International Journal of Research in Medical Sciences
Nadeak K. Int J Res Med Sci. 2019 Jul;7(7):2547-2552
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20192625

Original Research Article

Diagnostic test of urine sample, vaginal smear and combination of urine with vaginal smear to identify *Neisseria gonorrhoeae* with polymerase chain reaction method

Kristina Nadeak*

Department of Dermatology and Venereology, Faculty of Medicine Universitas Sumatera Utara, H. Adam Malik General Hospital, Medan, Sumatera Utara, Indonesia

Received: 09 May 2019
Revised: 05 June 2019
Accepted: 10 June 2019

***Correspondence:**
Dr. Kristina Nadeak,
E-mail: kristinanadeak67@yahoo.com

ABSTRACT

Background: Gonorrhoea is a disease caused by *Neisseria gonorrhoeae* that is transmitted through sexual contact. There are several examinations performed on gonorrhoea infection, one of them is Polymerase Chain Reaction (PCR). The objective is to determine the diagnostic test of urine samples, vaginal smear and combination of urine and vaginal smear in identifying *Neisseria gonorrhoeae* using the PCR method.
Methods: This study is a diagnostic test with a cross-sectional design involving 58 female sex workers (FSW). All FSWs are carried out of history and physical examination. Urine sampling, vaginal smear, combination of urine and vaginal smear, and endocervical smear were obtained for identifying *Neisseria gonorrhoeae* using PCR method, then a diagnostic test analysis of each sample was performed.
Results: The diagnostic test of PCR for *Neisseria gonorrhoeae* from urine samples was found sensitivity 44.4%, specificity 20.0%, positive predictive value (PPV) 83.3%, negative predictive value (NPV) 3.8% and accuracy 42.0%. From vaginal smear, we obtained sensitivity 34.0%, specificity 66.7%, PPV 88.2%, NPV 12.1% and accuracy 38.0%. And from combination of urine and vaginal smear, we obtained sensitivity 51.1%, specificity 20.0%, PPV 85.2%, NPV 4.3% and accuracy 48.0%.
Conclusions: From these results the researchers suggested that urine, vaginal and combination of urine and vaginal smear could not be used as an alternative to examine the sensitivity and specificity of *Neisseria gonorrhoeae*, so the endocervical sample remained the reference sample for examination of nucleic acid amplification tests for *Neisseria gonorrhoeae.*

Keywords: Diagnostic tests, *Neisseria gonorrhoeae*, PCR

INTRODUCTION

Gonorrhoea is a disease caused by *Neisseria gonorrhoeae*. *Neisseria gonorrhoeae* is a gram-negative diplococcal bacteria that is transmitted through sexual contact. Until now, gonococcal infection is still a global health problem.[1,2]

The highest incidence is found in less developed countries. Adolescents and young adults are at high risk of this infection. Gonococcal infection causes increase local expression of viral RNA and loss of mucosal integrity, therefore it can cause an increased risk of type 1 HIV infection.[2]

Based on data from WHO in 2015, it was found that globally gonorrhoea in men was 25.5 per 100,000 adults.[1] In the United States, it is estimated that 820,000 new infections of *N. gonorrhoeae* occur annually.[3] Whereas in Indonesia, gonorrhoea in men is 7.7 cases per 100,000 adults.[1] Based on Survey Terpadu Biologis dan Perilaku (STBP) by the Ministry of Health in 2013, it was found that data on the prevalence of gonorrhoea and chlamydial infections in the population of female sex workers (FSWs) was 32%.[4]

Urethral infection by *N. gonorrhoeae* in men can cause symptoms that encourage them to get treatment before a sequel occurs but not nearly enough to prevent transmission to other people. Whereas, in women, most infections are asymptomatic until complications have occurred.[3] Complications that usually occur can be local or diseminated.[5] Early treatment of gonorrhoea can provide a good prognosis.[6] Therefore, good investigations are needed to detect gonorrhoea infection.

Specific diagnostic tests of *N. gonorrhoeae* should be performed on all people at risk or suspected of suffering from *gonorrhoea*. The right diagnosis can prevent complications, reinfection and transmission of *gonorrhoea*.[3] There are several tests that can be performed on *gonorrhoea* infection, including culture, microscopic examination and noncultural examination, such as the nucleic acid amplification test (NAAT). NAAT provides better specificity and sensitivity, so it is recommended for screening and diagnosis purposes.[3,7]

NAAT examination consists of polymerase chain reaction (PCR), transcription-mediated amplification (TMA), and other nucleic acid amplification technologies.[8] Polymerase Chain Reaction (PCR) is a method of multiplying DNA into thousands to millions of copies of certain DNA sequences.[9] Specimens for NAAT examination can be obtained from endocervical and vaginal swabs in women, urethral swabs in men and urine examination in both women and men.[3]

Based on the explanation above, the researchers wanted to conduct a study on the comparison of urine samples, vaginal smear, combination of urine and vaginal smear, and endocervical smear for identification of *Neisseria gonorrhoeae* with the PCR method in FSW.

METHODS

This study is a diagnostic test with a cross-sectional data collection method involving 58 female sex workers (FSWs) who were in voluntary counselling and testing (VCT) and in the work area of the exemplary health center in Medan, Petisah Health Center, Padang Bulan Health Center and Medan Helvethia Health Center in Medan. This research was conducted from July to September 2017. Each subject of research signed an informed consent was included in this study. Ethical permission was given by the research ethics committee for health at the University of North Sumatra, No. 410/TGL/KEPK FK USU-RSUP HAM/2017. All FSWs are carried out of history and physical examination. Urine sampling, vaginal smear, combination of urine and vaginal smear, and endocervical smear were obtained for identifying *Neisseria gonorrhoeae* using PCR method.

Data were analysed descriptively to determine the frequency distribution of the subjects based on the characteristics of age, education level, and length of time working as an FSW. In addition, diagnostic tests of urine samples, vaginal smears and a combination of urine and vaginal smears were compared with the endocervical smear as a standard sample recommended for diagnosing *Neisseria gonorrhoeae* using the PCR method.

RESULTS

In this study, it was found that based on the age, the youngest age of FSWs was 19 years old and the oldest was 52 years old, with an average age was 31.6 years old. Most of FSWs was in the age group of 25-29 years old and >34 years old (36.0%). Based on the education level, out of 50 FSWs, 33 women (66.0%) had education level of middle school, followed by high school as many as 11 women (22.0%) and primary school as many as 6 women (12.0%). Based on the length of time as an FSW, out of a total of 50 FSWs, 47 women (94.0%) have been an FSW for more than 12 months, followed by ≤12 months as many as 3 women (8.6%), as shown in Table 1.

Table 1: Characteristics of FSW based on age, education level and length of time working as an FSW.

Characteristics	n	%
Age (group)		
15-19	1	2.0
20-24	6	12.0
25-29	18	36.0
30-34	7	14.0
>34	18	36.0
Education level		
Primary school	6	12.0
Middle school	33	66.0
High school	11	22.0
Length of time working as an FSW		
≤ 12 months	3	6.0
> 12 months	47	94.0

In the urine samples, there were 24 samples positive for *Neisseria gonorrhoeae* (48.0%) and 26 samples negative (52.0%). In the vaginal smear, 17 samples (34.0%) were positive for *Neisseria gonorrhoeae* and 33 samples (66.0%) had negative result. The combination of urine and vaginal smear had 27 samples (54.0%) positive for *Neisseria gonorrhoeae* and 23 samples (46.0%) were negative. Whereas in the endocervical smear, there were

45 samples (90.0%) had positive results for *Neisseria gonorrhoeae* and 5 samples (10.0%) had negative results, as shown in Table 2.

Table 2: Results of PCR for *Neisseria gonorrhoeae*.

PCR	Positive	Negative	Total
	n (%)	n (%)	n (%)
Urine	24 (48.0)	26 (52.0)	50 (100.0)
Vaginal smear	17 (34.0)	33 (66.0)	50 (100.0)
Urine+ vaginal smear	27 (54.0)	23 (46.0)	50 (100.0)
Endocervical smear	45 (90.0)	5 (10.0)	50 (100.0)

From 50 samples, we got the positive results for urine and endocervix samples as many as 20 samples, positive in urine and negative in endocervix as many as 25 samples, negative in urine and positive in endocervix as many as 4 samples and negative results for urine and endocervix were found in 1 sample, as shown in Table 3.

Table 3: Results of PCR for *Neisseria gonorrhoeae* in urine samples.

PCR	Endocervix positive	Endocervix negative	Total
Urine Positive	20	4	24
Urine Negative	25	1	26
Total	45	5	50

In this study, from 50 samples, we found the positive results for vaginal and endocervical smear as many as 15 samples, positive in vaginal but negative in endocervical as many as 2 samples, negative in vaginal but positive in endocervical as many as 29 samples and negative results for both vaginal and endocervical samples as much as 4 samples, as shown in Table 4.

Table 4: Results of PCR for *Neisseria gonorrhoeae* in vaginal smear.

PCR	Endocervix positive	Endocervix negative	Total
Vaginal Smear Positive	15	2	17
Vaginal Smear Negative	29	4	33
Total	44	6	50

Authors got positive result for both combination of urine and vaginal samples and endocervix samples as many as 23 samples, positive result combination of urine and vaginal samples but negative in endocervix samples as many as 4 samples, negative result combination of urine and vaginal samples but positive in endocervix samples as many as 22 samples and negative results for both samples as many as 1 sample, as shown in Table 5.

Table 5. Results of PCR for Neisseria gonorrhoeae in combination of urine and vaginal smear.

PCR	Endocervix positive	Endocervix negative	Total
Urine+vaginal smear positive	23	4	27
Urine+vaginal smear negative	22	1	23
Total	45	5	50

Of the three samples compared with endocervical smears, a combination of urine and vaginal smear had the highest sensitivity level of 51.1%, with a low specificity value of 20.0%, a positive predictive value (PPV) of 85.2%, a negative predictive value (NPV) of 4.3% and accuracy 48.0%. Meanwhile from the urine sample the sensitivity value was 44.4%, specificity 20.0%, PPV 83.3%, NPV 3.8% and accuracy of 42.0%. as shown in Table 6.

Table 6. Diagnostic test results of urine samples, vaginal smears and a combination of urine samples and vaginal smears.

PCR	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Akurasi (%)
Urine	44.4	20.0	83.3	3.8	42.0
Vaginal smear	34.0	66.7	88.2	12.1	38.0
Urine+vaginal smear	51.1	20.0	85.2	4.3	48.0

DISCUSSION

In this study it was found that the youngest age of FSWs was 19 years old and the oldest was 52 years old with an average age of 31.6 years old. Most of the FSWs were in the age group of 25-29 years old and >34 years old with the amount of each equal to 18 women (36.0%). Pollet found in his study that the average age of most FSWs was 27 years old.[10] Silfanus et al, found in their study that most of the FSWs in Medan were in the age group of 25-29 years old with 67 women (27.0%).[11] Topik found in his study that most of the FSWs in Bandar Baru, Deli Serdang, were in the age group of 20-24 years old with 28 women (53.8%).[12]

It is known that there are risk factors based on demographic and behavioral situations associated with STIs, including those aged less than 21 years old (25 years old in some places), changing partners and FSWs. Although in this study the age distribution was not at the age of those who were at high risk of getting STIs but from their work as an FSW, they were at high risk in developing STI disease.[13]

Based on the level of education, most of the FSWs had middle school education that is 33 women (66.0%), followed by high school groups as many as 11 women (22.0%) and primary school groups as many as 6 women (12.0%). This result is in accordance with Sitorus' study which reported that most of FSWs in Bandar Baru, Deli Serdang area, had middle school level of education which was 10 women (50.0%).[14] Wahyudi reported that most of FSWs in Jakarta had low level of education, which was 37 women (67.27%).[15] Chen's study about the prevalence of FSWs in China found that most of them had middle school level of education, which was 2074 women (79.1%).[16]

So that it can be concluded that in this study most of the FSWs had a low education level which was 39 women (78.0%). The high number of workers with high school level of education in the community from data of Badan Pusat Statistik (BPS), followed by a lack of employment for women in that level of education, lead a high unemployment rate which causes economic difficulties.[17] It supported the higher rates of FSWs who had middle school level of education in this study.

Based on the length of time working as an FSW, from a total of 58 women, 54 women had been an FSW for more than 12 months (91.4%) followed by groups less than 12 months as many as 4 women (8.6%). The research conducted by Pollet regarding the prevalence of cervicitis among FSWs in Peru, stated that the average length of work as an FSW was between 24 to 36 months.[10] Topik in his study, which examined the proportion of FSWs co-infected with *Neisseria gonorhoeae* and *Chlamydia trachomatis* in Bandar Baru, Deli Serdang, had different results. He got the longest working time as an FSW was less than 12 months as many as 30 women (57.7%).[12]

According to WHO, it was known that the longer the woman worked as an FSW, the higher the level of exposure to STIs, but it was important to remember that sexual exposure which causes the occurrence of an FSW with STIs can occur at first sexual contact, if sexual partners suffer from STIs.[17]

In the urine sample there were 24 positive samples of *Neisseria gonorrhoeae* (48.0%) and 26 negative samples (52.0%). In the vaginal smear there were 17 positive samples (34.0%) of *Neisseria gonorrhoeae* and 41 samples with negative result (66.0%). The combination of urine and vaginal smear had 27 samples positive for *Neisseria gonorrhoeae* (54.0%) and 23 samples with negative results (46.0%), whereas in the endocervical smear there were 45 samples positive for *Neisseria gonorrhoeae* (90.0%) and 5 samples negative results (10.0%). Based on a study conducted by Shafer which examined 250 women aged 15-45 years old, from samples of endocervix, urine, vagina, a mixture of endocervix and urine, a mixture of endocervix and vagina, a mixture of vagina and urine. Positive results from samples of endocervix, urine, vagina were obtained respectively, 1.0% (17/1785), 0.6% (10/1727), 1.8% (31/1.7744), while there were no positive results in a mixture of endocervix and urine, a mixture of endocervix and vagina, a mixture of vagina and urine.[18]

From the results of the diagnostic test analysis in urine samples, urine sensitivity was 44.4%, specificity 20.0%, PPV 83.3%, NPV 3.8% and accuracy 42.0%. Based on research conducted by Gaydos et al, it was stated that the sensitivity level of examination of female urine samples with ABT m2000 technology, GP AC2 and BD Probe Tec were 93.8%, 84.4%, and 76.7% respectively. Meanwhile the level of specificity with the three technologies mentioned above is 99.7%, 99.6% and 95.6% respectively.[19] Compared with the above research, the results of the sensitivity of urine samples in this study showed lower results. From this result, urine samples have relatively low sensitivity and specificity with this PCR technique, so further research is needed using other NAAT methods to see the sensitivity and specificity of urine samples for the detection of *Neisseria gonorrhoeae*.

From the results of diagnostic test analysis in vaginal smear, the sensitivity was 34.0%, specificity 66.7%, PPV 88.2%, NPV 12.1% and accuracy 38.0%. A study conducted by Cosentino et al, on the relevance of nucleic acid amplification technology, Strand Displacement Amplification showed sensitivity, specificity, PPV and NPV of 100.0%, 99.8%, 97.5% and 100.0% respectively.[20] Compared with the study of Cosentino et al, the results of sensitivity of vaginal smear in our study showed lower results. From this result vaginal smear has a low sensitivity and moderate specificity so that vaginal smears cannot be used as a sample for identifying *Neisseria gonorrhoeae* with PCR method. This low yield can be caused by the tropism of *Neisseria gonorrhoeae* that is not in the vaginal mucosa which has squamous epithelium.

From the results of the diagnostic test analysis on the combination of urine and vaginal smear, the sensitivity was 51.1%, specificity 20.0%, PPV 85.2%, NPV 4.3% and accuracy 48.0%. According to Shafer's study, the sensitivity of a combination of urine and cervical smears was 49.0%, combination of endocervix and vaginal smear 93.0%, and combination of vaginal smear and urine 79.0%.[18] Compared with Shafer's study, in this study the results of sensitivity of combination of urine and vaginal smear showed lower results.

Of the three samples compared with the endocervical smear, combination of urine and vaginal smear had the highest sensitivity level of 51.1%, with a low specificity value of 20.0%, PPV of 85.2%, NPV of 4.3% and accuracy 49.0%. Meanwhile the urine sample in this study had low sensitivity and specificity, followed by a combination of urine and vaginal smear which also had low sensitivity and specificity. While vaginal smear samples had a low sensitivity (<75.0%) despite having moderate specificity.

CONCLUSION

From these results the researchers suggested that urine, vaginal and urine and vaginal blends could not be used as an alternative to examine the sensitivity and specificity of *Neisseria gonorrhoeae* because the three samples tended to have low and medium result. The endocervical sample remained a reference sample for examination of *Neisseria gonorrhoeae* by nucleic acid amplification test.

ACKNOWLEDGEMENTS

Authors would like to thank all patients in this study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee (410/TGL/KEPK FK USU-RSUP HAM/2017)

REFERENCES

1. World Health Organization. Report on global sexually transmitted infection surveillance 2015. 2016. Available at: https://www.who.int/medical_devices/diagnostics/selection_in-vitro/selection_in-vitro-meetings/00007_04_2015WHO_STI_surveillance_report.pdf?ua=1. Accessed 15 May 2019.
2. Edwards JL, Apicella MA. The molecular mechanisms used by Neisseria gonorrhoeae to initiate infection differ between men and women. Clin Microbiol Rev. 2004;17(4):965-81.
3. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64(3):60-8.
4. Nilasari H, Daili SF, Gonore. In: Daili SF, Nilasari H, Makes WIB, Zubier F, Romawi R, Pudjiati SR, eds. Infeksi menular seksual. 5th Ed. Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia;2017:75-83.
5. Sparling PF. Biology of Neisseria gonorrhoeae. In: Holmes KK, Sparling PF, Stamm WE, Piot P, Wasserheit JN, Corey L, et al. Sexually Transmitted Disease. 4th Ed. New York:McGraw Hill;2008:607-622.
6. Rosen T. *Gonorrhoea*, mycoplasma, and vaginosis. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DC, eds. Fitzpatrick's dermatology in general medicine. 8th Ed. New York:Mcgraw Hill companies;2012:2515-2519.
7. Walker CK, Sweet RL. *Gonorrhoea* infection in women: prevalence, effects, screening and management. Int J Women Health. 2011;3:197-206.
8. Hook EW, Handsfield HH. Gonococcal infections in the adult. In: Holmes KK, Sparling PF, Stamm WE, Piot P, Wasserheit JN, Corey L, Cohen MS, Watss DH, penyunting. Sexually Transmitted Disease. 4th Ed. New York:McGraw Hill;2008:607-622.
9. Joshi M, Deshpande JD. Polymerase chain reaction: methods, principles, and application. Int J Biomed Res. 2010;2(1):81-97.
10. Pollett S, Calderon M, Heitzinger K, Solari V, Montano SM, Zunt J. Prevalence and predictors of cervicitis in female sex workers in Peru: an observational study. BMC Infect Dis. 2013;13:195.
11. Silfanus FJ, Sedyaningsih ER, Daili SF, Tanudyaya FK, Anartati AS, Purnamawati KA, et al. Report on the results of research on the prevalence of reproductive tract infections in female sex workers in Medan, North Sumatra, Indonesia, 2005. Available at: http://docplayer.info/33645732-Laporan-hasil-penelitian.html. Accessed 3 June 2019.
12. Topik MM, Nadeak K, Rangkuti IF. Proportion and Characteristics of Servicitis Gonorrhea and Chlamydial Coinfection among Sexual Workers in Bandar Baru Localization in Deli Serdang Regency. Available at: https://jurnal.usu.ac.id/index.php/jms/article/download/21024/9180. Accessed 3 June 2019.
13. Daili SF. Gonorrhea. In: Daili SF, Makes WIB, Zubier, eds. Infeksi Menular seksual. 4th ed. Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia; 2011:65-72.
14. Sitorus. Effectiveness of Combipac Drug (Combination of Cefixime and Azithromycin Drugs) on Gonorrhea Servicitis and Suspect of Non-Specific Servicitis in Sexual Workers in the Bandar Baru Community Health Center Deli Serdang District. Available at: http://repository.usu.ac.id/bitstream/handle/123456789/57433/Cover.pdf?sequence=7&isAllowed=y. Accessed 3 June 2019.
15. Wahyudi DT, Pusponegoro EH, Daili SF. Sensitivity and Specificity of the Syndromic Management and Amount of Polymorphonuclear Cells (PMN) in Female Genital Chlamydia Trachomatis Infection Compared to Results of Examination of Poymerase Chain Reaction (PCR). Media Dermato-Venereologica Indonesiana. 2009;36(1):9-16.
16. Chen XS, Yin YP, Liang GJ, wang QQ, Jiang N, Liu Q, et al. The prevalence's of Neisseria gonorhoeae and Chlamydia trachomatis infections among female sex workers in China. BMC Public Health. 2013;13:121.
17. World Health Organization. Prevention and Treatment of HIV and other Sexually Transmitted

Infections for Sex Workers in Low-and Middle-income Countries. 2012. Available at: https://apps.who.int/iris/bitstream/handle/10665/77745/9789241504744_eng.pdf;jsessionid=865D08426D44DF6E9980788810FFAB76?sequence=1. Accessed 15 May 2019.
18. Shafer MA, Moncada J, Boyer CB, Betsinger K, Flinn SD, Schachter J, et al. Comparing First-Void Urine Specimens, Self-Collected Vagina and Endocervical Specimens To Detect Chlamydia trachomatis and Neisseria gonorrhoeae by a Nucleic Amplification Test. J Clin Microbiol. 2003;41(9):4395-9.
19. Gaydos CA, Cartwright CP, Colannino P, Welsch J, Holden J, Ho SY, et al. Performance of the Abott Real Time CT/NG for Detection of Chlamydia trachomatis and Neisseria gonorrhoeae. J Clin Microbiol. 2010;48(9):3236-43.
20. Cosentino LA, Landers DV, Hiller SL. Detection of Chlamydia trachomatis and Neisseria gonorrhoaeae by Strand Displacement Amplification and Relevance of the Amplification Control for Use With Vaginal Swab Specimens. J Clin Microbiol. 2003;41(8):3592-6.

Cite this article as: Nadeak K. Diagnostic test of urine sample, vaginal smear and combination of urine with vaginal smear to identify *Neisseria gonorrhoeae* with polymerase chain reaction method. Int J Res Med Sci 2019;7:2547-52.