Neisseria gonorrhoeae Bacterial DNA Load in the Pharynxes and Saliva of Men Who Have Sex with Men

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Neisseria gonorrhoeae can be cultured in the saliva of individuals with pharyngeal gonorrhea. The aim of this study was to quantify the gonococcal bacterial DNA loads in the pharynxes and saliva among men who have sex with men (MSM) with untreated pharyngeal gonorrhea. Untreated MSM who tested positive for pharyngeal gonorrhea by culture and returned for antibiotic treatment within 14 days at the Melbourne Sexual Health Centre between October 2014 and March 2015 were eligible for this study. The gonococcal bacterial DNA load was measured using real-time quantitative PCR. The median gonococcal bacterial DNA loads in the pharynxes and saliva were calculated and compared to culture positivity using the Mann-Whitney U test. A total of 33 men were included in this study. The median gonococcal bacterial DNA load did not differ between the pharynxes in men who were culture positive (2.5×10^5 copies/swab) and culture negative (2.9×10^4 copies/swab) ($P = 0.166$) and the saliva (culture positive, 2.2×10^5 copies/ml; culture negative, 2.7×10^5 copies/ml) ($P = 0.499$). The bacterial DNA load in the pharynxes ($P = 0.695$) and saliva ($P = 0.969$) did not differ between who men returned for treatment within 7 days and those who returned 8 to 14 days later. Substantial gonococcal bacterial DNA loads were detected in both saliva and pharynxes among MSM with pharyngeal gonorrhea. These findings suggest that gonorrhea can be transmitted via sexual practices involving exposure to saliva, such as oroanal practices (rimming) and saliva use as a lubricant for anal sex.

The high prevalence of gonococcal infection among men who have sex with men (MSM) is a public health issue in many developed countries (1–4). It is important to understand how gonorrhea is transmitted in order to effectively reduce the burden of this epidemic. An Australian-based mathematical model conducted by Hui et al. has shown that gonorrhea cannot be eliminated even if 100% condom use is practiced for anal sex among MSM (5), suggesting that gonorrhea has other routes of transmission other than penile-anal sex. Several observational studies have shown that kissing, oroanal (rimming) practices, oral-penile sex, and saliva use as a lubricant for anal sex are all associated with the detection of gonorrhea (6, 7). All of these sexual activities involve the use and transfer of saliva, indicating that saliva could potentially act as a vehicle for the transmission of gonorrhea during sex. There have been three published studies showing that gonorrhea can be cultured from saliva samples (8–10); however, none of these studies examined the gonococcal bacterial DNA load in saliva. If saliva can act as a medium for gonorrhea transmission, it is important to understand the relative concentrations of bacteria at different anatomical sites to provide further understanding on gonorrhea transmission. The aim of this study was to quantify the gonococcal bacterial DNA loads in saliva and the pharynx.

MATERIALS AND METHODS

This cross-sectional study was conducted at the Melbourne Sexual Health Centre (MSHC) between 13 October 2014 and 25 March 2015. MSHC is one of the major sexual health services in Melbourne, Australia; it provides about 40,000 consultations each year, and about 37% of the consultations were for MSM (11). All services are provided free of charge. All MSM at MSHC were offered for HIV/sexually transmitted infection (STI) screening. For gonorrhea, MSM were routinely screened for pharyngeal and rectal gonorrhea, whereas urethral gonorrhea testing was limited to symptomatic patients. MSHC only used culture for gonorrhea detection

as part of routine STI screening during the study period, including screening for multiple antibiotic resistances (MICs) of penicillin, ceftriaxone, ciprofloxacin, and azithromycin, as well as resistance mechanisms against beta-lactamase.

The sample recruitment and collection of the study has been described elsewhere (8). Briefly, MSM who had a positive culture result for pharyngeal gonorrhea were contacted by the follow-up nurses and asked to return to MSHC for treatment. All of the men who returned to MSHC within 14 days following the diagnosis were invited to participate in this study by the study nurses. Written consents were obtained from all participants if they agreed to participate in the study. Pharyngeal and saliva specimens were collected on the day the men returned for the treatment of pharyngeal gonorrhea. Pharyngeal specimens were taken from the tonsillar fossae and the posterior oropharynx using cotton-tipped swabs (BSN Medical Australia) by three experienced sexual health clinicians, and the swabs were plated immediately onto GC agar medium (Media Preparation Unit, University of Melbourne) for culture separately. An additional pharyngeal swab was taken for a nucleic acid amplification test (NAAT) and then placed in 0.4 ml of phosphate-buffered saline (PBS). The participants were then instructed to masticate and allow the saliva to accumulate in the mouth for 60 s. They then expectorated into a sterile plastic

Received 1 June 2016 Returned for modification 23 June 2016

Accepted 9 July 2016

Accepted manuscript posted online 13 July 2016

Citation Chow EPF, Tabrizi SN, Phillips S, Lee D, Bradshaw CS, Chen MY, Fairley CK. 2016. *Neisseria gonorrhoeae* bacterial DNA load in the pharynxes and saliva of men who have sex with men. J Clin Microbiol 54:2485–2490. doi:10.1128/JCM.01186-16.

Editor: E. Munson, Wheaton Franciscan Laboratory

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TABLE 1 Demographic characteristics and clinical information among 33 MSM

Participant	Age (yr)	Period (days) between diagnosis and treatment	Past history of gonorrhea	MIC (mg/liter) at the day of screening/diagnosis ^a								Beta-lactamase ^b
				Penicillin		Ceftriaxone		Ciprofloxacin		Azithromycin		
				MIC	Status	MIC	Status	MIC	Status	MIC	Status	
1	25	4	No	0.25	LS	0.016	S	≤0.03	S	0.125	S	ND
2	26	7	No	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
3	30	6	No	>2	R	≤0.006	S	2	R	≤0.06	S	D
4	43	9	No	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
5	26	6	Yes	0.25	LS	≤0.008	S	≤0.03	S	0.125	S	ND
6	40	8	No	0.125	LS	≤0.008	S	≤0.03	S	0.25	S	ND
7	21	8	No	0.25	LS	≤0.008	S	≤0.03	S	≤0.06	S	ND
8	50	7	Yes	0.5	LS	0.016	S	8	R	0.125	S	ND
9	25	3	No	0.5	LS	≤0.008	S	4	R	0.25	S	ND
10	22	7	No	0.5	LS	≤0.008	S	≤0.03	S	≤0.06	S	ND
11	23	4	No	1	R	0.03	S	16	R	0.125	S	ND
12	46	8	No	0.5	LS	0.016	S	4	R	0.125	S	ND
13 ^c	31	13	No	NA	NA	NA	NA	NA	NA	NA	NA	NA
14	30	6	No	0.5	LS	≤0.008	S	≤0.03	S	0.25	S	ND
15	43	7	No	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
16	24	7	No	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
17	31	8	Yes	0.5	LS	≤0.008	S	≤0.03	S	0.25	S	ND
18	27	9	No	0.25	LS	≤0.008	S	≤0.03	S	0.125	S	ND
19	26	4	No	0.125	LS	≤0.008	S	≤0.03	S	≤0.06	S	ND
20	26	7	Yes	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
21	25	12	No	0.125	LS	≤0.008	S	≤0.03	S	≤0.06	S	ND
22	31	11	No	0.25	LS	0.016	S	≤0.03	S	0.25	S	ND
23	21	10	No	0.5	LS	0.03	S	4	R	≤0.06	S	ND
24	49	7	Yes	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
25	25	11	Yes	0.25	LS	≤0.008	S	≤0.03	S	0.125	S	ND
26	44	7	No	0.25	LS	0.016	S	≤0.03	S	≤0.06	S	ND
27	34	7	No	0.5	LS	≤0.008	S	4	R	0.25	S	ND
28	24	7	No	1	R	≤0.008	S	8	R	0.125	S	ND
29	27	8	No	0.125	LS	≤0.008	S	≤0.03	S	0.125	S	ND
30	28	14	No	0.25	LS	≤0.008	S	≤0.03	S	0.125	S	ND
31	36	7	Yes	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
32	21	6	No	0.25	LS	≤0.008	S	≤0.03	S	0.25	S	ND
33	28	6	No	0.5	LS	0.016	S	8	R	0.125	S	ND

^a S, susceptible; R, resistant; LS, less susceptible.^b D, detected; ND, not detected; NA, not applicable.^c Participant 13 was diagnosed outside the MSHC, and thus data for the MIC and the presence of beta-lactamase were not available.

specimen jar (Sarstedt Australia Pty, Ltd.), and a cotton-tipped swab was then swirled in the saliva. The swab with saliva specimen was plated immediately onto GC agar medium for culture. The saliva was stored neat at -80°C until testing. Then, 200 μl of each sample (swab swirled in PBS and neat saliva) was extracted using a MagNA Pure 96 (Roche Diagnostic, Indianapolis, IN) and eluted in 100 μl of elution buffer. An aliquot of 5 μl of extracted DNA was amplified. Beta-globin control was used to assess the specimen adequacy. *N. gonorrhoeae* *porA* and *opa* qPCR assays were used to ensure that the relative bacterial DNA load was not extremely different, and the *opa* gene was used to express the quantitation of DNA load (12, 13). *N. gonorrhoeae* contains 11 copies of *opa* genes and a single copy of the *porA* gene. The *N. gonorrhoeae* load was calculated by comparing quantitative PCR (qPCR) crossing points to the standard curve constructed by the amplification of different copy numbers from an *opa* assay (14).

A total of 33 MSM at MSHC that had a positive culture for pharyngeal gonorrhea at screening and who returned to our center for antibiotic treatment within 14 days during the study period were included (8). The correlation between beta-globin and bacterial DNA load was calculated for the pharyngeal swabs and saliva specimens. The medians and the in-

terquartile ranges (IQR) for beta-globin and for the *N. gonorrhoeae* bacterial DNA load in the pharynxes and saliva were calculated and stratified based on NAAT results and culture positivity. The *N. gonorrhoeae* bacterial DNA loads were also stratified based on qPCR and culture positivity. The *N. gonorrhoeae* bacterial DNA load data were highly skewed, and hence the data were log transformed in base 10. A Mann-Whitney U test was used to compare the median bacterial DNA loads between NAAT/culture-positive and -negative samples. All statistical analyses were performed using Stata version 13.1 (Stata Corp., College Station, TX). Ethics approval for this study was obtained from the Alfred Hospital Ethics Committee, Melbourne, Australia (number 316/14).

RESULTS

Table 1 shows the demographic characteristics and clinical data for all 33 participants in the study. The ages of the 33 participants ranged from 21 to 50 years, with a median of 27 years and an IQR of 25 to 34 years. All men (100%) were HIV negative at the time of recruitment. Seven men (21%) had a history of gonorrhea diag-

TABLE 2 Positivity, as determined by culture/NAAT and DNA load in the pharynges and saliva among men returning for treatment

Participant	Period (days) between diagnosis and treatment	Test result					
		Pharyngeal swabs			Saliva samples		
		Culture	NAAT	DNA load (copies/swab)	Culture	NAAT	DNA load (copies/ml)
1	4	Positive	Positive	164,221	Positive	Positive	369,650
2	7	Positive	Positive	568	Negative	Positive	13,554
3	6	Negative	Positive	2,413	Negative	Positive	17,887
4	9	Negative	Positive	2,824	Negative	Positive	7,114
5	6	Positive	Positive	262	Negative	Positive	3,500,000
6	8	Positive	Positive	2,954	Positive	Positive	65,919
7	8	Negative	Equivocal	31	Negative	Positive	344
8	7	Negative	Positive	262	Negative	Positive	2,937
9	3	Negative	Positive	267,326	Negative	Positive	375,234
10	7	Negative	Positive	4,773	Negative	Positive	25,442
11	4	Negative	Positive	334,741	Negative	Positive	8,900,000
12	8	Positive	Positive	56,219	Negative	Positive	159,649
13	13	Positive	Positive	320,019	Positive	Positive	1,100,000
14	6	Negative	Positive	790	Negative	Positive	121
15	7	Positive	Positive	7,400,000	Negative	Positive	30,229
16	7	Negative	Positive	8,500,000	Negative	Positive	4,100,000
17	8	Negative	Positive	186	Negative	Positive	490
18	9	Positive	Positive	170,493	Negative	Positive	3,818
19	4	Negative	Positive	11,000,000	Negative	Positive	13,000,000
20	7	Negative	Positive	29,285	Negative	Positive	11,323
21	12	Negative	Positive	7,200,000	Negative	Positive	8,100,000
22	11	Positive	Positive	1,300,000	Negative	Positive	3,700,000
23	10	Positive	Positive	2,900,000	Positive	Positive	69,993
24	7	Negative	Positive	154,662	Negative	Positive	466,352
25	11	Negative	Equivocal	15	Negative	Negative	NA ^a
26	7	Positive	Positive	177,005	Negative	Positive	950,592
27	7	Negative	Positive	1,074	Negative	Positive	5,001
28	7	Positive	Positive	1,700,000	Negative	Positive	1,600,000
29	8	Positive	Positive	6,600,000	Positive	Positive	31,000,000
30	14	Negative	Positive	462,061	Negative	Positive	18,000,000
31	7	Negative	Positive	170,493	Negative	Positive	824,400
32	6	Negative	Positive	2,600,000	Negative	Positive	9,900,000
33	6	Positive	Positive	19,000,000	Positive	Positive	39,594

^a NA, not applicable.

nosis. Data for MICs and beta-lactamase were available for 32 of the 33 men (Table 1). Three men (9%) were resistant to penicillin, and nine men (28%) were resistant to ciprofloxacin. No subjects were resistant to either ceftriaxone or azithromycin. Resistance against beta-lactamase was detected in one subject (3%).

Beta-globin was detected in all pharyngeal and saliva specimens from the 33 participants (median, 4.5×10^5 copies/swab; IQR, 1.4×10^5 to 8.7×10^5). There was no significant difference in the quantity of beta-globin in the pharynges between 14 culture-positive (median, 3.7×10^5 copies/swab; IQR, 1.6×10^5 to 1.1×10^6) and 19 culture-negative (6.4×10^5 copies/swab; IQR, 5.5×10^4 to 8.2×10^5) samples (Mann-Whitney, $P = 0.636$). In addition, no significant difference was observed in the quantity of beta-globin in saliva between 6 culture-positive (6.6×10^5 copies/ml; IQR, 2.0×10^5 to 2.5×10^6) and 27 culture-negative (2.5×10^6 copies/ml; IQR, 1.7×10^6 to 6.8×10^6) samples (Mann-Whitney, $P = 0.06$). There was a strong positive correlation between beta-globin and bacterial DNA load in the pharynx ($r =$

0.4199; $P = 0.0150$); however, this correlation was not observed in the saliva specimens ($r = -0.0662$, $P = 0.7190$).

Table 2 shows the positivity determined by culture and NAAT and gonococcal bacterial DNA loads in the pharynges and saliva of the 33 men. The median bacterial DNA load did not differ between 14 men with a culture-positive pharyngeal specimen (2.5×10^5 copies/swab; log 5.4) and 19 men with a culture-negative pharyngeal specimen (2.9×10^4 copies/swab; log 4.5) (Mann-Whitney, $P = 0.166$). Of the 31 men who tested positive for pharyngeal gonorrhea by NAAT, the median bacterial DNA load was 1.7×10^5 copies/swab (log 5.2) (Table 3 and Fig. 1).

The median bacterial DNA load in saliva was similar among 6 men with a culture-positive saliva specimen (2.2×10^5 copies/ml; log 5.3) and 26 men with a culture-negative saliva specimen (2.7×10^5 copies/ml; log 5.4) (Mann-Whitney, $P = 0.499$) (Fig. 1). Of the 6 men with a culture-positive saliva specimen, all had a positive culture result in their pharynges, and the median bacterial DNA load in the pharynx was 1.6×10^6 copies/swab (log 6.2). Of

TABLE 3 Gonococcal bacterial DNA loads in pharynxes and saliva as determined by culture and the NAAT among 33 MSM

Specimen	n	Test results on day of treatment					P ^c
		Positive		Negative			
		Median <i>N. gonorrhoeae</i> load (IQR)	Log ₁₀ median <i>N. gonorrhoeae</i> load (IQR)	n	Median <i>N. gonorrhoeae</i> load (IQR)	Log ₁₀ median <i>N. gonorrhoeae</i> load (IQR)	
Pharyngeal swabs (load/swab)							
Detected by culture (<i>n</i> = 33)	14	248,512 (56,219–2,943,254)	5.4 (2.8–6.5)	19	29,285 (790–462,061)	4.5 (2.9–5.7)	0.166
Detected by NAAT (<i>n</i> = 31) ^a	31	170,493 (2,824–2,571,742)	5.2 (3.5–6.4)				
Saliva specimens (load/ml)							
Detected by culture (<i>n</i> = 32) ^b	6	219,821 (65,919–1,112,657)	5.3 (4.8–6.0)	26	267,441 (7,113–3,719,717)	5.4 (3.9–6.6)	0.499
Detected by NAAT (<i>n</i> = 33)	32	264,650 (12,438–3,585,395)	5.4 (4.1–6.6)	1	0		

^a Two samples were excluded because the results were indeterminate.

^b One saliva sample was excluded because the NAAT result was negative, and no gonococcal bacterial DNA loads were detected.

^c That is, the *P* value comparing the positive and negative medians, as determined with a Mann-Whitney *U* test.

33 men, 32 tested positive in their saliva by NAAT, and one man tested negative in his saliva by NAAT. The median bacterial DNA load was 2.6×10^5 copies/ml (log 5.4) among 32 men (Table 3 and Fig. 1).

A total of 20 men (61%) returned for treatment within 7 days, and the remaining 13 men (39%) returned after between 8 and 14 days. The median bacterial DNA load in the pharynx was 1.7×10^5 copies/swab among men who returned within 7 days and 3.2×10^5 copies/swab among men who returned 8 to 14 days later; there was no significant difference between the two groups ($P = 0.695$). The median bacterial DNA load in saliva was 3.7×10^5 copies/ml among men who returned within 7 days, and it was 1.1×10^5 copies/ml among men who returned 8 to 14 days later; there was no significant difference between the two groups ($p = 0.969$).

DISCUSSION

To our knowledge, this is the first study to quantify the gonococcal bacterial DNA load in saliva. We found a substantial load of gonococcal bacterial in both sample types (i.e., pharyngeal swabs and saliva), suggesting that both samples are theoretically infectious, particularly among individuals who are culture positive in the

pharynx and saliva. These findings are consistent with the substantial body of observational research suggesting that activities involving saliva may transmit gonorrhea, whether through kissing, rimming, or potentially as a part of oral sex. Previous research has not specifically focused on the role of saliva, but given these findings, further investigation of saliva during sex would be warranted.

There has been one Australian study quantifying the gonococcal bacterial DNA load in the pharynx and rectum. In 2013, Bissessor et al. reported that the gonococcal bacterial DNA load in the pharynx is about 2,100 copies/swab (14), which is almost 80-fold lower than the estimate in our study (1.7×10^5 copies/swab). It is possible that the sampling technique may explain the lower levels found in the Bissessor study compared to our study. First, pharyngeal swabs were taken by all clinicians as part of routine clinical care in the study by Bissessor et al., whereas in our study the sampling was restricted to three experienced sexual health clinicians to minimize any variation in the sampling technique. Sampling technique can vary across sexual health clinicians and is critical to successful sampling (15). Second, in the present study, we sampled both the tonsillar fossae and the posterior oropharynx, which is probably required for optimal detection (16); in contrast, in the Bissessor study, specimens were only collected at the tonsillar fossae. The median beta-globin level detected in the present study (4.5×10^5 copies/swab) is higher than that detected in the study by Bissessor et al. (3.8×10^5 copies/swab). It is therefore possible that our highly regimented sampling technique “artificially” elevated what would be found on routine swabs, which may have generally lower loads than saliva, which involves simple expectorating, which is a fairly consistent sampling approach. In addition, the Bissessor et al. study also showed that the bacterial DNA load in the pharynx is significantly higher among men who were culture positive than among men who were culture negative; we also found this to be true, but our difference was not statistically significant. A number of reasons may explain this, including the limited statistical power of our study, a higher bacterial load in our study (because only initially culture positive men were included), or differences in sampling between the two studies.

Our results showed that the bacterial DNA loads in the pharynx and saliva did not differ at 7 and 14 days. Previous studies have suggested that the duration of pharyngeal gonorrhea is short (about 3 to 4 months), and it could be self-limited (17, 18). Given

FIG 1 Distribution of *N. gonorrhoeae* bacterial DNA load in the pharynxes and saliva in 33 men, stratified by culture positivity.

that our observational period was short (14 days), we might not have seen the decline in DNA load over time.

Nonpenetrative sexual activities, such as kissing, rimming, and saliva use as a lubricant, are common among MSM and have generally been considered to present low risk for the acquisition of bacterial STIs; however, these activities have been identified as risk factors for gonorrhea in several observational studies. Given that a substantial gonococcal bacterial DNA load is present in saliva, it is plausible that gonorrhea can be transmitted through saliva across all three anatomical sites, that is, from the pharynx to the rectum via rimming (19), from the rectum to the pharynx via rimming (7), from pharynx to pharynx via wet kissing (7), from the pharynx to the urethra via oral sex (19), and/or via saliva use as a lubricant for masturbation (6). In addition, a substantial proportion of MSM (69%) use the partner's saliva as a lubricant for anal sex (6), suggesting that this might account for rectal gonorrheal infections in men who report condom use with receptive anal sex. This hypothesis is supported by a recent observational study showing that, after adjusting for potential confounders, including condom use for receptive anal sex, about 41% of the cases of rectal gonorrhea can be attributed to the use of a partner's saliva as a lubricant for anal sex (6).

Our study has several limitations. First, the wide IQRs of the gonococcal bacterial DNA loads in the pharynx and saliva must be interpreted cautiously and require further studies with greater precision. Second, we did not measure the volume of saliva collected, nor did we weigh the pharyngeal swabs. Individuals might produce different amounts of saliva during sex in which, presumably, gonococcal bacteria are diluted, and this may vary the bacterial DNA load per milliliter of saliva. Third, although we measured the organism load on swabs and per ml of saliva, neither of these measurements is easily translatable to different sexual acts. Further studies may choose sampling methods with a closer relationship to the risk of acquisition. Finally, it is not possible to determine whether the DNA detected was from viable gonococci and therefore infectious. All of the men had had a recent positive throat culture, but the lower rate of culture positivity in saliva suggests that a greater proportion may be nonviable.

Saliva is generally considered to be a low-risk factor in the transmission of STIs such as gonorrhea. However, we have shown here that a substantial bacterial DNA load can be detected in saliva, suggesting that saliva could potentially be an infectious medium for gonorrhea transmission. Further studies, such as partner notification or partner studies with molecular genotyping, are required to provide further understanding on the route of gonorrhea transmission.

ACKNOWLEDGMENTS

C.K.F. conceived the idea that saliva may play a key role in the transmission of gonorrhea in M.S.M. E.P.F.C. and C.K.F. designed the study. D.L. designed the clinical procedures of the study. E.P.F.C. performed the data analysis. S.N.T. and S.P. performed the laboratory testing and assisted with the interpretation of the laboratory data. E.P.F.C. prepared the first draft of the manuscript. All authors contributed to the interpretation of the study findings and contributed to the drafting of the manuscript.

This study was supported by the National Health and Medical Research Council (NHMRC) program grant 568971. E.P.F.C. is supported by the Early Career Fellowships from the Australian NHMRC (number 1091226).

FUNDING INFORMATION

This work, including the efforts of Eric P. F. Chow, was funded by Department of Health | National Health and Medical Research Council (NHMRC) (1091226). This work, including the efforts of Christopher K. Fairley, was funded by Department of Health | National Health and Medical Research Council (NHMRC) (568971).

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