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Cytobrush and cotton swab as sampling tools for molecular diagnosis of female genital schistosomiasis in the uterine cervix

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ABSTRACT

Female genital schistosomiasis (FGS) caused by *Schistosoma haematobium* is a neglected chronic parasitic disease. Diagnosis relies mainly on a colposcopy, which reveals non-specific lesions. This study aimed to assess the performance of two sampling methods for the molecular diagnosis of FGS in the uterine cervix. We conducted a descriptive cross-sectional study in women of reproductive age in Saint Louis, Senegal, who presented for cervical cancer screening. Cotton swab and cytobrush samples were collected from the cervix and examined by real-time PCR. The PCR results obtained using the cotton swabs were compared with those obtained using cytobrush. Of the 189 women recruited, 56 (30%) were found to be positive for *S. haematobium* infection *via* real-time PCR. Women aged 40–54 years were predominantly infected (45%) followed by those aged 25–39 years (36%). Numerically more PCR-positive specimens were identified using cytobrush sampling. Of the 89 women who underwent both cytobrush and cotton swab sampling, 27 were PCR-positive in the cytobrush sampling *vs* 4 in the swab sampling. The mean Ct-value was 31.0 ± 3.8 for cytobrush-based PCR *vs* 30.0 ± 4.4 for swab-based PCR. The results confirm that real-time PCR can detect *Schistosoma haematobium* DNA in the uterine cervix. The next step will be to compare PCR with the other diagnostic methods of FGS.

1. Introduction

Female genital schistosomiasis (FGS) is a neglected tropical disease that causes gynecological manifestations in the female genital tract. Young girls and women in contact with schistosome-infested water, for recreational or domestic purposes are at high risk of developing FGS (Norseth et al., 2014; Orish et al., 2022). The disease is caused by the deposition of eggs of *Schistosoma haematobium* in the genital tract of women. FGS can cause severe complications including infertility, ectopic pregnancy, and abortions, and has been associated with prevalent HIV infection as well (Kjetland et al., 2006; Livingston et al., 2021; Orish et al., 2022).

Data from the literature estimate the burden of FGS to 40 million cases in women and girls living in sub-Saharan Africa (Hotez et al., 2019; Krentel and Steben, 2021). However, this number is probably underestimated due to the poor diagnosis and the lack of knowledge among the healthcare staff. Several reports from East Africa have revealed that the prevalence of FGS ranged from 30% to 75% in women with urinary schistosomiasis (Renaud et al., 1989; Poggensee et al., 2000). However, there is little information available for West Africa. In Senegal, schistosomiasis is endemic in all regions of the country. The prevalence of urogenital schistosomiasis due to *S. haematobium* ranged from 57.7% (Senghor et al., 2016) in the central part to more than 80% in the northern part of the country (Léger et al., 2020). The Saint Louis region located in the north is one of the endemic regions with the highest prevalence of schistosomiasis. Recent reports have shown a prevalence of 79% in adults in this area (Léger et al., 2020). Therefore, women living in this endemic setting are at a very high risk of developing FGS.

FGS diagnosis is challenging. Most of the lesions including sandy patches (grainy and homogenous) and abnormal blood vessels are

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identified using a colposcopy. Recently, the World Health Organization has developed a pocket atlas to help health workers diagnose the disease (WHO, 2015). The presence of parasite eggs could also be confirmed using histology following a biopsy (Engels et al., 2020). Unfortunately, this diagnostic method is invasive and is not always feasible. The current urinary schistosomiasis techniques including urine filtration and the detection of circulating anodic antigen (CAA) in urine do not provide information on genital involvement.

Another diagnostic possibility is the detection of *S. haematobium* DNA in genital samples, but this method has only been applied recently (Kjetland et al., 2009; Pillay et al., 2016, 2020; Sturt et al., 2020; Rafferty et al., 2021; Archer et al., 2022; Ursini et al., 2023).

This study aimed to assess the performance of real-time PCR for the detection of *S. haematobium* DNA using two different genital sample collection tools, the cotton swab and cytobrush.

2. Materials and methods

2.1. Study area

The study was conducted in the communes of Richard Toll and Rosso located in the Dagana Department, Saint Louis Region, Senegal. The Commune of Richard Toll is located in the northern zone of Senegal. The hydrographic network of this city comprises the Senegal River with its two dams, Diama and Manantalli. The water supply network is precarious, absent in certain neighborhoods where the population uses water from canals and the river for all their activities. The commune of Rosso is also located in an area bordered by the Senegal River. There are a few rice-growing developments and part of the sugarcane plantation of the Compagnie sucrière sénégalaise. These sites have been selected based on recent reports showing a high prevalence of urogenital schistosomiasis reaching 79% in adults living in this area (Léger et al., 2020).

2.2. Study design

A community-based cross-sectional investigation was carried out to determine the burden of female genital schistosomiasis in women of reproductive age and to test different sample collection tools/techniques to detect *S. haematobium* DNA in female genital tracts. The FGS screening was coupled with community health campaigns for cervical cancer prevention.

2.3. Study population and eligibility criteria

Women who presented to the clinic for cervical cancer screening and met the inclusion criteria were invited to participate in the study. Eligible women were of reproductive age, provided written informed consent in line with legal regulations in Senegal, were sexually active, residents of the study area, and willing to provide cervical samples.

2.4. Sample collection

Following informed consent, the study health staff (gynecologists and midwives) collected sociodemographic (demographics, water contact, sexual behaviour) and clinical information (genital symptoms) using a questionnaire.

Female genital schistosomiasis screening was carried out as follows. Cervical samples were collected from each participant to detect *S. haematobium* DNA. The cervix was examined after the speculum was inserted into the vagina. The cervix was cleaned with normal saline solution and the excess of the cervical mucus was removed before visualization. An endocervical cytobrush and a cotton swab were used to collect samples from the cervix. A cytobrush is a plastic tool used in general to exfoliate the top layer of cervical epithelial cells during the Pap smear procedure while the cotton swab is a wad of cotton wrapped around a short wooden rod.

Due to budgetary constraints, cotton swabs were chosen over flocked swabs. Furthermore, there was a limited supply of cervical cytobrushes; therefore the number of participants in the cervical cytobrush and swab sampling was unequal.

Screening for pre-cancerous stages of cervical cancer was also performed. Participants were screened for cervical cancer using visual inspection with acetic acid (VIA); 3–5% acetic acid solution and Lugol's iodine solution were applied to the cervix - an opaque white reaction was classified as positive and the lack of reaction was considered as negative.

2.5. DNA extraction and real-time PCR assay

DNA was extracted from cytobrushes and vaginal cotton swabs using the E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Georgia, USA), purified, and eluted following the manufacturer's recommendations.

The real-time PCR assay was performed targeting the highly repetitive *Dra1* sequence of *S. haematobium* as previously described (Cnops et al., 2013) using the primers Sh-FW (5′-GAT CTC ACC TAT CAG ACG AAA C-3′) and Sh-RV (5′-TCA CAA CGA TAC GAC CAA C-3′); and the Sh-probe (5′-FAM-TGT TGG AAG TGC CTG TTT CGC AA-TAMRA-3′).

The real-time PCR reaction mix was prepared using 10 µl of the Roche® Master mix, 0.5 µl of each primer and probe, 3.5 µl of nuclease-free water, and 5 µl of the extracted template DNA giving a total volume of 20 µl. The real-time PCR assay was performed on a CFX96TM machine (BIO-RAD, Life Science, Marnes-la-Coquette, France). The cycling conditions were as follows: 5 min at 95 °C followed by 39 cycles of 30 s at 95 °C and 60 s at 60 °C.

2.6. Data analysis

Questionnaire data were entered into Excel spreadsheet. The analysis was done using the TM R2.15.0 software (R Foundation for Statistical Computing, Vienna, Austria). Quantitative variables were described in terms of means, whereas qualitative data were presented as numbers and percentages. Student's *t*-test and Chi-square test or Fischer's exact test were used to perform statistical comparisons. A test result with a $P < 0.05$ was considered significant. The PCR result was considered positive when the Ct value was ≤ 35 in cytobrush and/or the cotton swab.

3. Results

A total of 189 patients were included in this study. Most of the patients (74.1%) were enrolled in Rosso and 25.9% were enrolled in Richard Toll. The distribution of the study population and the PCR results according to the number of pregnancies, number of deliveries, and contraception used is provided in Table 1. A total of 89 women underwent cytobrush screening while 189 participants were screened using the swabs. The real-time PCR for detection of *S. haematobium* in cervical samples including both methods was positive in 56 participants giving a prevalence of 30%. The prevalence of detectable *S. haematobium* DNA according to the type of sampling tool was 30.3% for the cytobrush samples and 16.9% for the cotton swab samples.

The mean age of the participants was 44.4 ± 11.1 years (range of 18–63 years). The molecular prevalence was greater in the age groups 25–39 years (35.3%) and 40–54 years (28.2%). Most of the women (87.3%) were married and 50.8% of them were in a monogamous relationship.

In the study population, 101 women had at least three sequential singleton pregnancies, representing 57.8% (113/189) of the total number of women, while 69 of them (36.5%) had between five and nine pregnancies. Regarding the visual inspection after acetic acid (VIA), the result was negative in 158 patients (83.6%). However, among the 28 participants presenting a positive VIA result, there were 6 women (21.4%) also positive for schistosomiasis *via* real-time PCR.

A comparison of the PCR detection of *S. haematobium* by the two

Table 1
PCR results according to the characteristics of the study population.

	Total no. of samples (%)	PCR-positive using either sampling tool	PCR-negative	*P*-value
		n (%)	*n* (%)	
Residence				0.861
Richard Toll	49 (25.9)	15 (30.6)	34 (69.4)	
Rosso	140 (74.1)	41 (29.3)	99 (70.7)	
Mean age ± SD (years)	44.4 ± 11.1	38.8 ± 10.9	41.1 ± 11.2	0.253
Age group (years)				0.509
< 25	15 (7.9)	4 (26.7)	11 (73.3)	
25–39	68 (36.0)	24 (35.3)	44 (64.7)	
40–54	85 (45.0)	24 (28.2)	61 (71.8)	
≥ 55	21 (11.1)	4 (19.0)	17 (81.0)	
Marital status				0.673
Married	165 (87.3)	50 (30.3)	115 (69.7)	
Divorced	16 (8.5)	5 (31.3)	11 (68.8)	
Widowed	5 (2.6)	1 (20.0)	4 (80.0)	
Single	3 (1.6)	0 (0)	3 (100)	
Type of marriage				0.372
Polygamous	74 (39.1)	20 (27.0)	54 (73.0)	
Monogamous	91 (48.1)	30 (32.9)	61 (67.1)	
Mean no. of pregnancies ±SD	5.0 ± 2.7	4.0 ± 2.8	5.0 ± 2.6	0.047
No. of pregnancies				0.421
< 5	101 (53.4)	34 (33.7)	67 (66.3)	
5–9	79 (41.8)	20 (25.3)	59 (74.7)	
≥ 10	9 (4.8)	2 (22.2)	7 (77.8)	
Mean no. of deliveries ±SD	4.0 ± 2.5	3.6 ± 2.5	4.3 ± 2.5	0.052
No. of deliveries				0.289
< 5	113 (57.8)	38 (33.6)	75 (66.4)	
5–9	69 (36.5)	17 (24.6)	52 (75.4)	
≥ 10	7 (3.7)	1 (14.3)	6 (85.7)	
Contraception used				0.610
Yes	59 (31.2)	16 (27.1)	43 (72.9)	
No	130 (68.8)	40 (30.8)	90 (69.2)	
Sexually transmitted infection (STI)				0.472
Yes	92 (48.6)	25 (27.1)	67 (72.8)	
No	97 (51.3)	31 (32.0)	66 (68.0)	
Visual inspection with acetic acid (VIA)				0.586
Positive	28 (14.8)	6 (21.4)	22 (78.6)	
Negative	158 (83.6)	49 (31.0)	109 (67.0)	
Not done	3 (1.6)	1 (33.3)	2 (66.7)	

Abbreviation: SD, standard deviation.

techniques revealed that of the 89 participants examined using both cytobrush and cotton swabs, 28 (31.5%) were positive *via* real-time PCR. Of the 100 remaining female participants screened using the cotton swabs, 28 (28%) were positive *via* real-time PCR.

Regarding the positivity rate according to the type of sampling tool, proportionally more PCR-positive cases were identified using the cytobrush (Table 2). In terms of infection intensity, the mean Ct-values were quite similar between the cytobrush and cotton swab sampling techniques (31.0 ± 3.8 *vs* 30.0 ± 4.4).

For the 89 patients screened using both sampling tools, we evaluated their performance by calculating the kappa coefficient. Among the 28 participants positive for *S. haematobium* DNA *via* real-time PCR, 24 (26.9%) were positive only using the cytobrush, 3 (3.3%) were positive using both cytobrush and swabs, and 1 (1.1%) was positive only using swabs (Table 3). There was a concordance between cytobrush and cotton swab test results for 64 (71.9%) participants (3 testing PCR-positive and 61 testing PCR-negative). The kappa coefficient indicated only slight agreement between the two techniques (0.125 ± 0.080; 95% confidence interval: 0.033–0.283).

Table 2
Proportion of PCR-positive results and infection intensity by sampling tool.

Sampling tool	*N*	PCR-positive	Ct-value		
		n (%)	Mean	< 30 *n* (%)	≥ 30 *n* (%)
Cytobrush	89	27 (30.3)	31 ± 3.8	4 (14.8)	23 (85.2)
Swab	189	32 (16.9)	30 ± 4.4	10 (31.3)	22 (68.8)

4. Discussion

Recent evidence has shown an association between female genital schistosomiasis (FGS) and positivity upon visual inspection with acetic acid (VIA) (Rafferty et al., 2021) and it is urgent to set up a monitoring system for mapping the burden of FGS and find strategies for its management. In the context of the limited diagnosis tools, molecular methods have been proposed as a specific technique for FGS diagnosis. Some authors have reported the PCR analysis on a self-sampled vaginal swab among sexually active women (Sturt et al., 2020) while vaginal lavage has been proposed for virgin girls (Kjetland et al., 2009; Orish et al., 2022).

The performance of the PCR for the detection of *S. haematobium* DNA in the uterine cervix depends mainly on the specimen sampling method. In our study, the proportion of PCR-positive samples was numerically higher in the cytobrush compared to the cotton swab technique. Among the participants screened using both sampling tools, we observed that only one case was positive in the swab and negative in the cytobrush sampling. In a cross-sectional study carried out in Madagascar, Randrianasolo et al. (2015) showed that among participants presenting rubbery papules at colposcopy (lesions with viable-looking ova), the PCR analysis using the cervicovaginal lavage fluid and the mucosal swab was more effective for the detection of *S. haematobium* DNA followed by the test performed with cytobrush. The difference in the performance of the swab could be the approach of applying the mucosal sterile swab over the cervix. Another aspect that was observed in the Madagascar study is that the different sampling methods were compared on apparent lesions of FGS under colposcopy. The authors found that the PCR was not strong when diagnosing sandy patches and abnormal blood vessels (Randrianasolo et al., 2015). This could be explained by the fact that the rubbery papules are associated with live ova, while the sandy patches and the abnormal blood vessels are probably caused by dead ova (Kjetland et al., 2009; Randrianasolo et al., 2015). This is one of the limitations of our study as we did not screen FGS lesions using colposcopy. Another limitation of this study was that we used cotton swabs instead of flocked swabs; further studies are needed to compare flocked swabs with cytobrushes.

Our study detected *S. haematobium* DNA in the uterine cervix of female participants living in a high endemic risk area of schistosomiasis (north of Senegal). We observed a prevalence of 30% using the real-time PCR technique targeting the *S. haematobium*-specific *Dra1* sequence on cotton swabs and cytobrushes. The molecular prevalence of genital schistosomiasis varies according to the level of endemicity. For example, the *S. haematobium* PCR-positivity rate in genital specimens was very low (5.9%) in a study carried out in two schistosomiasis low-endemic communities in Zambia (Rafferty et al., 2021). The low prevalence detected by molecular techniques might also be due to false negative results because not all sample types work well in the PCR analysis.

Regarding the performance of the *Dra1*-targeted PCR used in this study, previous studies have shown that PCR is sensitive and specific. Cnops et al. (2013) demonstrated the specificity of the primers and

Table 3
Comparison of PCR results for cytobrush *versus* swab sampling tools.

		Swab			
		Positive	Negative	Total	Kappa
Cytobrush	Positive	3	24	27	0.125
	Negative	1	61	62	
	Total	4	85	89	

probe by testing on a panel of 23 stool samples containing different parasites. In another study, Guegan et al. (2019) showed an excellent specificity (100%) of the *Dra1*-targeted PCR in a cohort of subjects who have never visited known endemic countries. Regarding sensitivity, Ibironke et al. (2012) have shown the excellent performance of *Dra1*--targeted PCR in 401 samples tested. However, despite the good performance of the *Dra1*-targeted PCR, to the best of our knowledge, the primers and probes have not been tested on cotton swabs and cytobrushes. Therefore, more studies are needed to confirm the sensitivity and specificity of this PCR technique using these sample types.

Despite the robust performance of the PCR techniques, logistical or budgetary constraints often require the use the other conventional methods including colposcopy and histopathology, particularly for lesions in other locations; the lesions could be located in the uterus, fallopian or ovaries (Orish et al., 2022). For histopathology, one of the main challenges in the field is that biopsies are not always feasible; they are difficult to perform in many rural settings due to the absence of equipment and the lack of skilled health staff. Most of the biopsies are performed in large hospitals located in large cities and not in the peripheral health facilities near these populations.

The main objective of our study was to assess the performance of two different sampling techniques in detecting *S. haematobium* DNA. Based on our results, we have identified cytobrush sampling as another prospective method for *S. haematobium* DNA detection. This tool has been demonstrated to be more effective for sampling vaginal microbiota compared to the swab. Mitra et al. (2017) have shown that the cytobrush can collect higher total bacterial load and biomass compared to rayon swabs. In a screening of human papillomavirus (HPV) as a primary prevention of cervical cancer, the cytobrush was proposed as a good tool for specimen sampling in the uterine cervix (Kedzia et al., 2010). Following the recommendations for integrating the female genital schistosomiasis control strategies into the existing healthcare system (Nemungadi et al., 2022), the use of cytobrush could facilitate the integration of screening for the prevention of cervical cancer and *S. haematobium* infections.

5. Conclusions

Female genital schistosomiasis is a complication of urogenital schistosomiasis in women, which can cause cervical cancer. The disease is highly neglected due to a lack of information among the health staff and a lack of effective diagnosis tools. The cytobrush currently used for HPV screening has been demonstrated in this study to be a good sampling method for the molecular detection of *S. haematobium* DNA in the uterine cervix. The *DRA1*-targeted PCR has yielded a high number of positive cases using this technique. Therefore, the *S. haematobium*-specific *DRA1* sequence could be used as a good target for the PCR detection of schistosomiasis in this sample type. Further studies are needed to better assess the performance of molecular techniques for the detection of *S. haematobium* DNA in the uterine cervix using cytobrushes compared with flocked swabs in women presenting apparent lesions of FGS during the colposcopy examination.

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Ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Senegalese National Ethics Committee (Comité National d'Ethique pour la Recherche en Santé, CNERS), agreement number 000017-MSAS/DPRS/CNERS. Informed written consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Doudou Sow: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Coumba Nar Ndiour:** Investigation, Writing – review & editing. **Ousmane Thiam:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Magatte Ndiaye:** Conceptualization, Methodology, Writing – review & editing. **Pape Ndiole Diagne:** Investigation, Writing – review & editing. **Souleymane Doucouré:** Conceptualization, Writing – review & editing. **Bruno Senghor:** Conceptualization, Writing – review & editing. **Oumar Gaye:** Methodology, Supervision, Writing – review & editing. **Cheikh Sokhna:** Methodology, Writing – review & editing. **Babacar Faye:** Methodology, Supervision, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting the conclusions of this article are included within the article.

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