

Increased Sensitivity of DNA Amplification Testing for the Detection of Pharyngeal Gonorrhea in Men Who Have Sex with Men

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We compared ligase chain reaction (LCR) assay with standard culture for the detection of pharyngeal *Neisseria gonorrhoeae* infection in men who have sex with men (MSM) presenting at a sexually transmitted diseases clinic in San Francisco. Pharyngeal specimens were obtained from 200 MSM who reported performing fellatio during the previous 2 weeks. Confirmatory testing of discrepant specimens was conducted using *N. gonorrhoeae* pilin proteins. Prevalence of pharyngeal *N. gonorrhoeae* was 6% by culture or 11% by LCR. The sensitivity and specificity of LCR were 94.7% and 97.8%, respectively, compared with values of 47.4% and 100% for culture. Prevalence of pharyngeal *N. gonorrhoeae* infection, as determined by DNA amplification testing, was higher than that suggested by traditional culture. Results support the use of DNA amplification testing in the oropharynx. The high prevalence of pharyngeal *N. gonorrhoeae* infection among MSM suggests that routine screening should be considered in efforts to reduce the burden of gonorrhea in this population.

Fellatio is a recognized means of transmission of various sexually transmitted diseases (STDs), including gonorrhea; syphilis; chlamydia; chancroid; and STDs caused by herpes simplex viruses 1 and 2, human papillomavirus, Epstein-Barr virus, and, possibly, HIV [1–3]. The primary risk factor for oral acquisition of such infections is fellatio with exposure to infectious secretions. The potential for detection of pharyngeal *Neisseria gonorrhoeae* is high among men who have sex

with men (MSM), a population in which unprotected oral sex and STDs are prevalent [4–7].

Clinical and research interest in pharyngeal *N. gonorrhoeae* has been minimal for several reasons. First, because of evidence that pharyngeal *N. gonorrhoeae* rarely occurs independently of genital infection, it is thought that treatment of STDs at genital sites also will be of therapeutic benefit for pharyngeal infection, if present. Second, there are indications that pharyngeal *N. gonorrhoeae* infection has a high spontaneous cure rate [8–10]. Third, it is believed, despite evidence to the contrary, that pharyngeal *N. gonorrhoeae* is uncommonly transmitted [11–13]. Therefore, routine screening for pharyngeal STDs is inconsistent, possibly resulting in undetected and untreated infections.

Recent data have highlighted new characteristics of pharyngeal *N. gonorrhoeae* infection. All cases of pharyngeal *N. gonorrhoeae* that were detected in a study from Great Britain were asymptomatic [14]. In another study, which was conducted in Seattle, 84% of individuals who had pharyngeal *N. gonorrhoeae* infection

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had no symptoms [11]. Recent evidence suggests that a higher proportion of pharyngeal infection occurs independently of genital infection among MSM than has been documented previously. Studies done in the 1970s and 1980s found a very small proportion of cases of isolated pharyngeal infection [13, 15–17], yet researchers in Seattle recently found that 64% of pharyngeal *N. gonorrhoeae* infections occurred in individuals without genital *N. gonorrhoeae* infection [11]. The widespread changes in sexual behavior that have occurred among MSM in response to the HIV epidemic may account for the differences between these studies [18, 19].

DNA amplification tests have improved detection of gonorrhea, since they are more sensitive than culture for the detection of extragenital *N. gonorrhoeae* infection [20–23]. A further advantage of DNA amplification tests, in comparison with culture-based testing, is the ease of specimen collection and management and of laboratory performance of the assay. No studies have validated or used DNA amplification methods of detection at extragenital sites in MSM, a population with a high rate of STDs [5, 6]. Although the prevalence of pharyngeal *N. gonorrhoeae* consistently has been shown to be higher in studies comparing MSM with heterosexual populations [10, 11, 24], all such studies have relied on traditional culture methods and may have underestimated the true prevalence of infection. In this study, we report the sensitivity and specificity of LCR for the detection of pharyngeal *N. gonorrhoeae* infection in a sample of MSM attending a municipal STD clinic in San Francisco.

PATIENTS, MATERIALS, AND METHODS

Patients and collection of specimens. Patients were recruited at the San Francisco Municipal STD Clinic (“City Clinic”). City Clinic provides, for a nominal fee, confidential STD diagnostic services, treatment, and counseling to all residents >12 years of age. We evaluated a sample of 200 men who were consecutively evaluated at the clinic and who reported (1) a history of performing fellatio on other men, and (2) lack of antibiotic use during the 2 weeks before they were seen at the clinic. Two pharyngeal swab specimens were obtained from the posterior pharynx of each patient; 1 specimen was used for culture and 1 for LCR testing (LCX; Abbott Laboratories). *N. gonorrhoeae* was isolated using selective medium (modified Thayer-Martin medium; Microbiological Media), and its identification was confirmed by fluorescent antibody or carbohydrate utilization reactions [25]. History of sore throat during the previous 2 weeks and antibiotic use during the previous 30 days was collected as part of routine clinical evaluation. Results of testing for urethral and rectal *N. gonorrhoeae* infection, which was conducted concurrently with testing for pharyngeal infection, were reviewed.

Test performance. Specimens with culture-positive results

were considered to have true-positive results. Specimens that had LCR-positive/culture-negative results were retested at Abbott Laboratories by use of an LCR assay with probes derived from the *N. gonorrhoeae* pilin gene [26–28]. Specimens that had LCR-positive/culture-negative results and that repeatedly tested positive for *N. gonorrhoeae* pilin proteins were considered to have true-positive results. Calculation of sensitivity and specificity was based on a “gold standard” that included all specimens with positive culture results and all specimens with confirmed positive LCR results. Calculation of 95% CIs for estimates of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) was based on a binomial sample distribution.

RESULTS

The age range of participants was 18–61 years. The proportion of pharyngeal specimens that tested positive for *N. gonorrhoeae* was 4.5%, by culture, or 11%, by LCR (table 1). Participants who tested positive for pharyngeal *N. gonorrhoeae* were significantly more likely to test positive for *N. gonorrhoeae* at other sites (e.g., by rectal or urethral culture or by LCR testing of urine samples; OR, 12.8; 95% CI, 4.1–39.1). Of the 22 participants with positive pharyngeal results according to LCR, 11 (50%) tested positive for *N. gonorrhoeae* at other sites. Of the 177 participants with negative pharyngeal results according to LCR, 13 (7.3%) tested positive for *N. gonorrhoeae* elsewhere. All participants were asymptomatic for pharyngeal infection. Seven participants reported antibiotic use during the previous 30 days; all 7 tested negative for *N. gonorrhoeae* by both culture and LCR.

Additional testing confirmed the presence of *N. gonorrhoeae* in 10 (71.4%) of the 14 specimens with LCR-positive/culture-negative results. Four remaining specimens (1 that repeatedly had negative test results, 1 of which there was an insufficient amount available for testing, and 2 of which had indeterminate results) were categorized as having negative results. Thus, the presence of *N. gonorrhoeae* was confirmed in 18 of 22 specimens with positive LCR results (table 2).

Table 3 shows the sensitivity, specificity, PPV, and NPV of LCR and culture for *N. gonorrhoeae* detection. Sensitivity and

Table 1. Results of the LCR assay versus culture for the detection of *Neisseria gonorrhoeae* in pharyngeal specimens obtained from 200 men who have sex with men.

Culture result	LCR assay result		Total
	Positive	Negative	
Positive	8	1	9
Negative	14	177	191
Total	22	178	200

NOTE. LCR, ligase chain reaction.

Table 2. Results of ligase chain reaction (LCR) assay and culture versus those of the "gold standard" for detection of *Neisseria gonorrhoeae* in pharyngeal specimens from 200 men who have sex with men.

LCR or culture result	Result of gold standard for detection		Total
	Positive	Negative	
LCR			
Positive	18	4	22
Negative	1	177	178
Total	19	181	200
Culture			
Positive	8	1	9
Negative	10	180	191
Total	18	181	200

NOTE. Gold standard denotes a positive culture result or positive results of both culture and LCR with *N. gonorrhoeae* pilin confirmation.

specificity were 94.7% and 97.8%, respectively, for LCR testing, and they were 47.4% and 100%, respectively, for culture. In our study population, for which the estimated true prevalence of pharyngeal *N. gonorrhoeae* infection was 9.5% (19 of 200 patients), the PPV was 81.8% and the NPV was 98.9% for LCR, compared with a PPV of 100% and an NPV of 94.8% for culture.

DISCUSSION

This study showed that the DNA amplification method (i.e., LCR) detected more than twice as many cases of pharyngeal *N. gonorrhoeae* infection than did culture (11% vs. 4.5%). As another group has found, the sensitivity of LCR for the diagnosis of infection was much higher than that of culture, but the specificity of LCR was slightly less than that of culture [21]. The PPV of LCR will be lower for populations with a lower prevalence of pharyngeal *N. gonorrhoeae* infection, but it will be $\geq 75\%$ for populations with a prevalence of $\geq 5\%$. These results show that screening for pharyngeal *N. gonorrhoeae* infection can be done reliably using LCR. Of significance, we found that one-half of the participants who tested positive for pharyngeal *N. gonorrhoeae* by LCR did not have infection identified at other sites. Thus, for the 200 men screened in this study, pharyngeal testing resulted in a 32% increase in the number of *N. gonorrhoeae* infections identified.

All patients reviewed in this study were asymptomatic, a finding that raises the possibility that, for some patients, infection may have gone undiagnosed and untreated if screening for pharyngeal infection had not been done. Thus, the prevalence of pharyngeal *N. gonorrhoeae* infection is likely to be

significantly underestimated, given its asymptomatic nature and the infrequency of routine screening.

For the 19 cases of pharyngeal *N. gonorrhoeae* infection that were considered to have true-positive results, LCR assay was superior to culture for the diagnosis of infection. The false-positive designation that resulted from the 3 pairs with LCR-positive/culture-negative results being classified as having negative results (because of unresolved confirmatory testing) reduced the specificity and the PPV of LCR.

It is unknown to what extent pharyngeal *N. gonorrhoeae* contributes to maintaining or increasing the spread of urethral or rectal *N. gonorrhoeae* among MSM. Fellatio has been shown to be an independent risk factor for urethral gonorrhea among MSM who denied performing insertive anal sex [11]; this finding suggests that pharyngeal gonococcal infection may be more transmissible than previously was believed. A similar study of heterosexual patients with STDs did not confirm this observation; however, the prevalence of pharyngeal gonorrhea is much lower among women than among MSM [29]. If the conclusion of Lafferty et al. [11] is correct, the importance of the present findings—especially the high proportion of isolated cases of pharyngeal *N. gonorrhoeae* infection—is elevated. Given the high prevalence of oral sex and the increases in cases of both urethral and rectal *N. gonorrhoeae* infection among MSM in San Francisco and elsewhere [4–7], pharyngeal infection remains an important factor to consider in the overall efforts to prevent STDs in this population.

We acknowledge the limitations of this study. The MSM who attended the STD clinic may not represent all MSM in San Francisco. The study's modest sample size influences the precision of the point estimates. In addition, it is impossible to know whether some of the LCR-positive/culture-negative results indicated resolving infections, despite the fact that we limited our sample of men to include only those who denied having recently used antibiotics. Because others have found inconsistencies between self-reports and detection of antibiotics in urine samples [30], further studies are warranted to determine whether the additional infections detected by LCR are infectious.

Table 3. Performance characteristics of the ligase chain reaction (LCR) assay versus those of culture for the detection of *Neisseria gonorrhoeae* in pharyngeal specimens from 200 men who have sex with men.

Performance characteristic	LCR assay, % (95% CI)	Culture, % (95% CI)
Sensitivity	94.7 (74.0–99.9)	47.4 (24.4–71.1)
Specificity	97.8 (94.4–99.4)	100.0 (98.0–100)
Positive predictive value ^a	81.8 (59.7–94.8)	100.0 (66.4–100)
Negative predictive value ^a	98.9 (96.9–100)	94.8 (90.6–97.5)

^a At a prevalence of 11%.

Advances in diagnostic testing challenge the role of culture as the gold standard for the diagnosis of pharyngeal *N. gonorrhoeae* infection. Because, in general, DNA amplification assays have not been approved by the US Food and Drug Administration for use in the testing of extragenital specimens, local laboratories must perform validation studies to offer these tests clinically. As the clinical and epidemiological significance of pharyngeal *N. gonorrhoeae* infection becomes better understood, manufacturers should consider obtaining approval from the US Food and Drug Administration for use of these important diagnostic tests at extragenital sites. For now, STD control programs and clinicians should work with local laboratories to validate the use of DNA amplification assays for extragenital testing for STDs, so as to provide improved clinical care and screening. By reducing the prevalence of disease—either by shortening the duration of infection or by reducing the likelihood of transmission—targeted screening for pharyngeal *N. gonorrhoeae* infection among high-risk populations could contribute to a reduction in the overall population burden of *N. gonorrhoeae* infection.

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