Original Article

Endourethral Swab versus Urine Collection for Real-Time PCR with TaqMan Probe Based Detection of Gonorrheal Infection among Men Who Have Sex with Men

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Objective: To compare endourethral swab and urine collection as diagnostic specimens for the detection of urethral gonorrhea using the polymerase chain reaction (real-time PCR with TaqMan probes).

Materials and Methods: Endourethral swabs and urine specimens were collected from 268 men who have sex with men [MSM] attending two walk-in clinics, a sexually transmitted diseases mobile clinic [STDs mobile clinic] and an antiretroviral clinic [ARV clinic]. The two specimens were processed for Neisseria gonorrhea [NG] DNA detection using real-time PCR with TaqMan probes. The main outcome was a positive result of gonorrhea from either sample.

Results: The 268 MSM had urine collections, but only 267 had taken endourethral swabs for PCR. NG DNA was detected in 104 (38.9%) of the 267 participants. The detection of NG indicated by a positive result from urethral swab was 73 (27.3%, 95% CI 22.1 to 33.1) with negative results in 194 men (72.6%). The detection of NG with a positive result from urine specimens was 54 (20.2%, 95% CI 15.9 to 25.9) with a negative result in 213 men (79.4%). In 23 of the 267 participants (8.6%), both specimens were positive (agreement 69.66%, kappa 0.169). In addition, discrepancies were found in 80 of the 267 (29.9%) subjects, in which only one of the genital specimens had positive results.

Conclusion: The high discrepancy between positive results of gonorrhea between urethral swab and urine collection suggests that both of specimens should be combined for the highest detection rate of urethral gonorrhea in male population.

Keywords: Endourethral swab, Urine collection, Urethral gonorrhea, Men who have sex with men

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The Centers of Diseases Control and Prevention [CDC] recommends several annual screening tests, including urethral swab and/or urine collection DNA testing, for men who have sex with men [MSM] who have had insertive anal intercourse [IAI] or receptive anal intercourse [RAI], during the preceding year, whether they are symptomatic or not[1]. Asymptomatic gonorrhea in men is very common[2,3]. Urogenital gonorrhea of the male population can be diagnosed by test using both a traditional method with an endourethral swab, or a non-invasive method with urine collection using both culture and nucleic acid amplification testing [NAAT][4]. Many studies using the polymerase chain reaction [PCR] assay for gonorrhea detection among male showed 96.1% (95% CI 94.4 to 97.7) and 99.0% (95% CI 98.2 to 99.8) for urethral samples, while 90.4% (95% CI 87.9 to 92.9) and 99.7% (95% CI 99.4 to 100) for the alternative method of urine collection, as the pooled sensitivities and pooled specificities respectively[5-8]. However, no widely accepted guidelines exist for screening of gonorrheal infection to replace invasive screening with non-invasive screening. Furthermore, the guidelines have not specifically addressed the question of whether tests on non-invasively obtained samples are as accurate as those obtained by urethral swab samples among MSM presenting with no symptoms or with a culture-negative result[9].

More data are needed to clarify whether any of these specimens are effective in screening for gonorrhea in asymptomatic men. Therefore, the present study compared the diagnostic accuracy of endourethral swab and urine collection for the detection of urethral gonorrhea using the polymerase chain

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reaction (real-time PCR with TaqMan probes specific to pseudogene).

Materials and Methods

Study population

Between August and December 2015, a prospective study of MSM considered to be at high-risk for gonorrhea infection was initiated in a research clinic. MSMs aged 18 years or older were eligible if they reported having anal intercourse either IAI or RAI[10] in their lifetime, and did not take any antibiotics in the previous two weeks. Participants were categorized as symptomatic if they presented with one or more symptoms such as dysuria, urogenital bleeding, pelvic or genital pain, urethral discharge, genital lesions or warts, genital itching or rash, or urethritis. Participants not exhibiting any of these symptoms were classified as asymptomatic.

Recruitment

MSM were recruited from two walk-in clinics. The first clinic was a sexually transmitted diseases mobile clinic [STDs mobile clinic], and the second clinic was an antiretroviral clinic [ARV clinic]. They both were in Khon Kaen, in the northeast of Thailand. In addition, identification and recruitment data of participants were collected by the first author while approaching individuals via personal networks and at social venues. Recruitment activities encompassed a region of the Khon Kaen municipality area. Meetings were held with local M-REACH teams (a non-government organization supported by the collaboration between Thai and United State to prevent sexual infection diseases among MSM) to enlist support for the ongoing research and prevent misunderstanding in the study population. Verbal informed consent was obtained. The study protocol was approved by the Khon Kaen Hospital Ethics Committee for Human Research (KE57061) and the Khon Kaen University Ethics Committee for Human Research (HE571153). Two hundred sixty-seven MSM presenting to the STDs mobile clinic and the ARV clinic were entered into the study. The sample size was calculated as follows (assuming the prevalence of rectal gonorrhea infection in the MSM group is about 6.1% from Bangkok, Thailand 2013)[11] as follow:

$$n = \frac{Z^2 (P)(1 - P)}{d^2}$$

$$= \frac{(1.96)^2 (0.061)(1-0.061)}{0.0252}$$

Screening for gonorrhea

Specimen collection: Urethral swab: Discharge from the meatus is the preferred specimen for the detection of Neisseria gonorrhea [NG]. If there is no meatal exudate in the postpubertal male, an endourethral swab can be used for the detection of gonococci. To increase the chance of detecting the organisms, swab samples should be collected from participants who have not voided for at least two hours. The swabs are suitable for smear preparation, culturing on appropriate media, and then kept in an appropriate tube for NAATs testing.

Urine collection: Urine collection is one of the specimen types suitable for nucleic acid tests for diagnosing NG infections in males. Leak-proof containers were provided to participants for the collection of urine specimens. Ten to 30 ml of first-catch urine was also collected from each subject after the swab.

In each participant, sterile Dacron swabs were collected to conduct the respective tests or assays. An endourethral swab was taken by a physician gently passing each cotton tipped swab 1 to 4 cm inside the urethral meatus and rotating it by 360°. The swab was placed in two sucrose phosphate [SP] transport mediums, and then processed according to each laboratory's standard procedure.

For real-time PCR analysis, 2 µl of extracted DNA samples were performed. The TaqMan real-time PCR[12] reaction mixture contained variable amounts of total DNA, the forward primer, reverse primer, TaqMan® probe, and Taqman Universal PCR Master Mix. Forward primer was 5'-CAG CAT TCA ATT TGT TCC GAG TC-3', reverse primer was 5'-GAA CTG GTT TCA TCT GAT TAC TTT CCA-3', and the specific TaqMan® probe for NG detection was 5'-CGC CTA TAC GCC TGC TAC TTT CAC GC-3'. The thermal cycle conditions for TaqMan® assay were as follows: 10 minutes at 95°C, 10 seconds at 95°C, 30 seconds at 60°C and 10 seconds at 72°C, for 40 cycles. The amplification plot used to define the threshold cycle [Ct] for a sample. Gel electrophoresis was confirmed the real-time PCR product with 89 bp.

A diagnosis of urethral gonorrheal infection was made with either a positive urethral swab or a positive urine specimen or both, by real-time PCR with TaqMan probes specific to pseudogene. The results were considered concordant if both the endourethral swab and urine collection gave the same results by culture or gonococcal real-time PCR with TaqMan probes. When one of the diagnostic specimens was negative

and other positive, results were considered discrepant. Data cleaning, recoding, and analysis were performed using Stata (v.11; StataCorp, College Station, Texas). Descriptive data were present in frequency and percentage. The overall detection rates were calculated. Cohen's kappa was used to measure the agreement of two specimen tests.

Results

Table 1 indicated the characteristic of both negative and positive result of urethral gonorrhea, and positive results were categorized by subgroup of characters. Two hundred sixty-eight participants were enrolled in the present study. All of them had a urine collection taken but only 267 participants had

Table 1. Characteristics of MSM participants were tested for urethral gonorrhea infection (n = 268)

Characteristics	Negative (n = 163)	Positive (n = 105)	Positive (n = 105)			*p*-value
			Endourethral swab (n = 50)	Urine (n = 32)	Both (n = 23)	
Age (years)						0.981
18 to 24	60 (36.81)	36 (34.29)	17 (34.00)	11 (34.38)	8 (34.78)	
≥25	103 (63.19)	69 (65.71)	33 (66.00)	21 (65.63)	15 (65.22)	
Median (IQR)	27 (18, 60)	28 (18, 55)	27.5 (18, 51)	27 (18, 51)	30 (18, 55)	
Occupation						0.929
Employed	91 (55.83)	66 (62.86)	32 (64.00)	17 (53.13)	17 (73.91)	
Non-employed	72 (44.17)	39 (37.14)	18 (36.00)	15 (46.88)	6 (26.09)	
Payment for sex						0.377
No	106 (65.03)	73 (69.52)	31 (62.00)	27 (84.38)	15 (65.22)	
Yes	48 (29.45)	26 (24.76)	15 (30.00)	5 (15.63)	6 (26.09)	
Unknown	9 (5.52)	6 (5.71)	4 (8.00)	0 (0.00)	2 (8.70)	
Receipt for sex						0.689
No	39 (23.93)	21 (20.00)	9 (18.00)	6 (18.75)	6 (26.09)	
Yes	114 (69.94)	79 (75.24)	37 (74.00)	26 (81.25)	16 (69.57)	
Unknown	10 (6.13)	5 (4.76)	4 (8.00)	0 (0.00)	1 (4.35)	
HIV status						0.015
Negative	58 (35.58)	25 (23.08)	11 (22.00)	4 (12.50)	9 (39.13)	
Positive	57 (34.97)	48 (46.15)	18 (36.00)	19 (59.38)	11 (47.83)	
Unknown	48 (29.45)	32 (30.77)	21 (42.00)	9 (28.13)	3 (13.04)	
Number of partners in previous 3 months						0.338
No	64 (39.26)	37 (35.24)	20 (40.00)	12 (37.50)	5 (21.74)	
1 partner	52 (31.90)	39 (37.14)	21 (42.00)	10 (31.25)	8 (34.78)	
>1 partner	47 (28.83)	29 (27.12)	9 (18.00)	10 (31.25)	10 (43.48)	
Having soft tissue injury during sex						0.372
No	83 (50.92)	51 (48.57)	23 (46.00)	20 (62.50)	8 (34.78)	
Yes	27 (16.56)	16 (15.24)	6 (12.00)	4 (12.50)	6 (26.09)	
Unknown	53 (32.52)	38 (36.19)	21 (42.00)	8 (25.00)	9 (39.13)	
Alcohol before having sex						0.558
No	84 (51.53)	62 (59.04)	31 (62.00)	19 (59.38)	12 (52.17)	
Yes	79 (48.47)	43 (40.95)	19 (38.00)	13 (40.63)	11 (47.83)	
Illicit drug use before having sex						0.674
No	136 (83.44)	93 (88.57)	44 (88.00)	28 (87.50)	21 (91.30)	
Yes	27 (16.56)	12 (11.42)	6 (12.00)	4 (12.50)	2 (8.70)	
Previous diagnosed STDs						0.189
No	125 (76.69)	72 (68.57)	31 (62.00)	25 (78.13)	16 (69.57)	
Yes	38 (23.31)	33 (31.43)	19 (38.00)	7 (21.88)	7 (30.43)	
Condom use						0.144
No	23 (14.11)	25 (23.80)	14 (30.00)	7 (21.88)	4 (17.39)	
Yes	140 (85.89)	80 (76.19)	36 (72.00)	25 (78.13)	19 (82.61)	
Having gonorrhea symptoms* at least one day in past 3 months						0.312
No	154 (94.48)	99 (94.29)	45 (90.00)	31 (96.88)	23 (100)	
Yes	9 (5.52)	6 (5.71)	5 (10.00)	1 (3.13)	0 (0.00)	

MSM = men who have sex with men; IQR = interquartile range; HIV; STDs = sexually transmitted diseases

* Discharge in urethra and anus, dysuria, anal pruritus pruritus, or sore throat

the endourethral swab available for PCR because one MSM refused this diagnostic test. Most of positive results of urethral gonorrhea (n = 69, 65.71%) were older MSM aged more than 25, and urethral swab was the main positive test for gonorrhea (n = 33, 66.00%). Participants with positive results were employed (n = 66, 62.86%) and urethral swab was the most effective specimen diagnosed gonorrhea (n = 32, 64.00%). One-third of gonorrhea infected MSM informed that they had no payment for sex (n = 73, 69.52%), whereas, more than one-third indicated they were paid for sex (n = 79, 75.24%). Urethral swab was the main route to diagnose gonorrhea infection in both characteristics (n = 31, 62.00% and n = 37, 74.0%, respectively). Nearly half of urethral gonorrhea was HIV positive (n = 48; 46.15%) and urine was the main specimen to diagnose gonorrhea (n = 19; 59.38%). Surprisingly, having one partner in previous three months was the most common in gonorrhea infection (n = 39, 37.14%), and urethral swab was the main diagnostic specimen for this infection (n = 21, 42.0%).

Less than half (n = 51, 48.57%) of MSM with positive result of gonorrhea indicated they had no history of soft tissue injury during sex, and urethral swab was the most important specimen to diagnose urethral gonorrhea (n = 23, 46.00%). Among gonorrhea infection, more than half of them did not consume alcohol before sex (n = 61, 58.65%) or illicit drug before having sex (n = 92, 88.46%), and urethral swab was the main diagnosing specimen in this group (n = 31, 62.00% and n = 44, 88%, respectively). More than one third said that they used condom before having sex (n = 80, 76.92%), and as similar as other characteristics, urethral swab was the main diagnosing type of specimen in this subgroup (n = 36, 72%). Only six (5.71%) of 104 positive tests of gonorrhea said that they had gonorrhea symptoms and most of symptomatic MSM were tested positive by urethral swab (n = 5, 10.00%). In addition, only four (3.81%) of them stated that they had history of STDs.

The pattern of results obtained from the 267 patients are shown in Figure 1. NG DNA was detected in 104 (38.9%) out of the 267 patients. Twenty-three (8.6%) of these had concordant positive results (agreement 69.66%, kappa 0.169); however, the results were discrepant in 80 patients (29.9%).

In 267 participants, the detection of NG that indicated a positive result from urethral swab was 73 (27.3%, 95% CI 22.1 to 33.1) and a negative result was 194 (72.6%). While the detection of NG with a positive result from a urine specimen was 54 (20.2%, 95% CI 15.9 to 25.9) and negative result was 213 (79.4%). Out of the 267 patients enrolled in the present study, one was a partner (contact) of a gonorrhea positive patient. NG could be detected in 104 of these participants (38.9%). In all gonorrhea positive participants, we did not examine microscopic evidence of urethritis, and surprisingly there was no positive result detected by traditional culture.

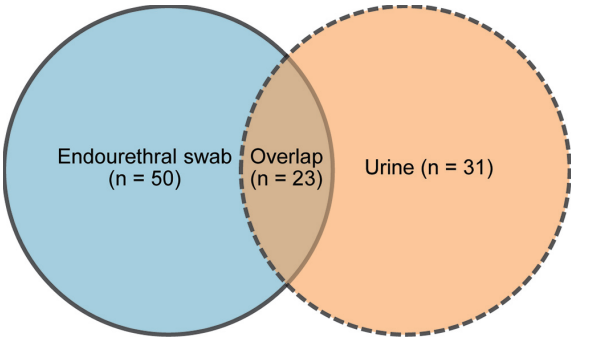

Figure 1. Positive results of NG detection in endourethral swabs and urine collection (n = 267).

Discussion

The main purpose of the current study was to evaluate the relative performance of endourethral swab and urine specimens for detection of NG in MSM. The number of participants studied had allowed for some detailed demographic description. For example, the age-specific detection rate of NG infection in high sexual activity population indicated slight differences between the distribution of urethral swab-positive and urine-positive men. However, from these two diagnostic specimens, older aged MSM was the major group of urethral infection (n = 33 of 50, and n = 21 of 32). This finding was similar as other study that indicated that older MSM was the main group of STDs infection[13]. The other characteristics indicated that urethral swab was the main specimen for diagnosis of urethral gonorrhea in men[14].

At first, we aimed to use two methods, traditional culture and real-time PCR with TaqMan probes, for the detection of gonorrhea. Surprisingly, there were no positive result from culture due to the small numbers of cell detected. This finding was concordant with a multicenter study among asymptomatic males that indicated low positive results by culturing (prevalence 1.6)[7]. Therefore, this traditional method could not be the gold standard for screening or even detecting gonorrhea in asymptomatic MSM. Real-time PCR with TaqMan probes can detect this infection even in low

level copies of bacterial (less than 103 copies/20 µl reaction) in asymptomatic participants[15] but there was not for the culture. As a result, we could not investigate the antibiotic sensitivities in the present study according to our plan. The low sensitivities of PCR and culture probably reflect the low bacterial burden in infected men who remain asymptomatic. When there are relatively few bacteria, it is more likely that an infected individual will test negative by culture and will have positive PCR results for only one specimen type. Thus, only a subset of infected individuals is detected as infected by any one test alone[7].

Males with asymptomatic urethritis are important reservoirs for transmission and increased risk for developing complications[16]. Therefore, a dual regimen that is a combination of ceftriazone 250 mg intramuscular [IM] as a single dose for gonorrhea and azitromycin 1 gram orally as single dose or doxycyclin 100 mg orally twice a day for seven days for treatment for chlamydial, co-infection of infected MSM[17] has been recommended, including those with low copies bacteria who are carriers, to prevent the spread of the infection[14]. Antibiotics can successfully cure gonorrhea in adolescents and adults. However, multidrug-resistant strains of gonorrhea are increasing globally[1]. According to the CDC report, there are two reasons for the likely increase in incidence. First, people may stay infected longer, which increases the chances of spreading. Second, and even more problematic, they noted that drug-resistant gonorrhea might have mutated to infect people even more easily[18].

The results from the present study suggest that when using real-time PCR with TaqMan probes, neither swab nor urine alone could represent all positives. Participants whose samples yield discrepant results, one negative and one positive for gonorrhea, may have a lower level of infection, which is near to the limit of detection by the test. Among the discrepant samples, the endourethral swab appears to be a significantly better diagnostic specimen than urine collection for detecting gonorrhea. However, the failure rate of gonorrhea detected by urethral specimen was 31/80 (38.7%). The urine specimen, which is alternatively used for the diagnosis of genital gonorrhea infection had failed to pick up gonorrhea in 49/80 (61.2%) of the discrepant specimens. This finding indicated that the urine specimen was found to be a less sensitive diagnostic specimen than urethral swab. There are two main reasons to explain this phenomenon. The first is NG tightly attaches with endourethral cell and it could still be appended to the human cell for a few minutes after urination. It is not completely washed out by urination, but might be temporarily removed by a urethral swab, just before the urine passing through the urethra. The second is, for the negative swab but positive urine collection, discrepancy might be caused by the location of infection, which is deeper inside the urethra than where the swab can reach (4 cm)[5].

In current clinical practice, we count on the endourethral swabs for the detection of gonorrhea and chlamydia. However, results from the present study indicate that urine collection could also be used for the diagnosis of these infections using DNA amplification methods[19]. In the present study, we found high discrepancy between swab PCR and urine PCR. If these specimens were used in combination, the detection rate in the population should be highest. Screening test by culture method may not be beneficial in this population or settings. Nowadays, the cost of real-time PCR with TaqMan probes is very expensive (1,200 baht per specimen), therefore, this method is not routinely used to screen gonorrhea infection in asymptomatic men.

Strength

The strength of our study is that it is the first study that included a large number of MSM to be screened for urethral gonorrhea using real-time PCR with TaqMan probes method. The population attending the two clinics are similar to many other clinic population, both in Thailand and in other countries, meaning that our findings are widely applicable.

Limitation

The limitations are that, although there were a large number of participants, this was a single center study. In all gonorrhea positive participants, we did not examine microscopic evidence of urethritis. Therefore, we could not conclude which cases had a disease of gonorrhea.

Conclusion

The high discrepancy between positive result of gonorrhea from urethral swab and urine collection suggests that both specimens should be combined for the highest detection rated of urethral gonorrhea in a male population.

What is already known on this topic?

The CDC recommends several annual screening tests including urethral swab and urine collection DNA testing for MSM who have had IAI or RAI, during

the preceding year, whether they are symptomatic or not. However, no widely accepted guidelines state that one should replace urethral swab with urine collection when screening for gonorrheal infection, and the guidelines have not specifically addressed the question of whether non-invasively obtained samples are as accurate as those obtained with urethral swab samples among MSM presenting with no symptoms or who have a culture-negative result.

What this study adds?

In this study, there was high discrepancy in terms of diagnostic performances for urethral gonorrhea between using endourethral swab and urine collection. Therefore, both endourethral swab and urine collection should be combined to get highest diagnostic performances for urethral gonorrhea in a male population

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Potential conflicts of interest

The authors declare no conflict of interest.

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