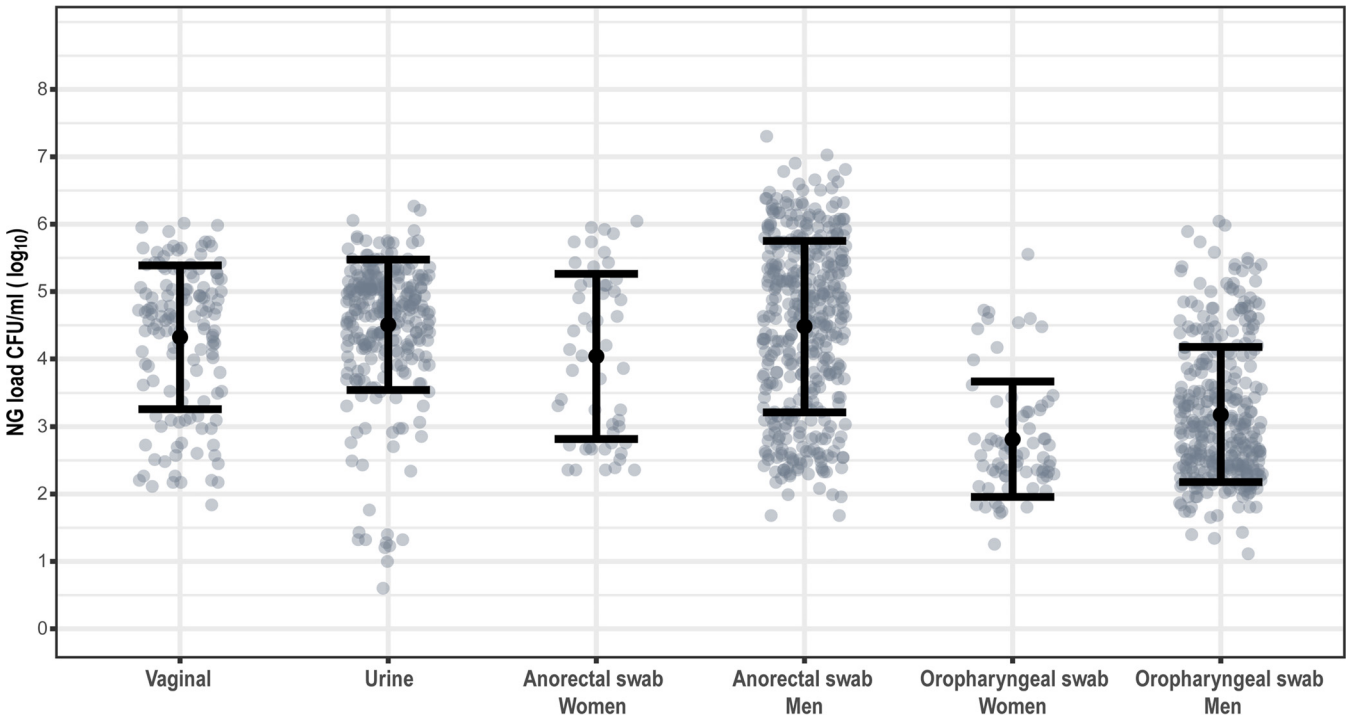

FIG 1 *Neisseria gonorrhoeae* bacterial loads stratified by sample material and sex. Oropharyngeal NG bacterial load was lower than genital and anorectal *N. gonorrhoeae* bacterial loads ($P < 0.001$). No significant difference was observed between the other sample materials. The small differences between women and men can be explained by differences in age, symptoms, and number of sex partners.

TABLE 3 Mean *N. gonorrhoeae* bacterial load and association of symptoms with *N. gonorrhoeae* bacterial load in women per sample material

Category	*n*	CFU/ml			Factor load
		Mean $\log_{10}$ (SD)	$\Delta\log_{10}$ (95% CI)[a]	Adjusted $\Delta\log_{10}$ (95% CI)	
Vaginal					
Asymptomatic	43	4.1 (±1.1)	Ref[b]	Ref	Ref
Symptomatic	93	4.4 (±1.0)	0.28 (−0.11–0.66)	0.22 (−0.17–0.61)	1.7
Anorectal					
Asymptomatic	48	3.9 (±1.2)	Ref	Ref	Ref
Symptomatic	4	5.4 (±0.8)	1.42 (0.20–2.65)[c]	1.67 (0.47–2.87)[d]	46.8
Oropharyngeal					
Asymptomatic	67	2.8 (±0.9)	Ref	Ref	Ref
Symptomatic	6	3.0 (±0.9)	0.16 (−0.57–0.89)	0.01 (−0.69–0.71)	1.0

[a]CI, confidence interval.
[b]Ref, reference.
[c]$P < 0.05$.
[d]$P < 0.01$.

respectively. However, these differences were not statistically different when corrected for age, symptoms, and number of sex partners ($P = 0.09$ and $P = 0.06$, respectively).

Association between symptoms and *N. gonorrhoeae* bacterial load. Most anorectal (75%) and oropharyngeal (89%) samples were from asymptomatic infections, but only a minority of the genital samples (23% to 32%) were from asymptomatic infections (Table 2). Genital symptoms were associated with a 3- to 5-fold higher bacterial load in urine samples from men (heterosexual men, $P < 0.001$; MSM, $P = 0.03$) but not in vaginal swabs from women ($P = 0.26$), in both univariable and multivariable linear regression models (Tables 3 to 5). Associations of oropharyngeal symptoms with *N. gonorrhoeae* bacterial load could not be assessed in heterosexual men, because no symptoms were reported (Table 4). In both women ($P = 0.007$) and MSM ($P < 0.001$), anorectal symptoms were associated with a higher *N. gonorrhoeae* bacterial load, 4- and 47-fold, respectively. In contrast, oropharyngeal symptoms were not associated with a higher *N. gonorrhoeae* load in either women ($P = 0.99$) or MSM ($P = 0.06$). The results remained similar in analyses that were restricted to women (97/197) or MSM (595/603) who were tested at both genital and extragenital sites, including in an analysis corrected for the *N. gonorrhoeae* load at the concurrent sites (see Tables S1 and S2 in the supplemental material).

DISCUSSION

This is the first study that determined the *N. gonorrhoeae* bacterial load in a large group of women, heterosexual men, and MSM at three different anatomic sample sites (urogenital, anorectal, and oropharynx). Oropharyngeal swabs had a significantly lower bacterial load (16- to 25-fold) than all other sample materials, whereas the other sample

TABLE 4 Mean *N. gonorrhoeae* bacterial load and association of symptoms with *N. gonorrhoeae* bacterial load in heterosexual men

Category	*n*	CFU/ml			Factor load
		Mean $\log_{10}$ (SD)	$\Delta\log_{10}$ (95% CI)[a]	Adjusted $\Delta\log_{10}$ (95% CI)	
Urine					
Asymptomatic	33	3.9 (±1.3)	Ref[b]	Ref	Ref
Symptomatic	78	4.7 (±0.9)	0.72 (0.31–1.14)[c]	0.74 (0.31–1.16)[c]	5.4
Oropharyngeal					
Asymptomatic	35	3.1 (±1.0)	NA[d]	NA	NA
Symptomatic	0	NA	NA	NA	NA

[a]CI, confidence interval.
[b]Ref, reference.
[c]$P < 0.001$.
[d]NA, not applicable, as no oropharyngeal symptoms were reported.

TABLE 5 Mean *N. gonorrhoeae* bacterial load and association of symptoms with *N. gonorrhoeae* bacterial load in MSM per sample material

Category	*n*	CFU/ml			Factor load
		Mean $\log_{10}$ (SD)	$\Delta\log_{10}$ (95% CI)[a]	Adjusted $\log_{10}$ (95% CI)	
Urine					
Asymptomatic	29	4.3 (±1.0)	Ref[b]	Ref	Ref
Symptomatic	129	4.6 (±0.8)	0.36 (0.00–0.72)[c]	0.41 (0.04–0.77)[c]	2.5
Anorectal					
Asymptomatic	292	4.3 (±1.3)	Ref	Ref	Ref
Symptomatic	109	4.9 (±1.2)	0.57 (0.29–0.84)[d]	0.57 (0.30–0.85)[d]	3.8
Oropharyngeal					
Asymptomatic	260	3.1 (±1.0)	Ref	Ref	Ref
Symptomatic	39	3.5 (±1.0)	0.34 (0.00–0.67)	0.31 (−0.02–0.64)	2.1

[a]CI, confidence interval.
[b]Ref, reference.
[c]$P < 0.05$.
[d]$P < 0.001$.

materials had comparable bacterial loads. Also, *N. gonorrhoeae* bacterial load was comparable in women and men per anatomical sample site. Genital symptoms were associated with a higher *N. gonorrhoeae* bacterial load in urine samples from men but not in vaginal swabs from women. Furthermore, anorectal symptoms were associated with a higher bacterial load in anorectal swabs from both women and MSM, but no difference was seen in oropharyngeal swabs. More than 70% of *N. gonorrhoeae* infections were single site infections, of which, 31% were anorectal only and 35% oropharyngeal only. This implies a need for extragenital testing, at least in MSM. Moreover, >60% of *N. gonorrhoeae* infections were asymptomatic, with a wide range between genital (23% to 32%), anorectal (75%), and oropharyngeal (89%) infections.

We demonstrated that oropharyngeal swabs have on average a 16- to 25-fold lower bacterial load than all other sample materials, which is in line with previous studies (16, 17). Next to sample material, *N. gonorrhoeae* load was associated with genital symptoms (18-fold) in men and anorectal symptoms in MSM (20-fold) in previous studies (16, 17). We observed associations of only 3- to 5-fold higher *N. gonorrhoeae* loads in urine samples from men with genital symptoms and 4- to 47-fold higher *N. gonorrhoeae* loads in anorectal swabs from women and MSM with anorectal symptoms. No significant association in women reporting genital symptoms was found, possibly due to the difficulty of recognizing genital symptoms compared to that for men (7). The large difference in results between our study and previous studies could be explained by differences in analytical methods, sample materials, and study population. The strong association of *N. gonorrhoeae* load with symptoms in anorectal swabs from women in our study (47-fold) is likely due to the low number of samples from women reporting anal symptoms ($n = 4$), resulting in a large variation in the estimates (Table 3).

To interpret the *N. gonorrhoeae* load of this study, in our opinion, the only relevant comparison could be with the reported infectious doses of experimental gonococcal infection studies. Hobbs et al. described that a high *N. gonorrhoeae* inoculum load is associated with higher infection probability in experimental gonococcal infections in male volunteers (15). *N. gonorrhoeae* was inoculated in the urethra by instillation of 0.2 to 0.3 ml *N. gonorrhoeae* suspension. A 50% infectious dose (ID_{50}), defined as the concentration of an infectious dose of a single exposure needed to infect half of the volunteers, of 3.3 $\log_{10}$ CFU for the MS11mkC strain and one of 5 $\log_{10}$ CFU for the FA1090 strain were estimated. To compare the *N. gonorrhoeae* loads of the present study with the reported ID_{50}, the ID_{50} dose was divided by the volume of the suspension (average of 0.25 ml), resulting in 3.9 $\log_{10}$ CFU/ml for the MS11mkC strain and 5.6 $\log_{10}$ CFU/ml for the FA1090 strain. This would imply that all three anatomical sites have sufficient transmission potential, as the mean *N. gonorrhoeae* load (3.1 to 4.5 $\log_{10}$ CFU/ml) is comparable with the ID_{50} doses. It would also mean that asymptomatic

N. gonorrhoeae infections would have a *N. gonorrhoeae* load that is sufficient for transmission, as the mean bacterial load was 2.8 to 4.3 $\log_{10}$ CFU/ml in both women and men (Tables 3 to 5). These comparisons and ID_{50} doses should be interpreted with caution, because the reported inocula of the experimental infection needed for infection varied between studies, most likely because of the inoculation procedures (21). Also, the large difference in ID_{50}s between the MS11mkC and FA1090 strains found in the study of Hobbs et al. suggests that infection is not solely driven by bacterial load (15, 22). In addition, we determined the *N. gonorrhoeae* load of an anatomical site, but this does not necessarily reflect the transmitted *N. gonorrhoeae* load per sexual act.

In our study, we observed that 65% of *N. gonorrhoeae* infections in MSM were single site infections, of which, 33% were anorectal only and 25% oropharyngeal only, confirming the need for extragenital testing in MSM. Potentially, we demonstrate the need for testing in women and heterosexual men, as high proportions of oropharyngeal-only infections were found (Table 1). However, women and heterosexual men were not systemically tested at genital and extragenital sites but on indication such as a history of direct exposure by anal or oral sex or symptoms. This could introduce bias and elevate the proportion of extragenital-only infections in women and heterosexual men. Moreover, >60% of *N. gonorrhoeae* infections are asymptomatic, with a wide range between genital (23% to 32%), anorectal (75%), and oropharyngeal (89%) infections. Asymptomatic infections may potentially lead to unnoticed transmission of *N. gonorrhoeae* and would be unnoticed when routine testing is not applied (as in heterosexual men and in women) (23). Even though the oropharyngeal site might have a lower transmission potential per sexual exposure due to the lower mean *N. gonorrhoeae* bacterial load, an oropharyngeal *N. gonorrhoeae* infection could have a profound role in transmission (16, 24, 25). First, oral sex is a common sexual practice in women, heterosexual men, and MSM (11). Second, kissing and saliva use as lubricant have also been proposed as a plausible route of transmission, as substantial amounts of *N. gonorrhoeae* were detected in the saliva of MSM (24, 26). In addition, oropharyngeal infections are thought to have a key role in the AMR development in *N. gonorrhoeae* because of the common presence of commensal *Neisseria* species that readily exchange DNA (14). As the estimated median duration of oropharyngeal *N. gonorrhoeae* infection is at least 15 weeks, it could be a reservoir for onward transmission and antimicrobial-resistant strains (14, 27, 28).

One of the strengths of this study is the diversity of the study population, achieved by including women and heterosexual men along with MSM and the routine extragenital testing in MSM. One of its weaknesses is that women and heterosexual men were not routinely tested extragenitally, thereby potentially underestimating the relative number of extragenital *N. gonorrhoeae* infections. Generalizability might be difficult, as only high-risk individuals for acquiring an STI were studied (10). In addition, the volume of first-void urine taken per consultation can vary, and that could have an impact on the determined *N. gonorrhoeae* bacterial load in a sample. This could also explain the large range of observed bacterial load in urine samples in Fig. 1.

In conclusion, our results suggest that all anatomical sample sites may have a role in transmission because of the substantial *N. gonorrhoeae* load. Oropharyngeal *N. gonorrhoeae* infections might have a smaller role, as the mean bacterial load is lower. It would also imply that a *N. gonorrhoeae* infection is equally relevant in women and men, as there is no difference in bacterial load. Oropharyngeal infections should be monitored, since they are frequently single infections, mostly asymptomatic, and could be involved in the AMR development of *N. gonorrhoeae*. Furthermore, extragenital testing should be systematically implemented in routine diagnostics, at least in MSM, to reduce missed infections and help improve *N. gonorrhoeae* control.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.

REFERENCES

1. Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, Chico RM, Smolak A, Newman L, Gottlieb S, Thwin SS, Broutet N, Taylor MM. 2019. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ 97:548P–562P. https://doi.org/10.2471/BLT.18.228486.
2. World Health Organization. 2012. Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*. World Health Organization, Geneva, Switzerland. https://www.who.int/reproductivehealth/publications/rtis/9789241503501/en/.
3. Centers for Disease Control and Prevention. 2014. Sexually transmitted disease surveillance 2014. Centers for Disease Control and Prevention, Atlanta, GA.
4. Reed JL, Huppert JS, Gillespie GL, Taylor RG, Holland CK, Alessandrini EA, Kahn JA. 2015. Adolescent patient preferences surrounding partner notification and treatment for sexually transmitted infections. Acad Emerg Med 22:61–66. https://doi.org/10.1111/acem.12557.
5. Theunissen KA, Bos AE, Hoebe CJ, Kok G, Vluggen S, Crutzen R, Dukers-Muijrers NH. 2015. *Chlamydia trachomatis* testing among young people: what is the role of stigma? BMC Public Health 15:651. https://doi.org/10.1186/s12889-015-2020-y.
6. Bignell C, Unemo M, European STI Guidelines Editorial Board. 2013. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. Int J STD AIDS 24:85–92. https://doi.org/10.1177/0956462412472837.
7. Workowski KA, Bolan GA, Centers for Disease Control and Prevention. 2015. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep 64:1–137.
8. Centers for Disease Control and Prevention. 2014. Recommendations for the laboratory-based detection of Chlamydia trachomatis and Neisseria gonorrhoeae–2014. MMWR Recomm Rep 63:1–19.
9. LeFevre ML, U.S. Preventive Services Task Force. 2014. Screening for chlamydia and gonorrhea: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 161:902–910. https://doi.org/10.7326/M14-1981.
10. Dukers-Muijrers NH, Schachter J, van Liere GA, Wolffs PF, Hoebe CJ. 2015. What is needed to guide testing for anorectal and pharyngeal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women and men? Evidence and opinion. BMC Infect Dis 15:533. https://doi.org/10.1186/s12879-015-1280-6.
11. Garner AL, Schembri G, Cullen T, Lee V. 2015. Should we screen heterosexuals for extra-genital chlamydial and gonococcal infections? Int J STD AIDS 26:462–466. https://doi.org/10.1177/0956462414543120.
12. Koedijk FD, van Bergen JE, Dukers-Muijrers NH, van Leeuwen AP, Hoebe CJ, van der Sande MA, Dutch STIc, Dutch STI centres. 2012. The value of testing multiple anatomic sites for gonorrhoea and chlamydia in sexually transmitted infection centres in the Netherlands, 2006–2010. Int J STD AIDS 23:626–631. https://doi.org/10.1258/ijsa.2012.011378.
13. Chan PA, Janvier M, Alexander NE, Kojic EM, Chapin K. 2012. Recommendations for the diagnosis of Neisseria gonorrhoeae and Chlamydia trachomatis, including extra-genital sites. Med Health R I 95:252–254.
14. Unemo M, Shafer WM. 2014. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev 27:587–613. https://doi.org/10.1128/CMR.00010-14.
15. Hobbs MM, Sparling PF, Cohen MS, Shafer WM, Deal CD, Jerse AE. 2011. Experimental gonococcal infection in male volunteers: cumulative experience with *Neisseria gonorrhoeae* strains FA1090 and MS11mkC. Front Microbiol 2:123. https://doi.org/10.3389/fmicb.2011.00123.
16. Bissessor M, Tabrizi SN, Fairley CK, Danielewski J, Whitton B, Bird S, Garland S, Chen MY. 2011. Differing *Neisseria gonorrhoeae* bacterial loads in the pharynx and rectum in men who have sex with men: implications for gonococcal detection, transmission, and control. J Clin Microbiol 49:4304–4306. https://doi.org/10.1128/JCM.05341-11.
17. Priest D, Ong JJ, Chow EP, Tabrizi S, Phillips S, Bissessor M, Fairley CK, Bradshaw CS, Read TR, Garland S, Chen M. 2017. *Neisseria gonorrhoeae* DNA bacterial load in men with symptomatic and asymptomatic gonococcal urethritis. Sex Transm Infect 93:478–481. https://doi.org/10.1136/sextrans-2016-052950.
18. National Institute for Public Health and the Environment. 2018. Draaiboek Consult seksuele gezondheid. National Institute for Public Health and the Environment, Bilthoven, the Netherlands.
19. Parra-Sánchez M, Palomares JC, Bernal S, González MT, Sivianes N, Pérez L, Pueyo I, Martín-Mazuelos E. 2012. Evaluation of the cobas 4800 CT/NG Test for detecting *Chlamydia trachomatis* and *Neisseria gonorrhoeae* DNA in urogenital swabs and urine specimens. Diagn Microbiol Infect Dis 74:338–342. https://doi.org/10.1016/j.diagmicrobio.2012.08.004.
20. Marlowe EM, Hardy D, Krevolin M, Gohl P, Bertram A, Arcenas R, Seiverth B, Schneider T, Liesenfeld O. 2017. High-throughput testing of urogenital and extragenital specimens for detection of *Chlamydia Trachomatis* and *Neisseria Gonorrhoeae* with Cobas CT/NG. Eur J Microbiol Immunol (Bp) 7:176–186. https://doi.org/10.1556/1886.2017.00018.
21. Cohen MS, Cannon JG, Jerse AE, Charniga LM, Isbey SF, Whicker LG. 1994. Human experimentation with Neisseria gonorrhoeae: rationale, methods, and implications for the biology of infection and vaccine development. J Infect Dis 169:532–537. https://doi.org/10.1093/infdis/169.3.532.
22. Schmidt KA, Schneider H, Lindstrom JA, Boslego JW, Warren RA, Van de Verg L, Deal CD, McClain JB, Griffiss JM. 2001. Experimental gonococcal urethritis and reinfection with homologous gonococci in male volunteers. Sex Transm Dis 28:555–564. https://doi.org/10.1097/00007435-200110000-00001.
23. Whittles LK, White PJ, Didelot X. 2017. Estimating the fitness cost and benefit of cefixime resistance in *Neisseria gonorrhoeae* to inform prescription policy: a modelling study. PLoS Med 14:e1002416. https://doi.org/10.1371/journal.pmed.1002416.
24. Fairley CK, Cornelisse VJ, Hocking JS, Chow EPF. 2019. Models of gonorrhoea transmission from the mouth and saliva. Lancet Infect Dis 19:E360–E366. https://doi.org/10.1016/S1473-3099(19)30304-4.
25. van Liere G, Dukers-Muijrers N, Wessel SK, Gotz HM, Hoebe C. 26 September 2019. What is the optimal testing strategy for oropharyngeal Neisseria gonorrhoeae in men who have sex with men? Comparing selective testing versus routine universal testing from Dutch STI clinic data (2008–2017). Clin Infect Dis https://doi.org/10.1093/cid/ciz964.
26. Chow EP, Tabrizi SN, Phillips S, Lee D, Bradshaw CS, Chen MY, Fairley CK. 2016. *Neisseria gonorrhoeae* bacterial DNA load in the pharynges and saliva of men who have sex with men. J Clin Microbiol 54:2485–2490. https://doi.org/10.1128/JCM.01186-16.
27. Chow EP, Camilleri S, Ward C, Huffam S, Chen MY, Bradshaw CS, Fairley CK. 2016. Duration of gonorrhoea and chlamydia infection at the pharynx and rectum among men who have sex with men: a systematic review. Sex Health 13:199–204. https://doi.org/10.1071/SH15175.
28. Barbee L, Soge O, Haglund M, Yeung W, Hughes J, Khosropour C, Golden M. 2019. O02.4 Incidence and duration of pharyngeal and rectal gonorrhea and chlamydia among high-risk men who have sex with men (MSM). Sex Transm Infect 95 (Suppl 1):A40.2-A40.