Original Article

Prevalence of *Trichomonas vaginalis* by polymerase chain reaction-based molecular method among symptomatic women from Northern India

Rajneesh Dadwal, Nandita Sharma[1], Rimjhim Kanaujia[1], Sakshi Malhotra[2], Hemant Chaudhry[1], Shivali Rathore[1], Aastha Saini[3], Rashmi Bagga[4], Abhishek Mewara[5], Sumeeta Khurana[5], Rakesh Yadav[1], Sunil Sethi[1]
Clinical Microbiology and Bioactive Screening Laboratory, CSIR-IMTECH, Chandigarh, Departments of [1]Medical Microbiology, [3]Pulmonary Medicine, [4]OBS and Gynaecology and [5]Medical Parasitology, PGIMER, [2]AIIMS, New Delhi, India

Address for correspondence:
Dr. Sunil Sethi, Department of Medical Microbiology, PGIMER, Chandigarh - 160 012, India.
E-mail: sunilsethi10@hotmail.com

Abstract

Introduction: Trichomoniasis remains one of the most common sexually transmitted infections, which is curable. To prevent complications and transmission, prompt and correct diagnosis is essential to treat *Trichomonas vaginalis*. The present study was done to evaluate polymerase chain reaction (PCR) with other conventional techniques for the diagnosis of *T. vaginalis* infection and determine the prevalence of *T. vaginalis* in women with vaginal discharge based on PCR assay. **Methods:** Vaginal swabs were collected by the trained health-care professional using FLOQSwabs™ (Copan, Italy) during routine pelvic examinations among 1974 symptomatic females. The wet microscopy, culture, and PCR were performed. **Results:** The sensitivity of wet mount and culture in comparison to PCR was 60.87% and 56.52%, respectively. The kappa inter-rater agreement of *T. vaginalis* PCR showed substantial agreement with wet mount microscopy ($\kappa = 0.742$) and culture ($\kappa = 0.707$). The PCR detected an additional 17 cases that were missed by conventional techniques. **Discussion:** The study highlights the importance of PCR for *T. vaginalis* screening among symptomatic females.

Key words: Diagnosis, polymerase chain reaction, *Trichomonas vaginalis,* trichomoniasis

Introduction

Among common sexually transmitted infections (STI), trichomoniasis which is caused by the parasitic protozoan *Trichomonas vaginalis* remains one of the curable STIs. The estimates of the World Health Organization account for nearly half of all curable STIs as trichomoniasis.[1] Worldwide, the prevalence ranging from 1.3% to 16.5% has been reported.[2,3] According to the 2016 global prevalence data of *T. vaginalis*, an incidence of 156.0 million cases was reported. The adults in the age group of 15–49 years were most affected, with an incidence of 5.3% and 0.6% in females and males, respectively.

T. vaginalis infection is asymptomatic in 25%–50% of the cases.[4] However, symptomatic females present with clinical features such as copious purulent vaginal discharge, pruritis, burning micturition, dyspareunia, lower abdominal pain, and rarely with a punctate hemorrhagic lesion of the cervix known as "strawberry cervix." In males symptoms such as urethritis, prostatitis, balanitis, and epididymitis are seen. If untreated, it leads to complications such as infertility and pelvic inflammatory disease.[5,6] In pregnant females, adverse outcomes such as preterm rupture of membranes, preterm birth, and low birth weight babies are also described.[1] The association of *T. vaginalis* with other STIs and cervical neoplasia has been implicated. *T. vaginalis* infection causes changes in the local vaginal and cervical microbiome causing the accumulation of HIV-infected cells such as lymphocytes and monocytes[1] which leads to increased transmission of HIV.

To prevent complications and transmission, prompt and correct diagnosis is essential to treat *T. vaginalis*. The syndromic approach to treating STIs is limited by the fact that most of the patients are asymptomatic. Furthermore, the overlapping of clinical symptoms of *T. vaginalis* and other STIs makes a diagnosis based on clinical symptoms

Access this article online

Quick Response Code:

Website: www.ijstd.org

DOI: 10.4103/ijstd.ijstd_21_22

How to cite this article: Dadwal R, Sharma N, Kanaujia R, Malhotra S, Chaudhry H, Rathore S, *et al*. Prevalence of *Trichomonas vaginalis* by polymerase chain reaction-based molecular method among symptomatic women from Northern India. Indian J Sex Transm Dis 2023;44:40-4.

Submitted: 21-Feb-2022 **Revised:** 21-Jul-2022
Accepted: 04-Mar-2023 **Published:** 06-Jun-2023

unreliable. Traditionally, wet mount microscopy is used to diagnose trichomoniasis. The delay in sample transportation leads to low sensitivity and nearly 50% of the cases can be missed.[1] The culture techniques are more sensitive but are time-consuming, cumbersome, and require expensive culture media. In comparison to wet microscopy, nucleic acid amplification tests have shown higher sensitivity for *T. vaginalis* detection.[7]

In India, the prevalence has been evaluated based on only wet mount microscopy. The present study was done to evaluate polymerase chain reaction (PCR) with other conventional techniques for the diagnosis of *T. vaginalis* infection and determine the prevalence of *T. vaginalis* in women with vaginal discharge based on PCR assay.

Methods

Study design and recruitment

The present prospective study was conducted from January 2017 to December 2018 at the regional STI reference, research, and training center which caters to 14 peripheral centers in Chandigarh. A total of 1974 clinical samples were collected from females (16–65 years of age) with vaginitis. Females presenting with chief complaints of genitourinary symptoms such as discharge, burning micturition, itching, lower abdominal pain, and infertility were enrolled in this study. Pregnant and menstruating females were excluded from the study.

Vaginal swabs were collected by the trained health-care professional using FLOQSwabs™ (Copan, Italy) during routine pelvic examinations. Two swabs were obtained from each patient. The study was approved by the Institutional Ethics Committees, PGIMER, Chandigarh (Ethics approval no. INT/IEC/2019/00222,2), and informed consent from all participants was obtained.

Extraction of genomic DNA

One vaginal swab was used to prepare wet mount and culture inoculation. The wet mount was reported within 4 h of sample collection for the presence of motile trichomonads by the trained microbiologist. Additionally, a Gram stain was also prepared from the same swab for the presence of yeast and bacterial vaginosis (BV) (Nugent's scoring). The second swab was squeezed in 1 ml normal saline, and aliquots were made. These samples were used for DNA extraction, and the remaining aliquots were stored at −20°C.

DNA extraction and *Trichomonas vaginalis* conventional polymerase chain reaction

The DNA extraction was performed according to the previously published protocol by Ong and Rivera using Chelex 100 (Sigma-Aldrich, St Louis, MO, USA).[7] The tvk3 and tvk7 primers were used as the targets specifically to amplify a 261 bp sequence of the 18S SS-rRNA gene segments of *T. vaginalis*. The conventional PCR was performed as per the published protocol.[8]

Trichomonas vaginalis culture

Clinical samples of *T. vaginalis* were cultured in the Diamond's trypticase-yeast extract-maltose medium which was supplemented with 10% horse serum (Gibco, Grand Island, NY, USA), streptomycin (250 µg/mL), penicillin (250 U/mL), and amphotericin B (50 µg/mL) (Thermo Fisher Scientific, Waltham, MA, USA).

Data analysis and statistics

The data were entered into the Statistical Package for the Social Sciences (SPSS) 26.0 for Mac OS (SPSS, Inc., Chicago, IL, USA). Continuous variables were compared using the Mann–Whitney *U*-test or Student's *t*-test as appropriate. Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. $P < 0.05$ was used to analyze epidemiological data.

Results

Epidemiological characteristics of *Trichomonas vaginalis*

In total, 1974 samples from female patients were included in this study, with 65.2% (1325) and 31.9% (647) in the years 2017 and 2018, respectively. The mean age of the study population was 31.58 years (range: 16–65). The age distribution is described in Figure 1. The highest number of positive cases belonged to the age group of 31–40 years ($n = 23$) followed by 21–30 years ($n = 14$). Among all samples, 46 were *T. vaginalis*-positive samples, yielding a positive rate of 2.34%.

Overall, vaginal discharge (68.1%, $n = 1384$) was the most common clinical presentation. The presence of infertility, itching, and lower abdominal pain was present in 13.9% ($n = 282$), 3.6% ($n = 73$), 2.33% ($n = 47$), and 0.7% ($n = 15$) of the patients, respectively. Nearly 11.1% ($n = 225$) of the females were asymptomatic. Among 46 *T. vaginalis* PCR-positive patients, vaginal discharge was the most common clinical presentation (87%, $n = 40$). The presence of infertility and itching was seen in 2.2% ($n = 1$) of the patients. The presence of vaginal discharge was observed to be significantly associated with *T. vaginalis* infection [$P = 0.013$, Table 1] Concomitant *Neisseria gonorrhoeae* was present in 4.3% (2, $P = 0.026$) while BV was associated in 30.4% (14, $P = 0.000$). Concomitant syphilis was present in 6.5% ($n = 3$), and candidiasis was observed in 8.6% ($n = 4$) of the patients.

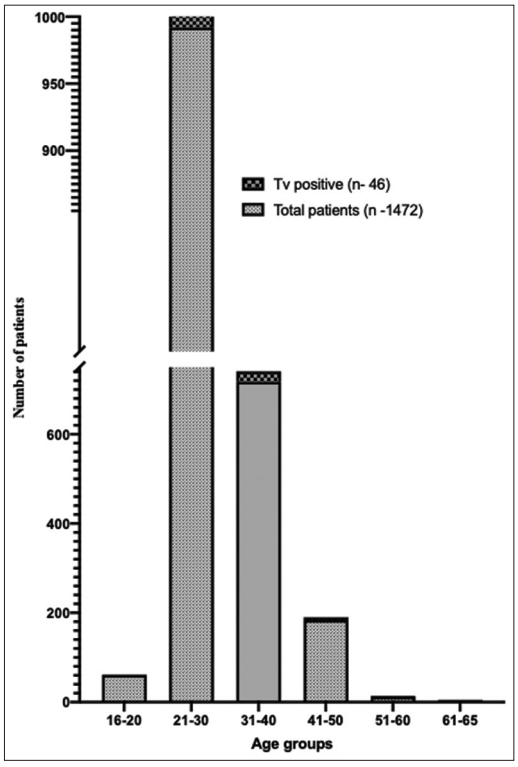

Figure 1: Age distribution of *Trichomonas vaginalis* infection among all patients

The sensitivity of wet mount and culture in comparison to PCR was 60.87% and 56.52%, respectively. The specificity of both wet mount and culture was >99%. The results of 28 microscopy-positive cases were observed to be nonconflicting to/with the results of PCR. In the 1946 microscopy-negative cases, 18 were found to be positive by PCR [Table 2]. The kappa inter-rater agreement of *T. vaginalis* PCR showed substantial agreement with wet mount microscopy (κ = 0.742) [Table 2]. The kappa inter-rater agreement of *T. vaginalis* PCR with culture was observed to be 0.707. The nomogram shown in Figure 2 displays the probability that a patient has a *T. vaginalis* infection after diagnosis by all three methods. The blue line indicates the positive likelihood ratio (LR+) for the positive test which was 0.39, 0.44, and 0.00 for wet mount, culture, and PCR, respectively. The red line indicates the negative LR for negative test results which was: >1000 for all methods.

Discussion

In the current study, *T. vaginalis* was detected more in the adult age group (21–40 years). This was similar to a study by Muthusamy *et al.*, where *T. vaginalis* were detected more frequently in females in the age group of 20–39 years.[9] The prevalence of trichomoniasis was found to be 2.34% using the PCR method as the gold standard. A similar burden (1.96%) of *T. vaginalis* was reported among sexually active symptomatic females.[10] Lower prevalence (1.96%–2.8%) comparable to the present study has also been reported from Bengaluru, Karnataka, and Gujarat.[10-12] However, higher prevalence ranging from 4.28% to 8.6% has also been reported in a few studies from Chandigarh, Punjab, Tamil Nadu, and Karnataka.[9,13-15] All these data display varying prevalence in both developing and developed countries. The use of different patient selection criteria and methods of trichomoniasis diagnosis can be the key difference in defining the prevalence in various studies. In countries where the incidence has decreased, increased awareness about STIs among people and prompt management in case of syndromic approaches may be accountable.

Table 1: Clinical complaints used in relation to polymerase chain reaction results

Symptoms	Negative (*n*=1928), *n* (%)	Positive (*n*=46), *n* (%)	*P*
Presence of discharge			
Yes	1344 (69.7)	40 (87.0)	0.013
No	584 (30.3)	6 (13.3)	
Asymptomatic/absence of discharge			
Yes	221 (11.5)	4 (8.7)	0.813
No	1707 (88.5)	42 (88.6)	
Burning micturition			
Yes	47 (2.4)	0	0.626
No	1881 (97.6)	46 (100)	
Itching			
Yes	72 (3.7)	1 (2.2)	1.000
No	1856 (96.3)	45 (97.8)	
Lower abdominal pain			
Yes	15 (0.8)	0	1.000
No	1913 (99.2)	46 (100)	
Infertility			
Yes	280 (14.5)	2 (4.3)	0.054
No	1648 (85.5)	44 (95.7)	

Table 2: The kappa inter-rater agreement of the diagnostic techniques

Diagnostic techniques	PCR		*P*	Kappa inter-rater agreement (k)
	Negative (*n*=1928)	Positive (*n*=46)		
Wet mount				
Negative	1927	18	0.000	0.742
Positive	1	28		
Culture				
Negative	1927	20	0.000	0.707
Positive	1	26		

PCR=Polymerase chain reaction

The presence of discharge was observed in 87.0% of the *T. vaginalis*-positive population and was found to be significantly associated with *T. vaginalis* patients ($P < 0.05$). Madhivanan *et al*. also reported abnormal vaginal discharge (37%) in most of the patients followed by genital itching and burning sensation in 18% and 16% of the females, respectively.[15]

The diagnosis of trichomoniasis should not be performed solely based on clinical symptoms due to overlapping presentation with other STIs. Furthermore, many patients with infection are asymptomatic and classical symptoms such as the strawberry cervix and discharge are present in 2% and 12% of the patients, respectively.[16] A wet mount is a simple and rapid method for trichomoniasis diagnosis. A minimum concentration of 10^4 organisms/ml in the sample is required for microscopic diagnosis. However, it should be performed within 10 min of the sample collection as the motility of the organism reduces with time.[14] In the present study, the microscopy (direct wet mount) and culture were positive in 1.46% (29/1974) and 1.31% (26/1974) of the patients, respectively. However, PCR detected additional 17 cases, increasing the positivity to 2.34%. The sensitivity of wet mount was observed to be 60% in the current study, which is comparable to other studies.[17-20]

Culture requires 300–500 viable organisms/ml in the sample and anaerobic condition for incubation for positivity. Media available for the cultivation of *T. vaginalis* are expensive and have a short shelf-life, and the technique itself is cumbersome. It requires days to weeks for a positive test result by culture. In a study by Cevahir *et al*., *T. vaginalis* infection was diagnosed in 10.5% and 11% of the females by culture in Diamond's medium and PCR, respectively.[19] Furthermore, the culture results require a minimum of 3–7 days. All these issues limit its use in resource-poor settings.

Thus, molecular techniques play a role in the diagnosis. A study from Belgium revealed comparable sensitivity and specificity of 88% and 97.3%, respectively, by PCR using the TVK3/TVK7 primer set as in the present study.[21] However, a decrease in the sensitivity, i.e., 63.9%, was noted when TVA5/TVA6 primer sets were used.[21] The TVK3 and TVK7 primers especially amplify a 300 bp sequence from the repetitive DNA in the *T. vaginalis* genome. A low level of extracted DNA and the presence of nonspecific inhibitors in the sample that could inhibit the amplification process resulting in a false-negative test are the limiting factors for PCR. In the current study, PCR positivity was higher (2.45%) as compared to culture or microscopy (1.96%). The ability of PCR to amplify nonviable or defective *T. vaginalis* could account for increased positivity as compared to culture or microscopy.[20,22,23]

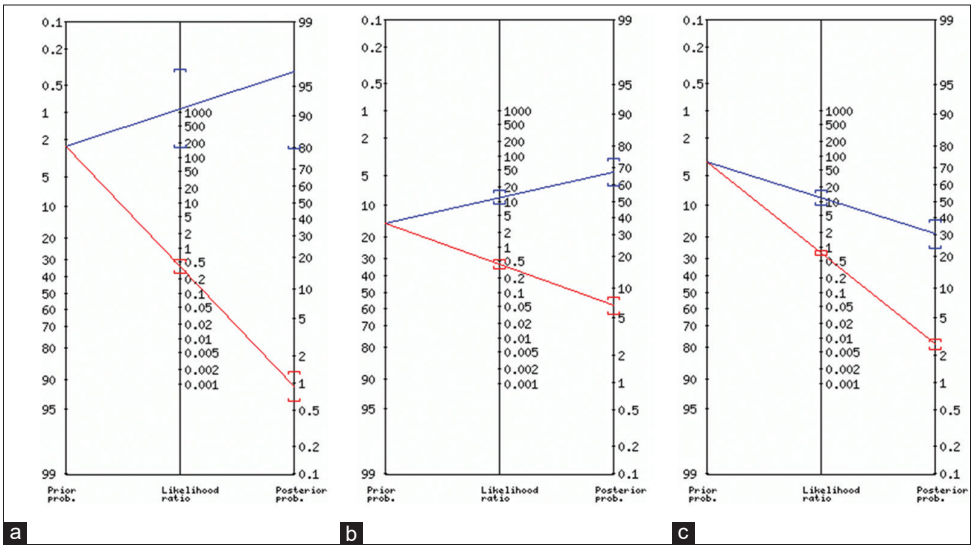

Figure 2: Nomogram of (a) wet mount; (b) culture; and (c) polymerase chain reaction

We observed a significant association of *T. vaginalis* infection in females with BV. This is crucial as females with BV are ignored in treatment guidelines. Madhivanan *et al*. also observed a significant association of BV (19.4% vs. 2.7%; $P < 0.0001$) with *T. vaginalis* infection.[15] In a recent meta-analysis, a nearly two-fold higher risk for acquiring *T. vaginalis* infection was observed in patients with BV infection.[24] *Lactobacillus* species produce substances with antimicrobial properties (hydrogen peroxide, lactic acid, and bacteriocin-like substances) that may play a key role in inhibiting the growth of cervicovaginal pathogens.[25] Bacteria frequently associated with BV produce sialidases and mucinases, damage genital epithelia, and disrupt innate immunity, compromising the physical and immune barriers to infection.[26]

Our study had few limitations. The present study determined the prevalence of *T. vaginalis* only in symptomatic patients. This does not estimate the true burden of *T. vaginalis* as approximately two-thirds of patients are known to be asymptomatic.[27]

The public health implication of *T. vaginalis* should be reconsidered as it is an easily treatable STI. The high-risk population should be focused, and strategies to test and treat these populations should be tailored. There is a need for further evaluation and implementation of molecular techniques to diagnose trichomoniasis, particularly in settings where young females seek health care.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Schwebke JR, Burgess D. Trichomoniasis. Clin Microbiol Rev 2004;17:794-803.
2. Anh PK, Khanh NT, Ha DT, Chien DT, Thuc PT, Luong PH, *et al.* Prevalence of lower genital tract infection among women attending maternal and child health and family planning clinics in Hanoi, Vietnam. Southeast Asian J Trop Med Public Health 2003;34:367-73.
3. Wilson C, Doniach S. A computer model to dynamically simulate protein folding: Studies with crambin. Proteins 1989;6:193-209.
4. Swygard H, Seña AC, Hobbs MM, Cohen MS. Trichomoniasis: Clinical manifestations, diagnosis and management. Sex Transm Infect 2004;80:91-5.
5. Viikki M, Pukkala E, Nieminen P, Hakama M. Gynaecological infections as risk determinants of subsequent cervical neoplasia. Acta Oncol 2000;39:71-5.
6. Sexually Transmitted Diseases Treatment Guidelines; 2015. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6403a1.htm. [Last accessed on 2023 Mar 10].
7. Ong VA, Rivera WL. Prevalence of *Trichomonas vaginalis* in vaginal swabs from sex workers in Angeles City, Pampanga, Philippines as detected by PCR. Trop Med Health 2010;38:29-34.
8. Kengne P, Veas F, Vidal N, Rey JL, Cuny G. *Trichomonas vaginalis*: Repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. Cell Mol Biol (Noisy-le-grand) 1994;40:819-31.
9. Muthusamy S, Elangovan S. A study on the prevalence of genital trichomoniasis among female outpatients attending sexually transmitted infection clinic in a tertiary care hospital. J Lab Physicians 2017;9:16-9.
10. Vijaya Mn D, Umashankar K, Sudha , Nagure AG, Kavitha G. Prevalence of the *Trichomonas vaginalis* infection in a tertiary care hospital in rural Bangalore, Southern India. J Clin Diagn Res 2013;7:1401-3.
11. Chauhan V, Shah M, Thakkar S, Patel SV, Marfatia Y. Sexually transmitted infections in women: A correlation of clinical and laboratory diagnosis in cases of vaginal discharge syndrome. Indian Dermatol Online J 2014;5:S1-5.
12. Singh S, Saha R, Suneja A, Das S. A hospital-based study on the prevalence of trichomoniasis and evaluation of accuracy of various diagnostic techniques. Trop Parasitol 2020;10:124-9.
13. Malla N, Kaur S, Khurana S, Bagga R, Wanchu A. Trichomoniasis among women in North India: A hospital based study. Indian J Sex Transm Dis AIDS 2008;29:76.
14. Bansal R, Jindal N, Chand B. Clinical and laboratory evidence of *Trichomonas vaginalis* infection among women of reproductive age in rural area of Malwa region of Punjab. Indian J Med Microbiol 2013;31:416-7.
15. Madhivanan P, Bartman MT, Pasutti L, Krupp K, Arun A, Reingold AL, *et al.* Prevalence of *Trichomonas vaginalis* infection among young reproductive age women in India: Implications for treatment and prevention. Sex Health 2009;6:339-44.
16. Vatanshenassan M, Rezaie S, Mohebali M, Niromand N, Kazemi B, Babaei Z, *et al. Trichomonas vaginalis*: Investigation of a novel diagnostic method in urine samples using cysteine proteinase 4 gene and PCR technique. Exp Parasitol 2010;126:187-90.
17. Patil MJ, Nagamoti JM, Metgud SC. Diagnosis of *Trichomonas vaginalis* from vaginal specimens by wet mount microscopy, in pouch TV culture

system, and PCR. J Glob Infect Dis 2012;4:22-5.
18. Khatoon R, Jahan N, Ahmad S, Khan HM, Rabbani T. Comparison of four diagnostic techniques for detection of *Trichomonas vaginalis* infection in females attending tertiary care hospital of North India. Indian J Pathol Microbiol 2015;58:36-9.
19. Cevahir N, Kaleli I, Kaleli B. Evaluation of direct microscopic examination, acridine orange staining and culture methods for studies of *Trichomonas vaginalis* in vaginal discharge specimens. Mikrobiyol Bul 2002;36:329-35.
20. Radonjic IV, Dzamic AM, Mitrovic SM, Arsic Arsenijevic VS, Popadic DM, Kranjcic Zec IF. Diagnosis of *Trichomonas vaginalis* infection: The sensitivities and specificities of microscopy, culture and PCR assay. Eur J Obstet Gynecol Reprod Biol 2006;126:116-20.
21. Crucitti T, Van Dyck E, Tehe A, Abdellati S, Vuylsteke B, Buve A, *et al.* Comparison of culture and different PCR assays for detection of *Trichomonas vaginalis* in self collected vaginal swab specimens. Sex Transm Infect 2003;79:393-8.
22. Caliendo AM, Jordan JA, Green AM, Ingersoll J, Diclemente RJ, Wingood GM. Real-time PCR improves detection of *Trichomonas vaginalis* infection compared with culture using self-collected vaginal swabs. Infect Dis Obstet Gynecol 2005;13:145-50.
23. Wendel KA, Erbelding EJ, Gaydos CA, Rompalo AM. *Trichomonas vaginalis* polymerase chain reaction compared with standard diagnostic and therapeutic protocols for detection and treatment of vaginal trichomoniasis. Clin Infect Dis 2002;35:576-80.
24. Seña AC, Goldstein LA, Ramirez G, Parish AJ, McClelland RS. Bacterial Vaginosis and its association with incident *Trichomonas vaginalis* infections: A systematic review and meta-analysis. Sex Transm Dis 2021;48:e192-201.
25. Borges S, Silva J, Teixeira P. The role of lactobacilli and probiotics in maintaining vaginal health. Arch Gynecol Obstet 2014;289:479-89.
26. Borgdorff H, Gautam R, Armstrong SD, Xia D, Ndayisaba GF, van Teijlingen NH, *et al.* Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier. Mucosal Immunol 2016;9:621-33.
27. García PJ, Chavez S, Feringa B, Chiappe M, Li W, Jansen KU, *et al.* Reproductive tract infections in rural women from the highlands, jungle, and coastal regions of Peru. Bull World Health Organ 2004;82:483-92.