Trichomonas vaginalis is Highly Prevalent in Adolescent Girls, Pregnant Women, and Commercial Sex Workers in Ndola, Zambia

Tania Crucitti, PHD, Vicky Jespers, MD,* Chanda Mulenga, MSc,† Shepherd Khondowe, MPH,† Judith Vandepitte, MD,* and Anne Buvé, PHD**

Objectives: The aim of the study was to assess the prevalence of *Trichomonas* sp. infection among adolescent girls, pregnant women, and commercial sex workers in Ndola, Zambia.

Methods: A cross-sectional study was conducted among 460 girls attending school, 307 pregnant women, and 197 commercial sex workers. Self-collected specimens from the vagina, rectum, and mouth were tested by polymerase chain amplification assays for the presence of *Trichomonas vaginalis*, *Pentatrichomonas hominis*, and *Trichomonas tenax*. Genotyping was performed on specimens that tested positive for *T. vaginalis*.

Results: The prevalence of vaginal infection with *T. vaginalis* was 24.6% among the adolescents, 32.2% among the pregnant women, and 33.2% among the commercial sex workers. Trichomonads other than *T. vaginalis* were rarely found in the vagina, rectum, and mouth. The presence of *T. vaginalis* in the rectum was associated with *T. vaginalis* in the vagina. *T. tenax* was also detected in the vagina. A total of 9 actin genotypes of *T. vaginalis* were identified. The distribution of the actin genotypes of *T. vaginalis* was similar in the 3 study groups.

Conclusion: We detected high prevalence rates of trichomoniasis among women in Ndola, Zambia. Prevalence among adolescent girls was lower than among pregnant women and commercial sex workers but was still high. We were not able to detect differences in *T. vaginalis* actin genotypes among the 3 study groups.

Trichomoniasis is the most common curable sexually transmitted infection (STI) worldwide.[1] The infection is frequently asymptomatic in both women and men. When symptomatic, it can cause vaginitis, urethritis, and cervicitis in women and urethritis in men.[2,3] There is growing evidence that *Trichomonas vaginalis* infection of the vagina is associated with an increased risk of acquisition and transmission of HIV infection.[4–10] Moreover, trichomoniasis has been implicated in adverse pregnancy outcome.[11]

Although infection rates in sub-Saharan African women are estimated to be among the highest in the world, clinical research on *T. vaginalis* infection in Africa has not received the same attention as other STI such as gonorrhoea and syphilis. Published data on the prevalence and incidence of trichomoniasis in Africa are limited, but it appears that there are large variations in prevalence between different regions in sub-Saharan Africa. For instance, in Benin, the prevalence of trichomoniasis in women in the general population was 3.2%, whereas it was 47% in Uganda.[12,13]

In the multicentre study on factors determining the differential spread of HIV in 4 African cities, including Cotonou (Benin), Yaoundé (Cameroon), Kisumu (Kenya), and Ndola (Zambia), a high prevalence (34.3%) of *T. vaginalis* infection was found among women in the general population in Ndola.[12,14] This finding was in line with the findings of a study performed 20 years earlier in Zambia, where 38.5% of antenatal clinic attendees and 31.4% of women at a gynecology clinic were infected with *T. vaginalis*.[15] In addition, both studies found a high prevalence of *T. vaginalis* infection in young girls. Hira found prevalence rates of 4.7% to 8.5% in girls younger than 15 years old, and Buvé et al found that 40% of young women who denied that they had ever had sexual intercourse had trichomoniasis, as assessed by culture.[14,15] This latter finding suggests that a certain proportion of women may have acquired *T. vaginalis* infection through transmission routes that do not involve penetrative sexual intercourse. Another possible explanation is that the intestinal flagellated parasite *Pentatrichomonas hominis*, which is morphologically very close to *T. vaginalis*, infects the vagina by cross contamination from the rectum.[14,15]

The aim of the current study was to assess the prevalence of infection with *T. vaginalis* and human trichomonads in 3 female populations with different sexual behavior patterns, in Ndola, Zambia.

MATERIALS AND METHODS

Study Design and Procedures

The study took place in Ndola in selected administrative areas that represent the different socioeconomic strata of town. We included in the study adolescent girls attending school, pregnant women attending antenatal clinic, and commercial sex workers (CSW). Adolescent girls aged between 13 and 16 years were recruited at school, and were not menstruating at the time of specimen collection. Informed consent was provided by a

From the *Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium; and †Department of Microbiology, Tropical Diseases Research Centre, Ndola, Zambia.

The authors thank all participants for their willingness to participate in this study. The authors are indebted to all recruiting staff from the TDRC who walked long distances day and night they also thank the laboratory staff and in particular Ireen Nyoni from the TDRC laboratory, and Said Abdellati, Vicky Cuylaerts, Bénédicte De Deken, and Hilde Smet from the ITM laboratory. They are also grateful to David Mwakazanga, the field and data entry supervisor at TDRC and they also thank J. Kulda (Charles university, Prague) for so generously providing the Trichomonad strains.

Supported by the Directorate General for Development Cooperation, Belgian Government (AIDS Impuls Fonds n°904100).

Correspondence: Tania Crucitti, PhD, HIV/STI Reference Laboratory, Institute of Tropical Medicine, 155 Nationalestraat, 2000 Antwerpen-Belgium. E-mail: trcucitti@itg.be.

Received for publication May 27, 2009, and accepted September 18, 2009.

DOI: 10.1097/OLQ.0b013e3181c21f93

parent or legal guardian and assent was given by the girl herself. CSWs were self acknowledged sex workers and were recruited at their places of work or another convenient location. They took part in the study after giving their informed consent, and provided they did not menstruate at the time of specimen collection. Pregnant women were recruited at antenatal clinics in Ndola.

All women were interviewed about their sociodemographic characteristics, sexual behavior, and genital hygiene practices. Interviews were conducted face-to-face by trained interviewers. After the interview, the women were instructed on how to collect a sample of the vagina, the rectum, and the mouth. EZ culturette swabs (Becton Dickinson BBL, Sparks, MD) were used and after sampling were kept in a cooler box until transport to the laboratory of Tropical Diseases Research Centre (TDRC).

Laboratory Methods

The self-administered samples were stored at the TDRC, at −20°C until shipment on dry ice to the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. The specimens were tested for *T. vaginalis*, *P. hominis*, and *Trichomonas tenax* by polymerase chain reaction assay (PCR). DNA was extracted from the clinical specimens using the QIAamp DNA minikit (Qiagen, Hilden, Germany). We used the TVK3/7 primer set, which targets a repetitive DNA fragment of *T. vaginalis*, and the PT3/7 and Th3/5 primers sets, to amplify a sequence of the 18SrRNA gene of *T. tenax* and *P. hominis*, respectively.[16–18] Positive results for *T. vaginalis* were confirmed using the IP1/IP2 primer set targeting an E 650 family repeat.[19] Positive results for *T. tenax* and *P. hominis* were confirmed through amplification of the internal transcribed spacer region of the 5.8S rRNA gene using the TFR1/2 primer set.[20]

T. vaginalis amplicons were detected using an enzyme immunoassay (EIA); the amplicons of *T. tenax* and *P. hominis* were detected using agarose gelelectrophoresis. The PCR testing procedures for *T. vaginalis* and *P. hominis* were described previously.[18,21] For the detection of *T. tenax* the assay was performed as described by Kikuta et al. [17] The specificity of the above listed primer sets, with the exception of the TFR1/2 primer set, was evaluated using reference strains summarized in Table 1. The TFR1/2 primer set amplifies *T. vaginalis*, *T. tenax,* and *P. hominis*. The amplicons of both *T. vaginalis* and *T. tenax* have the same size of 368 bp, but they differ in nucleotide sequence with 25 different nucleotides. The amplicon of *P. hominis* is shorter, 339 bp. The other primer sets were species specific.

Inhibition and adequacy of the vaginal and oral specimens was assessed by amplification of the β_2 microglobulin gene.[22,23] Adequacy of the rectal specimens was assessed by amplification of *Escherichia coli*.[24]

Strain typing of *T. vaginalis* was performed on clinical specimens with a positive TVK3/7 amplification result. A previously published PCR-restriction fragment length polymorphism method was applied.[25] Briefly, the actin gene target was amplified by a nested PCR, with outer primers Tv8S/Tv9R and inner primers Tv10S/Tv11R. The primers were synthesized by Eurogentec, Seraing, Belgium. Upon completion of PCR, 15 μL of each amplified specimen was analyzed by electrophoresis in a 2% agarose gel in Tris-acetate-EDTA buffer (pH 8.5). After visualization of the amplified product, 5 μL was digested for 4 hours at 37°C with restriction endonucleases *Hind*II, *Mse*I, and *Rsa*I, respectively. The restriction endonucleases *Hind II* and *Rsa I* were purchased from Roche Molecular Biochemicals, Mannheim, Germany. The restriction endonuclease *Mse*I was purchased from New England BioLabs Inc., Ipswich, MA.

TABLE 1. Strains Used for the Determination of the Analytical Specificity of the Primer Pairs

Species	Origin
Trichomonas vaginalis	ATCC 30001, ATCC 30240, ATCC 50141, ATCC 50144
Trichomonas gallinae	ATCC 30002
Tritrichomonas suis	ATCC 30169
Trichomonas tenax	ATCC 30207
Pentatrichomonas hominis	ATCC 30000, ATCC 30098
P. hominis	PHKT, received from J. Kulda (Charles University, Prague)
T. vaginalis	Tv 17–48, received from J. Kulda (Charles University, Prague)
Tritrichomonas foetus	KVCL, received from J. Kulda (Charles University, Prague)
T. gallinae	TGK, received from J. Kulda (Charles University, Prague)
Tetratrichomonas gallinarum	1–11 M2, received from J. Kulda (Charles University, Prague)

The strains with origin ATCC were obtained from the American Type Culture Collection.

Separation of the fragments was performed using 3% agarose gel in Tris-acetate-EDTA buffer (pH 8.5). The gel was stained with ethidium bromide 0.5 μg/mL (Sigma, Bornem, Belgium) and was photographed under short ultraviolet light. The sizes of the amplified products were assessed by comparing with a 100 bp commercial weight marker Smartladder SF (Eurogentec, Seraing, Belgium). The digestion of the amplified product with the restriction enzymes yielded distinct DNA fragments. Actin genotypes were assigned by combining the DNA fragments.

Each test run included 1 negative and 1 positive control. The negative control consisted of the reaction mixture with water instead of DNA extract, the positive control was a previously typed *T. vaginalis* ATCC reference strain, randomly selected from the ATCC reference strains at each run. Testing was considered valid if after nested PCR no amplified product was detected in the negative control and if after digestion the reference ATCC strain gave the previously determined actin genotype.

All assays were performed according to standard quality assurance guidelines for molecular diagnosis.[26,27]

Data Analysis

A sample was considered positive for DNA of *T. vaginalis*, *T. tenax*, or *P. hominis* if at least 2 different PCR assays with different targets were positive for respectively *T. vaginalis*, *T. tenax*, or *P. hominis*.

Prevalence rates are presented with 95% confidence intervals. Differences in prevalence were tested on their statistical significance using the chi-squared test for proportions.

Ethics

The study was approved by the Ethics Committees of the TDRC in Ndola and the ITM in Antwerp.

TABLE 2. Rates of *Trichomonas vaginalis*, *Trichomonas tenax,* and *Pentatrichomonas hominis* in Vaginal, Oral, and Rectal Specimens

	Vaginal Specimens			Oral Specimens			Rectal Specimens		
	ado	anc	csw	ado	anc	csw	ado	anc	csw
No. specimens	445	307	195	459	306	195	448	307	197
No. inadequate specimens (%)	6 (1.3)	0	5 (2.6)	1 (0.2)	3 (1.0)	7 (3.6)	132 (29.5)	81 (26.4)	53 (26.9)
T. vaginalis (%*)	108 (24.6)	99 (32.2)	63 (33.2)	0	2 (0.7)	2 (1.1)	4 (1.3)	5 (2.2)	1 (0.7)
T. tenax (%*)	3 (0.7)	1 (0.3)	0	0	16 (5.3)	8 (4.3)	0	0	0
P. hominis (%*)	0	0	0	0	0	0	1 (0.3)	2 (0.9)	0

ado indicates adolescents; anc, antenatal clinic attendees; csw, commercial sex workers; (%*), data are expressed as % of adequate specimens.
Vaginal and oral inadequate specimens: lack of amplification of the β_2 microglobulin gene.
Rectal inadequate specimens: lack of amplification of *E. coli.*

RESULTS

A total of 460 adolescent girls, 307 pregnant women, and 197 CSW gave their informed consent and were included in the study. The adolescent girls were 13 to 16 years old, one-third of them being 13 years old. The age range for the pregnant women was 15 to 42 years and for the CSW 14 to 45 years. Of the pregnant women, 34.2% were aged 20 to 24 years; among the CSW this percentage was 45.7%. Laboratory results were available for 936 (97%) of the 964 participating women. For the remaining 28 women, the laboratory results were invalid, mostly due to inadequate or missing specimen.

T. vaginalis was found in vaginal specimens of 108 of 439 adolescent girls (24.6%, 95% confidence interval [CI]: 20.6–28.6); 99 of 307 pregnant women (32.2%, 95% CI: 27.0–37.4) and 63 of 190 CSW (33.2%, 95% CI: 26.5–39.9) (Table 2). Prevalence was lower in adolescent girls than in pregnant women and CSW and this difference was statistically significant ($\chi^2 = 7.31$ on 2 *df*, $P = 0.026$). Among adolescent girls who reported that they had ever had penetrative sex the prevalence of *T. vaginalis* infection was 35.6% (21/59, 95% CI: 23.6–49.1); among adolescents who denied that they ever had sexual intercourse the prevalence was 22.9% (87/380, 95% CI: 18.8–27.5). Table 3 summarizes the prevalence rates of vaginal trichomoniasis found in adolescents by age and reported sexual activity.

T. vaginalis was also found in the mouth and the rectum (Table 2). The presence of *T. vaginalis* DNA in the rectum was strongly associated with the presence of *T. vaginalis* DNA in the vagina (odds ratio: 18.2, 95% CI: 4.0–82.5, $P < 0.001$).

TABLE 3. Prevalence Rates of Vaginal Trichomoniasis Among the Adolescent Girls

			Prevalence of Vaginal *T. vaginalis*	
Age	No. Participants	Prevalence of Vaginal *T. vaginalis*	Had Ever Had Penetrative Sex N = 59	Had Never Had Penetrative Sex N = 380
13	157	19.1%	30% (3/10)	18.4% (27/147)
14	109	26.6%	10% (1/10)	28.3% (28/99)
15	75	32%	38.5% (5/13)	30.6% (19/62)
16	98	25.5%	46.1% (12/26)	18.1% (13/72)

We had specimens from the 3 collection sites from 223 pregnant women. Of the 65 women who had *T. vaginalis* DNA in the vagina, 1 also had *T. vaginalis* DNA in the mouth. None of the women had *T. vaginalis* DNA in the mouth alone.

T. tenax DNA was detected in samples from the vagina and from the mouth. Two of the 4 women who harbored *T. tenax* DNA in the vagina also had *T. vaginalis* DNA in the vagina. Among 24 women with detectable *T. tenax* DNA in the mouth, none had *T. vaginalis* DNA in the mouth, but 6 had *T. vaginalis* DNA in the vagina. *P. hominis* DNA was detected in rectal specimens of 3 participants, 1 of whom also had *T. vaginalis* DNA in the vagina.

Out of 270 vaginal samples with *T. vaginalis* DNA, 231 (85.6%) were successfully genotyped, but in only 2 out of 15 positive samples from the mouth and the rectum genotyping was successful. Nine different actin genotypes of *T. vaginalis* in the vagina were identified in the 3 study groups. The distribution of the genotypes identified in the vagina was very similar in the 3 groups of women (Table 4). The most common genotypes were G, H, and P, making up 74.5% of trichomonas infections. Genotype B was detected in 1 adolescent girl and genotype K in 1 pregnant woman. Genotype R was not detected in the adolescents and genotype I was not identified in the CSW.

TABLE 4. Distribution of the *Trichomonas vaginalis* Actin Genotypes Among the 3 Study Groups

Genotype	Adolescents N = 96 (%)	Antenatal Clinic Attendees N = 85 (%)	Commercial Sex Workers N = 50 (%)
B	1 (1.0)	0	0
E	6 (6.3)	6 (7.1)	2 (4.0)
G	39 (40.6)	36 (42.4)	21 (42.0)
H	18 (18.8)	10 (11.8)	11 (22.0)
I	5 (5.2)	8 (9.4)	0
K	0	1 (1.2)	0
N	4 (4.2)	3 (3.5)	3 (6.0)
P	18 (18.8)	12 (14.1)	7 (14.0)
R	0	1 (1.2)	3 (6.0)
Mixed	5 (5.2)	8 (9.4)	3 (6.0)

Mixed: an isolate was considered to be a mixture of *T. vaginalis* strains if the total sum of the different fragments obtained with 1 specific restriction enzyme was superior to the target size of 1100 bp.

DISCUSSION

Using PCR assays, we found high prevalence rates of *T. vaginalis* infection in 3 groups of women in Ndola, Zambia, with different levels of sexual activity. We found no difference in prevalence between CSW and pregnant women. The prevalence in adolescent girls was lower, but was still substantial as nearly 1 in 4 girls aged 13 to 16 years was found to have *T. vaginalis* infection.

In the multicentre study, on factors determining the differential spread of HIV in 4 African cities, which was conducted in 1997–1998, the prevalence of trichomoniasis was 42% among CSW in Ndola, compared to 33.2% in this study.[28] A decrease in prevalence of *T. vaginalis* infection in CSWs was also observed in Kinshasa (Democratic Republic of Congo), Cotonou (Benin), Abidjan (Ivory Coast), and in Bobo-Dioulasso (Burkina Faso).[29–32] These changes in prevalence may be attributed to changes in sexual behavior of the study populations, in particular increases in condom use, and improved STI case management.[29]

We had postulated that a certain proportion of cases of vaginal trichomoniasis, especially in adolescent girls who denied that they had ever had sexual intercourse, are in fact caused by *P. hominis*, a human trichomonad that is found in the large intestine and that is morphologically similar to *T. vaginalis*.[14] We did not find evidence for the colonization of the vagina by *P. hominis* and our study confirms the conclusions of Adu-Sarkodie et al that *P. hominis* is not involved in the etiology of vaginal trichomoniasis.[33]

Inoculation studies conducted in the 1940s concluded that the trichomonads are body site specific and can not survive outside their natural habitat.[34,35] We detected DNA of *T. tenax*, a trichomonad that is normally found in the oral cavity, in 4 vaginal specimens. As we did not culture *T. tenax*, we are unsure whether the DNA that was extracted was from viable organisms. We postulate that the vagina was infected with this oral trichomonad through autoinoculation via hands or through oral sex.

We also found *T. vaginalis* DNA outside the vagina. The presence of *T. vaginalis* in the rectum was associated with the presence of *T. vaginalis* in the vagina. The rectum could have been infected through cross contamination with *T. vaginalis* present in the vagina. Besides, the rectum could act as a source for *T. vaginalis*, from where the vagina could almost constantly be reinfected. In order to test the latter hypothesis, we analyzed 100 stool samples from girls aged 5 to 8 years. The samples were analyzed by PCR as described above. None of the specimens harbored *T. vaginalis, T. tenax*, or *P. hominis* (data not shown). We concluded that it is very unlikely that *T. vaginalis* present in the intestine colonizes the vagina and contributes to the high rates of vaginal trichomoniasis we have found in Ndola. The presence of *T. vaginalis* in the mouth was associated with the presence of *T. vaginalis* in the vagina in pregnant women. As with *T. tenax*, this finding suggests autoinoculation and/or oral sexual activity.

In our study populations, we identified 9 different *T. vaginalis* actin genotypes. In the 3 groups of women most infections were caused by 3 genotypes, including G, H, and P. There was no evidence for differences in the distribution of genotypes between the different groups of women. This suggests that women with different sexual behavior patterns and different sexual networks are infected with the same strains of *T. vaginalis*. Further research is needed to investigate the usefulness of other markers or combination of markers.

In this study, we used a genotyping method applied on clinical specimens. Although we achieved a sensitivity of 85% on the self collected vaginal specimens, the method was not sensitive on the self collected specimens from the mouth and rectum. The method could be improved by applying it on *T. vaginalis* isolates. This implies the use of culture medium and additional logistical requirements, and costs. In addition, the culture of *T. vaginalis* may lead to selective growth and selection of isolates. However, further improvement of the sensitivity of the method is required.

In conclusion, we confirmed that the prevalence of *T. vaginalis* infection is very high among adolescent girls, pregnant women, and CSWs in Ndola, Zambia. *T. tenax* and *P. hominis,* however, were relatively uncommon and were rarely found in the vagina. The data on trichomoniasis in adolescent girls who denied that they had ever had penetrative sexual intercourse, suggest that nonsexual routes of transmission may be more important in certain populations than generally acknowledged. The high rates of *T. vaginalis* infection found in this study are worrisome considering the association of trichomoniasis with poor pregnancy outcome and with increased risk for HIV acquisition. Screening and treatment of vaginal *Trichomonas* infection can easily be included in antenatal care programmes, at relatively low cost. In addition, control programmes with screening and treatment should be put in place for adolescent girls who are highly vulnerable to HIV infection.

REFERENCES

1. World Health Organization. Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections, Overview and Estimates. WHO/CDS/EDC/2001.10.Geneva, Switzerland: WHO, 2001.
2. Fouts AC, Kraus SJ. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis 1980; 141:137–143.
3. Petrin D, Delgaty K, Bhatt R, et al. Clinical and microbial aspects of *Trichomonas vaginalis.* Clin Microbiol Rev 1998; 11:300–317.
4. Laga M, Manoka A, Kivuvu M, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS 1993; 7:95–102.
5. Røttingen JA, Cameron DW, Garnett GP. A systematic review of the epidemiologic interactions between classic sexually transmitted diseases and HIV: how much really is known? Sex Transm Dis 2001; 28:579–597.
6. Sexton J, Garnett G, Rottingen JA. Meta-analysis and metaregression in interpreting study variability in the impact of sexually transmitted diseases on susceptibility to HIV infection. Sex Transm Dis 2005; 32:351–357.
7. McClelland RS, Sangare L, Hassan WM, et al. Infection with *Trichomonas vaginalis* increases the risk of HIV-1 acquisition. J Infect Dis 2007; 195:698–702.
8. Van Der Pol B, Kwok C, Pierre-Louis B, et al. *Trichomonas vaginalis* infection and human immunodeficiency virus acquisition in African women. J Infect Dis 2008; 197:548–554.
9. Cohen MS, Hoffman IF, Royce RA, et al. Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1. AIDSCAP Malawi Research Group. Lancet 1997; 349:1868–1873.
10. Kissinger P, Amedee A, Clark RA, et al. *Trichomonas vaginalis* treatment reduces vaginal HIV-1 shedding. Sex Transm Dis 2009; 36:11–16.
11. Cotch MF, Pastorek JG, Nugent RP, et al. *Trichomonas vaginalis* associated with low birth weight and preterm delivery. Sex Transm Dis 1997; 24:353–360.
12. Buvé A, Caraël M, Hayes RJ, et al; for the Study Group on Heterogeneity of HIV Epidemics in African Cities. Multicentre study on factors determining differences in rate of spread of HIV in sub-Saharan Africa: methods and prevalence of HIV infection. AIDS 2001; (suppl 4):S5–S14.

13. Wawer MJ, McNairn D, Wabwire-Mangen F, et al. Self-administered vaginal swabs for population-based assessment of *Trichomonas vaginalis* prevalence. Lancet 1995; 345:131–132.
14. Buvé A, Weiss HA, Laga M, et al. The epidemiology of trichomoniasis in women in four African cities. AIDS 2001; 15:S89–S96.
15. Hira PR. Observations on *Trichomonas vaginalis* infections in Zambia. J Hygiene Epidemiol Microbiol Immunol 1977; 21:215–224.
16. Kengne P, Veas F, Vidal N, et al. *Trichomonas vaginalis*: repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. Cell Mol Biol 1994; 40:819–831.
17. Kikuta N, Yamamoto A, Fukura K, et al. Specific and sensitive detection of *Trichomonas tenax* by the polymerase chain reaction. Let Appl Microbiol 1997; 24:193–197.
18. Crucitti T, Abdellati S, Ross DA, et al. Detection of *Pentatrichomonas hominis* DNA in biological specimens by PCR. Lett Appl Microbiol 2004; 38:510–516.
19. Shaio M-F, Lin P-R, Liu J-Y. Colorimetric one-tube nested PCR for detection of *Trichomonas vaginalis* in vaginal discharge. J Clin Microbiol 1997; 35:132–138.
20. Felleisen RSJ. Comparative sequence analysis of 58S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. Parasitol 1997; 115:111–119.
21. Crucitti T, Van Dyck E, Tehe A, et al. Comparison of culture and different PCR assay for detection of *Trichomonas vaginalis* in self collected vaginal swab specimen. Sex Transm Infect 2003; 79:393–398.
22. Resnick RM, Cornelissen MT, Wright DK, et al. Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. J Natl Cancer Inst 1990; 82:1477–1484.
23. Tabrizi SN, Paterson B, Fairley CK, et al. A Self-administrated technique for the detection of sexually transmitted diseases in remote communities. J Infect Dis 1997; 176:289–292.
24. Sabat G, Rose P, Hickey WJ, et al. Selective and sensitive method for PCR amplification of *Escherichia coli* 16S rRNA genes in soil. Appl Environ Microbiol 2000; 66:844–849.
25. Crucitti T, Abdellati S, Van Dyck E, et al. Molecular typing of the actin gene of *Trichomonas vaginalis* isolates by PCR-restriction fragment length polymorphism. Clin Microbiol Infect. 2008; 14: 844–852.
26. Neumaier M, Braun A, Wagener C. Fundamentals of quality assessment of molecular amplification methods in clinical diagnostics. International federation of clinical chemistry scientific division committee on molecular biology techniques. Clin Chem 1998; 44:12–26.
27. Burkardt HJ. Standardization and quality control of PCR analyses. Clin Chem Lab Med 2000; 38:87–91.
28. Morison L, Weiss HA, Buvé A, et al. Commercial sex and the spread of HIV in four cities in sub-Saharan Africa. AIDS 2001; 15:S61–S69.
29. Vandepitte JM, Malele F, Kivuvu DM, et al. HIV anf other sexually transmitted infections among female sex workers in Kinshasa, Democratic Republic of Congo, in 2002. Sex Transm Dis 2007; 34:203–208.
30. Alary M, Mukenge-Tshibaka L, Bernier F, et al. Decline in the prevalence of HIV and sexually transmitted diseases among female sex workers in Cotonou, Bénin. AIDS 2002; 16:463–470.
31. Ghys PD, Diallo MO, Ettiegne-Traore V, et al. Increase in condom use and decline in HIV and sexually transmitted diseases among female sex workers in Abidjan, Côte d(Ivoire, 1991–1998. AIDS 2002; 16:251–258.
32. Nagot N, Meda N, Ouangre A, et al. Review of STI and HIV epidemiological data from 1990 to 2001 in urban Burkina Faso: implication for STI and HIV control. Sex Transm Infect 2004; 80:124–129.
33. Adu-Sarkodie Y, Opoku BK, Crucitti T, et al. Lack of evidence for the involvement of rectal and oral trichomonads in the aetiology of vaginal trichomoniasis in Ghana. Sex Transm Infect 2007; 83:130–132.
34. Stabler RM, Feo LG, Rakoff AE. Implantation of intestinal trichomonads (*T. hominis*) into the human vagina. Am J Trop Med Hyg 1941; 34:114–118.
35. Stabler RM, Feo LG. Inoculation of the oral trichomonad (*T. tenax*) into the human vagina. Am J Trop Med Hyg 1942; 22: 639–642.