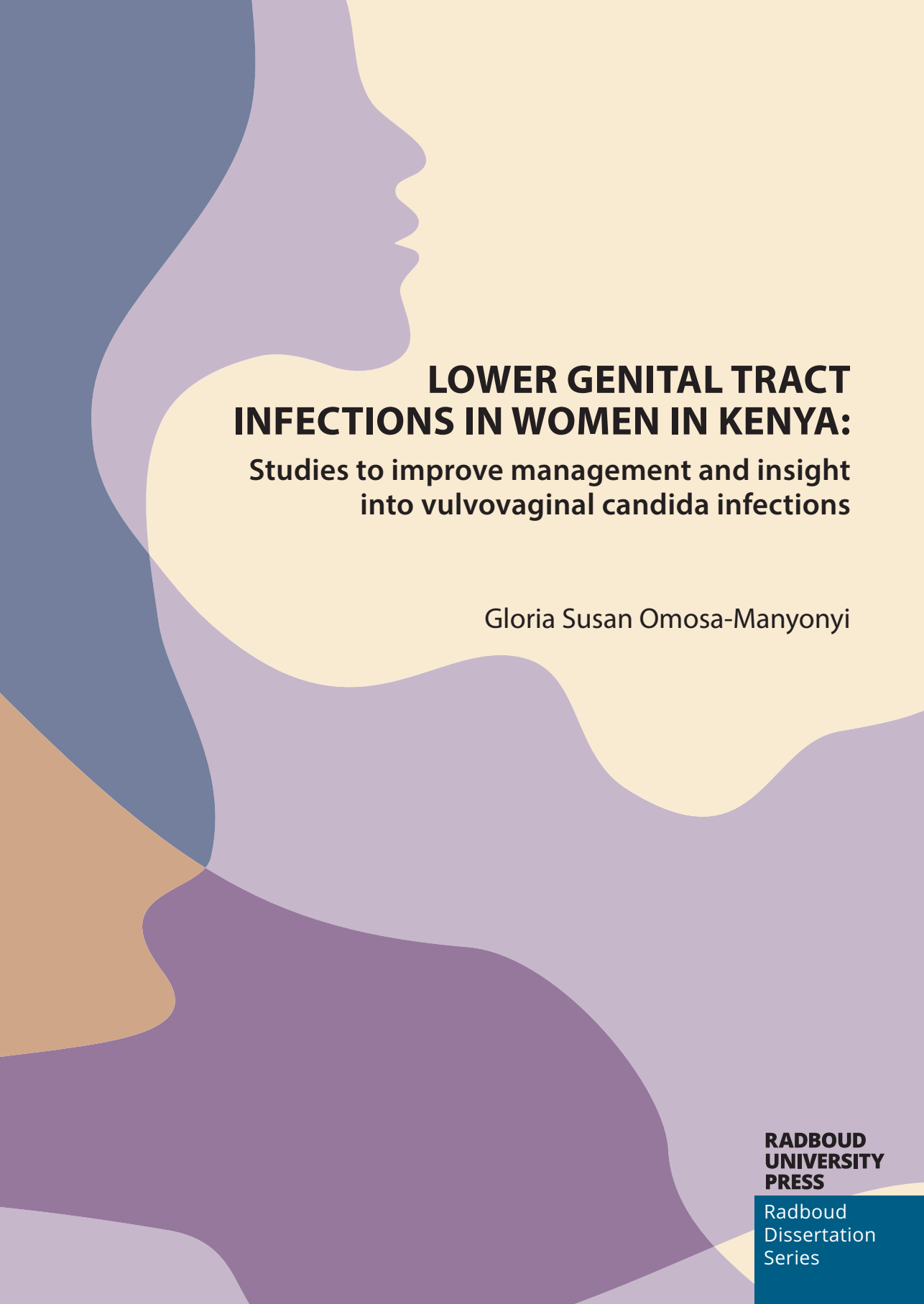

LOWER GENITAL TRACT INFECTIONS IN WOMEN IN KENYA:

Studies to improve management and insight into vulvovaginal candida infections

Gloria Susan Omosa-Manyonyi

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Gloria Susan Omosa-Manyonyi

The research in this thesis was performed at the the Department of Internal Medicine within the Radboud Institute for Health Sciences, Nijmegen, The Netherlands. Main collaborators were: Radboud University Medical Centre and the University of Nairobi School of Medicine, Nairobi, Kenya.

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Dr. L. Spinnewijn

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CHAPTER 1: GENERAL INTRODUCTION, AIM AND OUTLINE OF THE THESIS

GENERAL INTRODUCTION

Genital tract symptoms are a common reason for outpatient consultations in women and include vaginal discharge, dysuria, lower abdominal pain, dyspareunia, and pruritus. Vaginal discharge is the commonest of these symptoms [1, 2].

Genital tract symptoms are often a mark of genital infection; in some women however, this is not the case [3]. Indeed, from studies in sub-Saharan Africa (SSA), 27% to 49% of women with vaginal discharge did not have an infection [4-6]. Furthermore, women may not always distinguish between physiologic and abnormal leucorrhoea hence vaginal discharge may be overreported [7]. On the other hand, underreporting of genital infections may also occur as not all genital infections present with genital tract symptoms; for example, gonorrhoea and chlamydia in women may occur without any symptoms [8, 9], while HIV and Hepatitis B infections exhibit non-genital symptoms.

Genital tract infections in women constitute a significant public health problem with a high disease burden [6, 10, 11]. The infections are responsible for significant morbidity, including infertility in women, negatively affecting quality of life. Moreover, lower genital tract infections (LGTI) increase the risk of HIV transmission, and are responsible for adverse birth outcomes [12-14]. This is particularly so in women who experience repeat episodes of vulvovaginal candidiasis (VVC), which is associated with psychological, biological and social sequel including depression, misery, worry, anxiety, embarrassment and interference with normal sexual life [15-17]. Furthermore, VVC causes premature rupture of membranes in pregnancy, and also increases HIV transmission [12-16]. Despite this recognized role in HIV transmission, it is remarkable that vaginal candidiasis is not a target in containing the HIV epidemic.

Different classes of pathogens may cause genital infections, including several bacterial, fungal, viral and parasitic agents. Common pathogens seen in SSA include *Neisseria gonorrhoea* (NG), *Chlamydia trachomatis* (CT), *Treponema pallidum*, *Mycoplasma genitalium*, bacterial vaginosis (BV), *Candida* species, human papilloma virus, *Trichomonas vaginalis* (TV), and *Schistosoma haematobium* (Figure 1). Here, the most prevalent infections associated with lower genital tract symptoms (LGTS) are VVC and BV, followed by sexually transmitted infections (STI) [4-6, 18]. Up to 80% of women presenting with LGTS at outpatient clinics have at least one confirmed LGTI [2, 4, 5], and dual infections are not uncommon [19]. **Chapter 3** of this thesis determines the infections causing LGTS in women in Nairobi, Kenya.

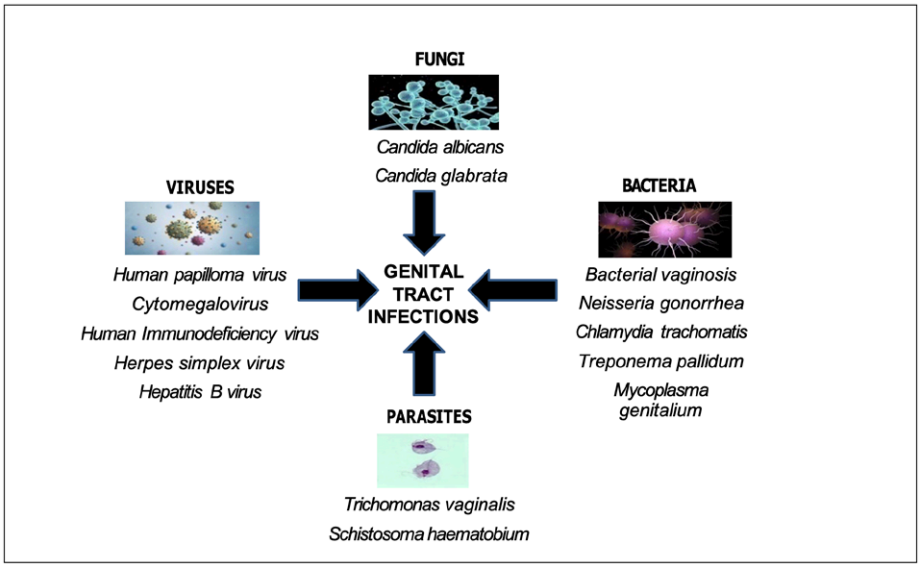

Figure 1. Common pathogens causing female genital infections in sub-Saharan Africa

Management of women with LGTS may be complicated as infectious or non-infectious causes may underlie symptoms. In addition, several pathogens can cause similar symptoms, sometimes more than one pathogen at a time [19]. Importantly, many infectious agents can only be treated by specific antimicrobial agents. Mismanagement and inappropriate use of antimicrobial agents may therefore easily occur. **Chapter 2** describes the inadequacies in service delivery for the diagnosis and treatment of vaginitis and vaginosis in Nairobi, Kenya. Accurate and early diagnosis of LGTI for appropriate antimicrobial treatment is important to prevent complications. Diagnostic tools are only rarely used in SSA to diagnose LGTI, hence treatment is often given empirically or based on syndrome-based treatment algorithms [20]. If these diagnostic algorithms are not correct or not implemented correctly, women may return for new treatment, which contributes to overconsumption of antimicrobial drugs and the development of antimicrobial resistance.

In Kenya, like in many low- and middle-income countries, the syndromic algorithms (Figure 2) based on genital tract symptoms guide treatment decisions for genital tract infections [21]. However, studies have shown that the syndromic approach is inadequate in the management of LGTI [18, 22-25]. The syndromic flowcharts are poor at classification of syndromes/diagnosis; besides, clinical detection is particularly more difficult in patients with dual/multiple infections. Additionally, asymptomatic

infections and infections with non- genital symptoms (e.g. HIV, Hepatitis B) are not catered for [26-28]. Furthermore, by the syndromic algorithms, recurrent vulvovaginal candidiasis (RVVC), defined as at least three VVC episodes per year, is not contemplated hence is neglected; plus, the algorithm often leads to unnecessary use of antibiotics further worsening RVVC. In **Chapter 3**, the optimization of the syndromic management of female LGTI in Nairobi, Kenya, is evaluated.

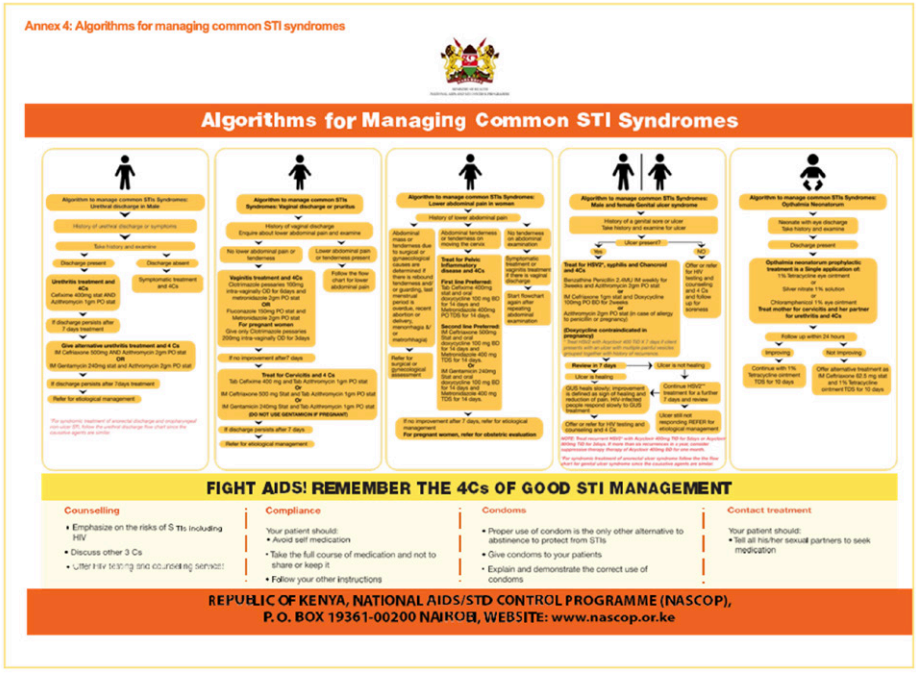

Figure 2. Syndromic flowcharts used in Kenya for management of genital infections [21]

The syndromic treatment guidelines were originally designed to be simplistic and for use by lower cadre health care workers without high level of training and skills, especially those based in remote hard-to-reach areas. This scenario has since evolved: health care workers with higher training and skills are now more available, access to resources online is now a reality, and there is more access to and availability of better diagnostics. I postulate that healthcare workers with higher training may find the syndromic charts too simplistic and hence may not always adhere to the guidelines. Another factor that needs to be considered is the pelvic examination, that is presently part of the guidelines. A pelvic examination is not always practical or acceptable: - the procedure is intrusive to the patient; resources such as examination rooms, vaginal speculums and gloves are scarce; and health care workers are not always available due to a high volume of patients and

competing tasks [29]. So, nonadherence to the guidelines, such as not performing pelvic examination, may occur and as such also lead to erroneous diagnosis and possible incorrect treatment. **Chapter 2** demonstrates the reality at health facilities in Nairobi, a likely representation of the practice in Kenya, and indeed other like environments.

Efforts to improve the performance of the LGTI syndromic guidelines have so far been unimpressive [24]. To improve diagnostic accuracy for better care, additional microbiological testing may be needed. The inclusion of vaginal specimen point-of-care (POC) testing may be a solution to the aforementioned challenges of the syndromic approach. Presently, there are rapid and accurate real-time PCR tests for STI which are relatively affordable for resource-limited settings [30]; no such tests are available yet for candidiasis. Self vaginal-sampling, if included, would remove the need for vaginal examination as a diagnostic procedure, and health care workers would be freed for other tasks. The need for advanced studies for better pathobiological understanding of LGTI remains, including improved and practicable vaginal specimen sampling. Also, to keep up with the need for better pathobiological understanding of LGTI, it is necessary to design improved and practicable vaginal specimen sampling techniques. **Chapters 4 and 5** include studies on vaginal mucosal sample collection and capacity building.

The *Candida* species of fungi can exist both as commensals and pathogens in various mucosal surfaces in humans including the vulvovaginal epithelium [31, 32]. *Candida* vaginal colonization is present in 20% to 30% of pre-menopausal women at a single point in time, by culture-based methods [33, 34]; by genome sequencing methods the single-time-point prevalence of Candida fungi is about 65% [35]. VVC occurs when *Candida* becomes an opportunistic pathogen following loss of the balance in the *Candida*-host commensal relationship. VVC is one of the most prevalent genital infections and affects about three quarters of women at least once in their lifetime [36]. Data from hospital-based studies from SSA show that up to about 50% of women with LGTS have VVC [37-39], and that the *Candida* fungus accounts for up to 84% of the genital infections detected [40]. In past studies from Nairobi Kenya, VVC was the most frequent infection in symptomatic women [41, 42].

In most women VVC is sporadic, in some it is a chronic disease [43], while others will have recurrent episodes of VVC, with more than 138 million women suffering from RVVC each year [44]. Few studies have measured the burden of RVVC in the general population and estimate the prevalence to be 5% to 9% [37, 45]. An online survey among women in Europe and America revealed that more than 20% of those with

VVC reported having RVVC [45], while in SSA up to about 50% of VVC are reported to be RVVC [46]. In **Chapter 3** we determine the frequency of RVVC in symptomatic women in Nairobi.

Remarkably, although vaginal Candida infections are very common, the pathogenesis of RVVC is not completely understood. Various factors contribute to occurrence RVVC in up to 70% of cases; the rest are idiopathic [47]. The identified susceptibility factors may be due to the *Candida* species and virulence, host vaginal dysbiosis precipitated by various factors such as antibiotic use, high oestrogen and diabetes mellitus, as well as intrinsic host influences including immunological, hormonal and genetic factors [47-49]. The risk for RVVC increases in women with Mannose-binding lectin polymorphism [50-52], while some individuals with RVVC showed less T-cell proliferation in response to *Candida* antigen [53]. On the other hand, there are no data that indicate that RVVC should be recognized as a manifestation of a HIV infection or that women with RVVC should be tested for HIV [54].

Data on the burden of RVVC and associated predisposing factors including genetic predisposition in women in Africa and indeed Kenya, are urgently needed. Despite having the biggest genetic heterogeneity and the largest infectious disease burden, African populations are underrepresented in studies of genetic susceptibility to infectious diseases [55]. **Chapter 6** explores the genetic susceptibility to RVVC in women in Nairobi, Kenya.

Being the main effector molecules of biological processes, proteins are targeted by many therapeutic agents [56]. Hence, there is a need to identify the systemic proteins associated with RVVC; these would be interesting as either drivers of disease or products of immune response to RVVC, towards distinguishing possible pathways of therapeutic importance. **Chapter 7** studies Kenyan women with RVVC to determine if they demonstrate a distinct plasma proteomic signature.

Likewise, the effects of the repeat and long-term changes in the vaginal milieu in RVVC are not known. Cervicovaginal proteins and metabolites are markers of various biological processes including cell organization and differentiation, enzyme activity, metabolism, and immune responses [57]. Untargeted analysis of the vaginal metabolome and proteome offers the opportunity to unravel these parameters, and relate these to the pathogenesis of genital tract infections and susceptibility to HIV infection. While the association with vaginal microbiome and metabolome has been studied in some genital tract infections [58], the role of the mucosal immune response in RVVC has been left out of the equation. Studying

this dysregulated immune response in RVVC requires various vaginal sampling techniques across heterogenous populations for maximum understanding, which calls for standardization of the mucosal sample collection and assay techniques. In **Chapter 5** we describe a regional mucosal training venture for multi-center collaboration in basic and clinical research in Eastern Africa.

In **Chapter 8**, I summarize the findings of this thesis, discuss their implications, and share recommendations for the future.

Aim of the thesis

To improve management and pathobiological understanding of LGTI and RVVC in Kenyan women.

Content of the thesis

Chapter 1	Introduction and aim of the thesis
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Chapter 8	Discussion, conclusion and recommendations

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CHAPTER 2: INADEQUACIES IN SERVICE DELIVERY FOR THE DIAGNOSIS AND TREATMENT OF VAGINITIS AND VAGINOSIS IN NAIROBI, KENYA

Gloria S Omosa-Manyonyi, Lucina N Koyio, Esther W. Mwangi, Hannah Gathura, Andre van der Ven, Jaap ten Oever

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Abstract

Vulvovaginal candidiasis (VVC), a common cause of vaginitis, affects 75% of women in their lifetime. In Kenya, vaginitis/VVC is managed using the vaginal discharge syndrome guidelines. We assessed how frequently healthcare workers consider the diagnosis of vaginitis/VVC in symptomatic women, and adherence to the syndromic guidelines, outpatient records at Nairobi City County health facilities, of non-pregnant symptomatic females aged ≥15 years were abstracted. Descriptive statistics were applied, and analysis of determinants of practice determined using multivariable logistic regression models. Of 6,516 patients, 4,236 (65%) (inter-facility range 11–92%) had vaginitis of which 1,554 (37%) were considered VVC (inter-facility range 0–99%). Vaginitis was associated with facility, adjusted odds ratio (aOR) 2.80 (95% confidence interval (CI) 1.64–4.76) and aOR 0.03 (95% CI 0.02–0.04); and month, aOR 0.33 (95% CI 0.25–0.43). Vaginal examination was in 53% (inter-facility range 0–98%). Adherence to syndromic treatment was 56% (inter-facility range 0–83%), better with older patients (aOR 7.73, 95% CI 3.31–18.07). Vaginitis and VVC are commonly diagnosed in symptomatic patients in Nairobi; adherence to the syndromic guidelines is low and differs across the health facilities. Interventions to improve adherence are needed.

Introduction

Vulvovaginal candidiasis is the most common cause of infectious vulvovaginitis worldwide, alongside bacterial vaginosis (BV).[1–5] Globally, around 75% of women experience at least one episode of vulvovaginal candidiasis (VVC) in their lifetime, while up to 9% develop recurrent vulvovaginal candidiasis (RVVC).[6] Behavioral and biological factors including hormonal changes during pregnancy or hormonal contraceptive use, diabetes mellitus, and antibiotic medication, predispose to sporadic VVC.[1,7] These factors are also prevalent in Sub-Saharan Africa (SSA), where VVC is also common.[2,8] Data from Kenya give VVC lifetime prevalence rates of up to 46% in non-pregnant symptomatic women, and up to 90% during pregnancy.[9–11]

Management of VVC at public/government-run health facilities (hospitals, health centers, and dispensaries) in Kenya is commonly carried out by nurses and clinical officers using the Kenyan guidelines for reproductive tract infections (RTI) that is based on the World Health Organization (WHO) Sexually Transmitted Infections Treatment Guidelines.[12] The Kenyan RTI guidelines of 2006[13] were in use up to the year 2018 when a revised guidelines version[14] was released. In the guidelines, lower genital tract symptoms (LGTS) associated with VVC, BV, and trichomoniasis are classified under the vaginal discharge syndrome and syndromically managed as vaginitis. The syndromic management includes inquiry on lower abdominal pain, abdominal and vaginal examinations, and then a short course treatment for VVC, BV, and trichomoniasis (if abdominal pain/tenderness is absent). Persistent cases are thereafter treated for sexually transmitted infections (STI). In this algorithm, RVVC is not contemplated, yet unnecessary use of antibiotics could worsen RVVC.[6]

Although the Kenyan guidelines recommend a syndromic approach for the treatment of LGTS, healthcare workers may not always adhere to this and instead clinically diagnose specific conditions and treat accordingly. For instance, the clinical assessment may be without a pelvic examination with the likelihood of an inaccurate syndromic diagnosis and hence a misguided treatment. Proper management of vaginitis is important; apart from the associated discomfort and negative impact on quality of life and pregnancy, VVC, BV, and trichomoniasis increase the risk for vertical and horizontal transmission of HIV.[15–20]

Most studies evaluating the syndromic approach have focused on how well it is applied in predicting STI;[4] similar data for vaginitis are scarce. We found no data from Kenya assessing the performance of the syndromic approach in the management of vaginitis syndrome. In the present study, a retrospective review of

records at healthcare facilities of Nairobi City County (NCC), Kenya, was done to determine how often healthcare workers consider the diagnoses of vaginitis and VVC in women with LGTS, and to assess the healthcare workers' adherence to the vaginal discharge syndromic management guidelines.

Definitions

Vaginitis
All records with a diagnosis term of vaginitis or synonyms including vaginosis, vaginal discharge/genital discharge, VVC, trichomoniasis, and bacterial vaginosis/BV.

Vulvovaginal candidiasis
Records with a specified diagnosis term of vulvovaginal candidiasis/VVC or synonyms.

Vulvovaginal candidiasis synonyms
Vaginal thrush, moniliasis, vaginal candidiasis, vulval candidiasis, and genital candidiasis.

Vaginal examination
Refers to any vaginal examination indicated in the clinical notes, that is, inspection of the external genitalia and digital or speculum vaginal examination, since our data could not qualify the type of vaginal examination performed for individual patients.

Vulvovaginal candidiasis treatment
Any intra-vaginal antifungal or short-term (<1 week) oral antifungal.

Adherence to the recommended treatment of vaginitis

Prescription of intravaginal clotrimazole plus oral metronidazole. For a sub-analysis, we considered oral in place of intravaginal antifungal treatment correct as well.

Adherence to the recommended management of vaginitis
The performance of a vaginal examination plus a prescription of intravaginal clotrimazole and oral metronidazole.

Diagnosis-treatment mismatch in Vulvovaginal candidiasis
Refers to specified cases of VVC as defined above not receiving any antifungal treatment; and use of sole antifungal treatment in the absence of documented vaginitis/VVC.

Methods

Study design and setting

In this retrospective study, outpatient records of all women fulfilling the inclusion criteria were reviewed, for the period January to December 2016. This study was a precursor to planned larger studies on VVC and RVVC in women, in Nairobi. Nine NCC health centers (Level 3 facilities) plus three NCC sub-county hospitals (Level 4 facilities) participated in the study; together these facilities covered nine of the 10 NCC health zones.

In Kenya, health facilities are categorized into six Kenya Essential Package for Health (KEPH) levels based on infrastructure, equipment, and staffing: LEVEL 1—Community Facilities; LEVEL 2—Health Dispensaries; LEVEL 3—Health Centers; LEVEL 4—Sub-county Hospitals; LEVEL 5—County Referral Hospitals; Level 6—National Referral Hospitals.[21] Level 3 health facilities are staffed by clinical officers and nurses; Level 4 facilities additionally have medical officers and medical specialists. All the health facilities use national guidelines for the management of various conditions along with standardized treatment; for example, the Kenyan guidelines of 2006 for RTI services.[13] All public health facilities also use standardized health records reporting tools/registers in either electronic or paper form. Outpatient data is summarized in various registers; the Ministry of Health (MOH) 204 B Outpatient Register is used to summarize data for outpatients older than 5 years (Supplementary material).[22] This form is designed to capture summary information such as patient name, outpatient number, age, sex, date of visit, diagnosis, and treatment. Accordingly, all NCC facilities use the national guidelines for the management of various conditions, as well as the standardized national reporting tools and treatment. The Kenyan guidelines of 2006 for RTI services,[13] that were in use in 2016, provided directions on the management of syndromes associated with abnormal vaginal discharge (Figure 1). In 2016, therefore, health workers in the NCC facilities were required to apply the syndromic approach prescribed in the 2006 RTI guidelines for the management of LGTS in women.

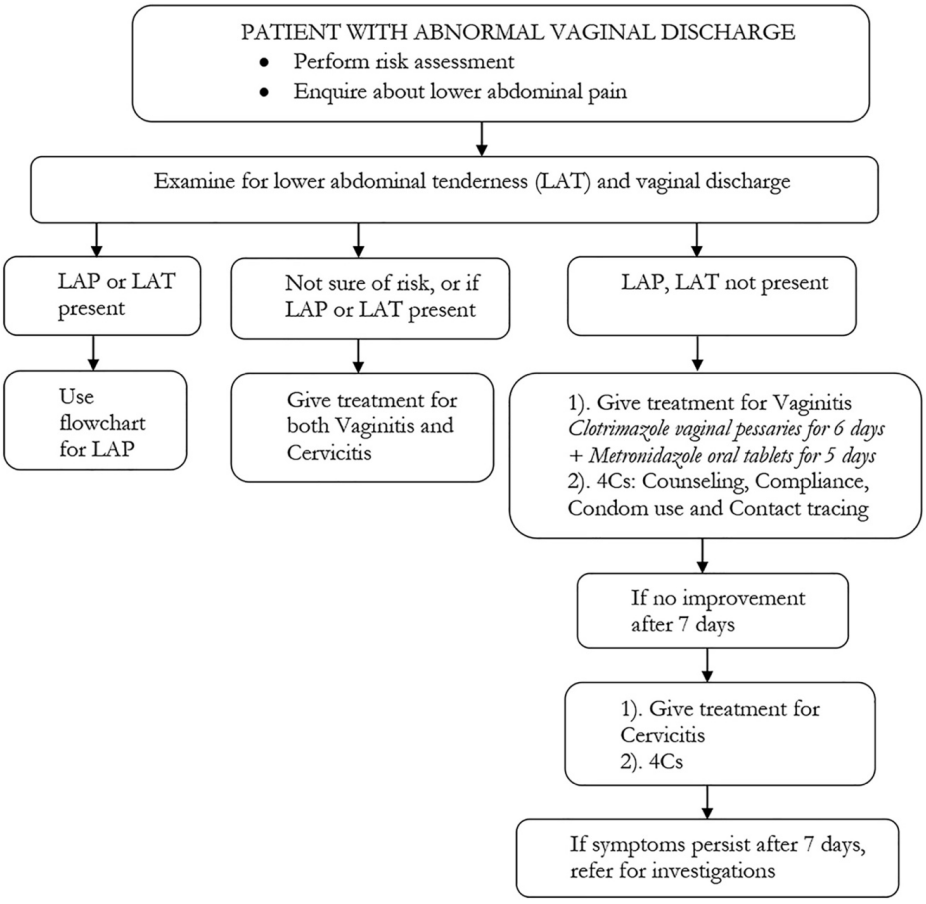

Figure 1. Summary of the Kenyan guidelines 2006 on syndromic management of vaginal discharge syndrome.

Inclusion and exclusion criteria

Included were records of all female patients aged 15 years and above, with a documented diagnosis of vaginitis as defined above, or any other diagnosis associated with LGTS, that is, pelvic inflammatory disease/PID, cervicitis, and sexually transmitted infection/STI. We excluded records of pregnant women, and those missing an outpatient number, age, or diagnosis.

Objectives

The objectives of this chart review were to assess the contribution of vaginitis and VVC to all diagnoses associated with LGTS, measure adherence to the recommended management of vaginitis, and evaluate treatment mismatch for documented vaginitis/VVC.

Outcome measures

Among women presenting with LGTS we assessed

- The relative contribution of vaginitis and specified VVC
- Guideline adherence for the management of vaginitis, and determinants
  - The performance of a vaginal examination
  - Correct antimicrobial treatment for vaginitis and specified VVC
  - Diagnosis–treatment mismatch in VVC.

Data collection

Following training, data personnel from each participating health facility abstracted data, excluding the patient's name, from the facility's MOH 204 B Outpatient Register (Supplementary material) and transcribed it onto the study's data collection forms. Where available, patients' clinic notes were consulted for data regarding vaginal examination. Monitoring of the data collection was performed weekly. The data were subsequently transferred onto an excel sheet by an independent data clerk. Data bearing the same outpatient number, age, facility, and visit date were treated as one entry.

Data analysis

Vaginitis and VVC were identified based on the diagnosis written in the chart, as defined above. The medications prescribed were categorized as antifungal, metronidazole/tinidazole, antibiotic, and others. Entries without data on vaginal examination and medicine prescribed were excluded from the analyses for vaginal examination and treatment, respectively.

Frequency tables and descriptive statistics were used to assess the contribution of vaginitis and VVC to LGTS, guideline adherence, and diagnosis–treatment mismatch. Analysis of determinants of practice performance was done by multivariable logistic regression models. The independent variables were the patient's age, health facility, and the month the patient was seen.

Ethical considerations

The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee granted ethical approval for this study (P980/12/2016). We also obtained research authorization from NCC. All data were stripped of patients' names and analyzed anonymously.

Results

We obtained a total of 6,890 records of patients with LGTS from 12 NCC health facilities comprising three sub-county hospitals and 9 health centers. Excluded were 374 entries, leaving a total of 6,516 records of patients with LGTS. The majority of patients (72%) were aged 18–32 years with a mean ± SD age of 29 ± 8.6 years (Figure 2 and Table 1).

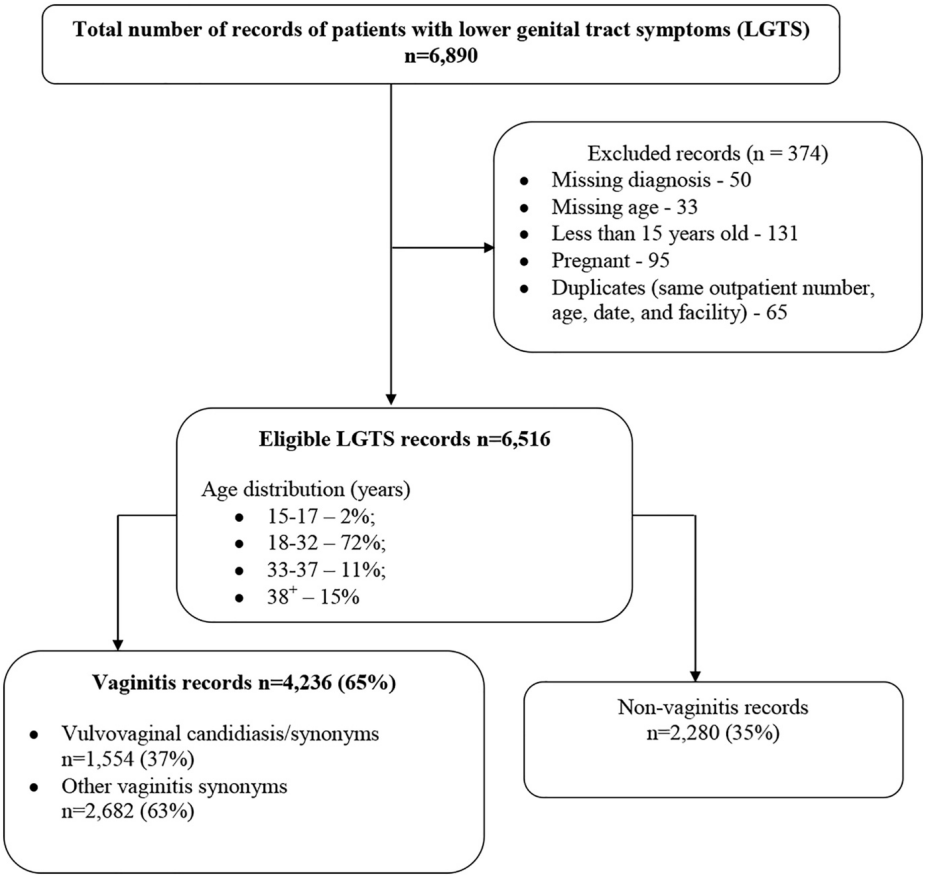

Figure 2. Patients with LGTS seen at outpatient clinics of Nairobi City County health facilities, year 2016.

Table 1. Distribution of patients with lower genital tract symptoms by diagnosis and rates of vaginal examination, at health facilities of Nairobi City County, year 2016.

Facility	Overall data (LGTS patients)		Age		Vaginitis		VVC (of the vaginitis)		LGTS missing VE data		Vaginal examination performed (VE)[a]					
											LGTS		Vaginitis[b]		VVC[d]	
	n	%	Mean	SD	*N*	%	*n*	%	*n*	%	*N*	%	*n*	%	*n*	%
HealthCenter-1	2,396	36.8	30.3	8.87	1,959	81.8	0	0	140	5.8	1,841	81.6	1,556	84.5	c	c
HealthCenter-2	255	3.9	27.3	7.47	67	26.3	51	76.1	23	9.0	0	0.0	0	0.0	0	0.0
HealthCenter-3	319	4.9	27.3	6.39	221	69.3	37	16.7	319	100.0						
HealthCenter-4	194	3.0	26.9	6.26	130	67.0	118	90.8	0	0.0	98	50.5	76	77.6	69	90.8
HealthCenter-5	535	8.2	26.8	6.11	61	11.4	6	9.8	525	98.1	0	0.0	0	0.0	0	0.0
Hospital-1	839	12.9	30.5	9.26	344	41.0	309	89.8	0	0.0	13	1.5	5	38.5	4	80.0
HealthCenter-6	434	6.7	27.0	7.46	314	72.4	307	97.8	0	0.0	427	98.4	312	73.1	306	98.1
Hospital-2	382	5.9	30.4	9.48	206	53.9	190	92.2	88	23.0	25	8.5	16	64.0	12	75.0
HealthCenter-7	294	4.5	26.0	5.65	228	77.6	225	98.7	294	100.0						
Hospital-3	100	1.5	27.8	7.82	76	76.0	70	92.1	1	1.0	1	1.0	0	0.0	0	0.0
HealthCenter-8	191	2.9	28.5	8.54	176	92.1	127	72.2	12	6.3	1	0.6	1	100.0	1	100.0
HealthCenter-9	577	8.9	28.5	10.46	454	78.7	114	25.1	577	100.0						
All	6,516	100.0	29.0	8.59	4236	65.0	1,554	36.7	1,979	30.4	2,406	53.0	1,966	81.6	392	19.9

LGTS: lower genital tract symptoms; VVC: vulvovaginal candidiasis; SD: standard deviation; VE: vaginal examination. Empty cells–––VE data missing.

[a]Excludes those missing VE data.

[b]Vaginitis of the LGTS who had VE performed.

[c]No documented VVC cases.

[d]VVC of the vaginitis who had VE performed.

Table 2. Factors associated with a diagnosis of vaginitis in patients with LGTS at health facilities of Nairobi City County, year 2016.

	Total (*n* = 6,516)	Vaginitis (*n* = 4,236, 65%)		Bivariate analysis				Multivariate analysis			
				OR	*p*-value	[95% CI]		OR	*p*-value	[95% CI]	
	n	*n*	%			LCL	UCL			LCL	UCL
Facility											
HealthCenter-1	2,396	1,959	81.76	Ref							
HealthCenter-2	255	67	26.27	0.079	0.000	0.059	0.107	0.072	0.000	0.053	0.097
HealthCenter-3	319	221	69.28	0.503	0.000	0.388	0.652	0.489	0.000	0.376	0.636
HealthCenter-4	194	130	67.01	0.453	0.000	0.330	0.622	0.459	0.000	0.331	0.637
HealthCenter-5	535	61	11.40	0.029	0.000	0.022	0.038	0.031	0.000	0.023	0.042
Hospital-1	839	344	41.00	0.155	0.000	0.130	0.184	0.158	0.000	0.132	0.188
HealthCenter-6	434	314	72.35	0.584	0.000	0.462	0.738	0.504	0.000	0.392	0.648
Hospital-2	382	206	53.93	0.261	0.000	0.208	0.327	0.216	0.000	0.171	0.273
HealthCenter-7	294	228	77.55	0.771	0.081	0.575	1.033	0.751	0.055	0.560	1.007
Hospital-3	100	76	76.00	0.706	0.148	0.441	1.131	0.727	0.201	0.446	1.185
HealthCenter-8	191	176	92.15	2.617	0.000	1.529	4.479	2.797	0.000	1.642	4.764
HealthCenter-9	577	454	78.68	0.823	0.090	0.658	1.031	0.767	0.023	0.610	0.964
Month seen											
January	642	492	76.64	Ref							
February	628	469	74.68	0.899	0.417	0.696	1.162	0.949	0.725	0.711	1.268
March	703	540	76.81	1.100	0.938	0.784	1.301	1.068	0.641	0.811	1.405
April	611	436	71.36	0.760	0.033	0.590	0.979	0.769	0.069	0.579	1.021
May	580	360	62.07	0.499	0.000	0.389	0.639	0.557	0.000	0.416	0.746

Table 2. Continued

	Total (*n* = 6,516)	Vaginitis (*n* = 4,236, 65%)		Bivariate analysis				Multivariate analysis			
				OR	*p*-value	[95% CI]		OR	*p*-value	[95% CI]	
	n	*n*	%			LCL	UCL			LCL	UCL
June	597	401	67.17	0.624	0.000	0.486	0.801	0.990	0.948	0.737	1.331
July	562	278	49.47	0.298	0.000	0.233	0.382	0.449	0.000	0.335	0.602
August	504	310	61.51	0.487	0.000	0.377	0.629	0.655	0.008	0.480	0.895
September	467	267	57.17	0.407	0.000	0.314	0.527	0.447	0.000	0.330	0.606
October	500	241	48.20	0.284	0.000	0.220	0.365	0.326	0.000	0.246	0.433
November	524	311	59.35	0.445	0.000	0.346	0.573	0.579	0.000	0.433	0.774
December	198	131	66.16	0.596	0.003	0.422	0.843	0.721	0.152	0.461	1.128
Age (years)											
15–17	115	73	63.48	Ref							
18–22	1,327	880	66.31	1.133	0.538	0.762	1.683				
23–27	1952	1250	64.04	1.024	0.903	0.693	1.514				
28–32	1,403	895	63.79	1.014	0.946	0.683	1.504				
33–37	740	491	66.35	1.135	0.545	0.754	1.708				
38–42	502	341	67.93	1.161	0.360	0.798	1.861				
43–47	218	139	63.76	1.012	0.959	0.633	1.619				
48–52	134	83	61.94	0.936	0.803	0.559	1.568				
53+	125	84	67.20	1.179	0.545	0.692	2.008				

LGTS: lower genital tract symptoms; OR: odds ratio; CI: confidence interval; LCL: lower confidence limit; UCL: upper confidence limit.

The relative contribution of vaginitis and VVC to LGTS

Of the eligible 6,516 patients' records, we classified 4,236 (65%) as cases of vaginitis with an inter-facility proportion range of 11–92%. Of the vaginitis, 1,554 (37%) were judged to be VVC by clinical diagnosis (inter-facility range of 0–99%); suspected VVC diagnosis was high at eight of 12 facilities (>72%), while one health center reported none. (Table 1)

The likelihood of vaginitis cases was associated with health facility and month in the year; there was no association with patient's age. Concerning HealthCenter-1, vaginitis was about thrice as likely in one other health center (adjusted odds ratio (aOR) 2.80 (95% confidence interval (CI) 1.64–4.76), while this diagnosis was less likely at eight other facilities (including two sub-county hospitals) with the least aOR being 0.03 (95% CI 0.02–0.04). Compared to January vaginitis cases were less likely during May, July, August, September, October, and November; aOR 0.33 (95% CI 0.25–0.43) (Table 2).

Guideline adherence in management of vaginitis

Performance of vaginal examination

After excluding those with missing data (n = 1,979, 30%), the rate of vaginal examination overall was 53% (n = 2,406). Three facilities contributed almost all the vaginal examinations performed with an inter-facility range of 0–98%. Vaginal examination rates in those classified as vaginitis and in the subset of suspected VVC were 82% and 20%, respectively (Table 1).

At bivariate analysis, health facility was significantly associated with the performance of vaginal examination. Due to the skewing and considerable missing data we did not assess this association at multivariate analysis. The vaginal examination was also associated with month in the year; compared to January, vaginal examination was least likely in July (aOR of 0.38 (95% CI 0.28–0.50)), but up to twice as likely during September and December (aOR of 2.22 (95% CI 1.46–3.39). The patient's age was not associated with the performance of vaginal examination (Table 3).

Table 3. Factors associated with performance of vaginal examination in patients with LGTS at health facilities of Nairobi City County, year 2016.

	Vaginal examination			Bivariate analysis				Multivariate analysis			
	Total (*n* = 6,516)	Yes (*n* = 2,406; 53%)		OR	*p*-value	[95% CI]		OR	*p*-value	[95% CI]	
		n	%			LCL	UCL			LCL	UCL
Facility											
HealthCenter-1	2,256	1,841	81.60	Ref							
HealthCenter-2	232	0	0.00								
HealthCenter-3	0	0		0.230	0.000						
HealthCenter-4	194	98	50.52			0.170	0.311				
HealthCenter-5	10	0		0.004	0.000						
Hospital-1	839	13	1.55	13.751	0.000	0.002	0.006				
HealthCenter-6	434	427	98.39	0.021	0.000	6.467	29.239				
Hospital-2	294	25	8.50			0.014	0.032				
HealthCenter-7	0	0		0.002	0.000						
Hospital-3	99	1	1.01	0.001	0.000	0.000	0.017				
HealthCenter-8	179	1	0.56			0.000	0.009				
HealthCenter-9	0	0									
Month seen											
January	456	244	53.51	0.960	0.752						
February	484	254	52.48	1.130	0.348	0.743	1.240	0.954	0.721	0.738	1.234
March	497	281	56.54	0.949	0.691	0.875	1.459	1.125	0.368	0.871	1.454
April	456	238	52.19	0.765	0.045	0.731	1.230	0.947	0.680	0.730	1.228
May	440	206	46.82	1.128	0.384	0.588	0.995	0.764	0.044	0.587	0.993
June	393	222	56.49	0.379	0.000	0.860	1.480	1.133	0.368	0.863	1.488

Table 3. Continued

	Vaginal examination			Bivariate analysis				Multivariate analysis			
	Total (*n* = 6,516)	Yes (*n* = 2,406; 53%)		OR	*p*-value	[95% CI]		OR	*p*-value	[95% CI]	
		n	%			LCL	UCL			LCL	UCL
July	375	114	30.40	0.988	0.934	0.285	0.506	0.379	0.000	0.284	0.504
August	327	174	53.21	2.027	0.000	0.743	1.314	0.983	0.907	0.739	1.308
September	300	210	70.00	0.762	0.063	1.490	2.758	2.003	0.000	1.472	2.726
October	319	149	46.71	1.366	0.030	0.572	1.014	0.762	0.063	0.571	1.015
November	355	217	61.13	2.218	0.000	1.031	1.811	1.354	0.035	1.021	1.795
December	135	97	71.85			1.460	3.368	2.222	0.000	1.457	3.389
Age (years)											
15–17	71	35	49.30	1.118	0.653						
18–22	843	439	52.08	1.126	0.625	0.689	1.814				
23–27	1,299	679	52.27	1.078	0.759	0.699	1.816				
28–32	1,020	522	51.18	1.450	0.142	0.666	1.744				
33–37	535	313	58.50	1.310	0.296	0.883	2.381				
38–42	391	219	56.01	1.174	0.567	0.789	2.173				
43–47	182	97	53.30	1.435	0.244	0.678	2.032				
48–52	103	60	58.25	0.847	0.599	0.781	2.636				
53+	93	42	45.16	Ref		0.456	1.573				

LGTS: lower genital tract symptoms; OR: odds ratio; CI: confidence interval; LCL: lower confidence limit; UCL: upper confidence limit.

Table 4. Distribution of treatment by diagnosis category in patients with LGTS at health facilities of Nairobi City County, year 2016.

Facility	Vaginitis[a]	Treatment of vaginitis						Syndromic management of vaginitis (vaginal examination plus correct syndromic treatment)[b]		Treatment of suspected VVC				Non-vaginitis	Sole antifungal use in non-vaginitis	
		Correct syndromic (vaginal antifungal plus metronidazole)		Modified syndromic (oral or vaginal antifungal plus metronidazole)		Antifungal received (oral or vaginal)				Antifungal received (oral or vaginal)		Sole antibiotic treatment				
	n	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	*n*	%
HealthCenter-1	1,791	1,477	82.5	1,483	82.8	1,728	96.5	1,284	84.9	c	c	c	c	159	152	95.6
HealthCenter-2	38	0	0.0	0	0.0	38	100.0	0	0.0	27	100.0	0	0.0	209	38	18.2
HealthCenter-3	220	121	55.0	122	55.5	210	95.5			33	89.2	4	10.8	26	22	84.6
HealthCenter-4	124	0	0.0	0	0.0	119	96.0	0	0.0	108	96.4	1	0.9	173	119	68.8
HealthCenter-5	35	2	5.7	9	25.7	26	74.3	0	0.0	2	100.0	0	0.0	88	1	1.1
Hospital-1	125	3	2.4	5	4.0	61	48.8	0	0.0	59	50.4	39	33.3	46	35	76.1
HealthCenter-6	161	0	0.0	1	0.6	106	65.8	0	0.0	103	66.0	47	30.1	72	67	93.1
Hospital-2	122	8	6.6	24	19.7	94	77.0	0	0.0	88	80.0	11	10.0	72	57	79.2
HealthCenter-7	227	52	22.9	55	24.2	219	96.5			218	97.3	5	2.2	85	80	94.1
Hospital-3	63	4	6.3	6	9.5	59	93.7	0	0.0	55	94.8	3	5.2	51	45	88.2
HealthCenter-8	57	1	1.8	1	1.8	57	100.0	0	0.0	52	100.0	0	0.0	70	56	80.0
Total	2,963	16,68	56.3	1706	57.6	2,717	91.7	1,284	73.0	745	83.2	110	12.3	1,051	672	63.9

LGTS: lower genital tract symptoms; VVC: vulvovaginal candidiasis.

[a]Excluded 1273 entries which were missing treatment data.

[b]Excluded 1204 entries which were missing vaginal examination data.

[c]No documented VVC cases.

Empty cells - All vaginal examination data missing.

Treatment of vaginitis and VVC

A total of 1,273 (30%) vaginitis entries without treatment data were excluded from this analysis. Of those with complete data (*n* = 2,963), adherence to the recommended syndromic prescription for vaginitis (clotrimazole vaginal pessaries plus metronidazole) was overall 56% (*n* = 1,668) with 89% of these being from one facility; inter-facility range of 0–83%. Using a less strict definition of guideline adherence (including oral antifungal), the overall adherence rate increased to 58% (*n* = 1,706), inter-facility range 0–83%. Antifungal treatment was prescribed in 92% (*n* = 2,717) of the vaginitis, inter-facility range 49–100%; and in 83% of those suspected to have VVC, inter-facility range 50–100%. Compliance with the guideline recommendation of vaginal examination plus syndromic treatment for vaginitis was 73% (*n* = 1,284), all from one facility (HealthCentre-1) (Table 4).

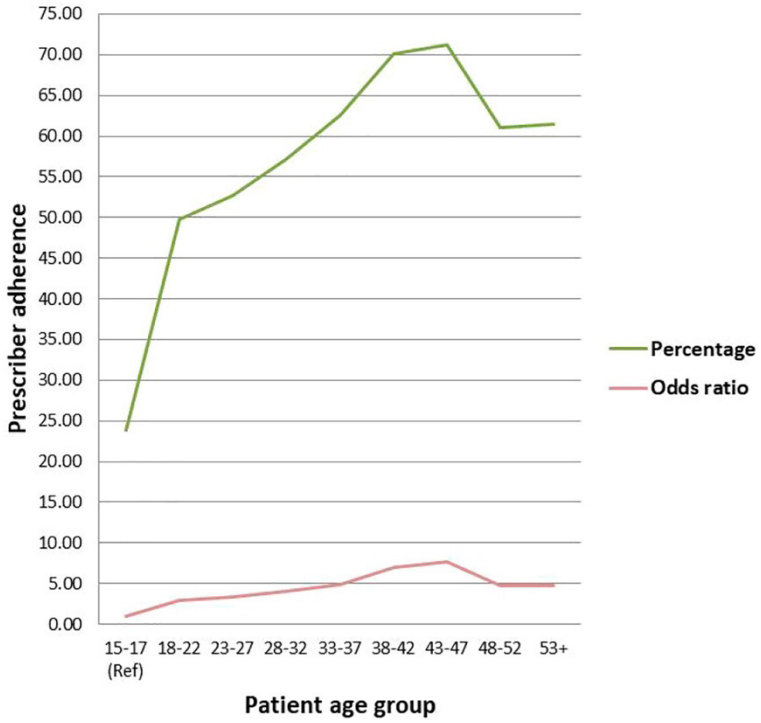

Figure 3. Relationship between prescriber adherence to syndromic treatment guideline and patient age group, at outpatient clinics of Nairobi City County health facilities, year 2016.

Table 5. Factors associated with syndromic treatment in patients with vaginitis at health facilities of Nairobi City County, year 2016.

	Total vaginitis (*n* = 2,963)	Syndromic treatment given (*n* = 1,668, 56%)		Bivariate analysis				Multivariate analysis			
		n	%	OR	*p*-value	[95% CI]		OR	*p*-value	[95% CI]	
						LCL	UCL			LCL	UCL
Facility											
HealthCenter-1	1,791	1,477	82.47	Ref							
HealthCenter-2	38	0	0.00								
HealthCenter-3	220	121	55.00	0.260	0.000	0.194	0.348				
HealthCenter-4	124	0	0.00								
HealthCenter-5	35	2	5.71	0.013	0.000	0.003	0.054				
Hospital-1	125	3	2.40	0.005	0.000	0.002	0.017				
HealthCenter-6	161	0	0.00								
Hospital-2	122	8	6.56	0.015	0.000	0.007	0.031				
HealthCenter-7	227	52	22.91	0.063	0.000	0.045	0.088				
Hospital-3	63	4	6.35	0.014	0.000	0.005	0.040				
HealthCenter-8	57	1	1.75	0.004	0.000	0.001	0.028				
Month seen											
Jan	309	181	58.58	Ref							
Feb	325	183	56.31	0.911	0.564	0.665	1.249	0.912	0.576	0.662	1.258
March	328	186	56.71	0.926	0.633	0.676	1.269	0.941	0.708	0.683	1.295
Apr	305	170	55.74	0.891	0.477	0.647	1.226	0.896	0.507	0.647	1.240
May	220	130	59.09	1.021	0.906	0.719	1.452	1.035	0.849	0.727	1.472
Jun	285	137	48.07	0.655	0.010	0.473	0.905	0.674	0.020	0.484	0.939

Table 5. Continued

	Total vaginitis (*n* = 2,963)	Syndromic treatment given (*n* = 1,668, 56%)		Bivariate analysis				Multivariate analysis			
		n	%	OR	*p*-value	[95% CI]		OR	*p*-value	[95% CI]	
						LCL	UCL			LCL	UCL
Jul	190	92	48.42	0.664	0.027	0.462	0.955	0.693	0.053	0.478	1.005
August	239	114	47.70	0.645	0.011	0.459	0.906	0.651	0.016	0.460	0.922
September	220	143	65.00	1.313	0.135	0.918	1.878	1.336	0.114	0.932	1.915
October	188	118	62.77	1.192	0.355	0.821	1.730	1.212	0.316	0.832	1.767
November	243	160	65.84	1.363	0.081	0.962	1.932	1.358	0.088	0.956	1.929
December	111	54	48.65	0.670	0.072	0.433	1.036	0.684	0.093	0.439	1.066
Age (years)											
15–17	42	10	23.81	Ref							
18–22	576	287	49.83	3.178	0.002	1.534	6.585	2.981	0.004	1.407	6.317
23–27	867	457	52.71	3.567	0.001	1.732	7.346	3.345	0.001	1.588	7.044
28–32	667	381	57.12	4.263	0.000	2.062	8.814	4.006	0.000	1.895	8.469
33–37	358	224	62.57	5.349	0.000	2.548	11.230	4.903	0.000	2.286	10.519
38–42	241	169	70.12	7.511	0.000	3.507	16.089	6.973	0.000	3.185	15.265
43–47	101	72	71.29	7.945	0.000	3.462	18.232	7.734	0.000	3.311	18.067
48–52	59	36	61.02	5.009	0.000	2.073	12.100	4.702	0.001	1.922	11.499
53+	52	32	61.54	5.120	0.000	2.074	12.637	4.764	0.001	1.897	11.966

OR: odds ratio; CI: confidence interval; LCL: lower confidence limit; UCL: upper confidence limit.

At bivariate analysis, all three independent variables showed an association with prescriber adherence to the syndromic treatment guideline. The patient's age increase showed a positive correlation with prescriber adherence to the recommended syndromic treatment for vaginitis (Figure 3); at multivariate analysis, this association was statistically significant, peaking in the age group 43–47 years (aOR 7.73, 95% CI 3.31–18.07). Adherence to the syndromic treatment guideline was significantly less likely at five facilities compared to HealthCenter-1 at bivariate analysis; but due to the highly skewed data, that is, eight facilities contributing less than 0.5% of the data and one facility contributing 88% of the data, we did not assess for this association at multivariate analysis. The month patient was seen was associated with prescriber adherence to syndromic treatment; in relation to January, adherence was less likely during June and August (aOR 0.67, 95% CI 0.48–0.94) (Table 5).

Diagnosis–treatment mismatch in VVC

We omitted records lacking treatment data from this analysis. Of 894 records of women considered to have VVC, 12% received antibiotic-only prescriptions. Antifungal-only treatment was given in 64% (n = 672) of the non-vaginitis entries; the majority (48%) of these were judged to be urinary tract infections, and were concentrated in two facilities (Table 4 and Figure 4).

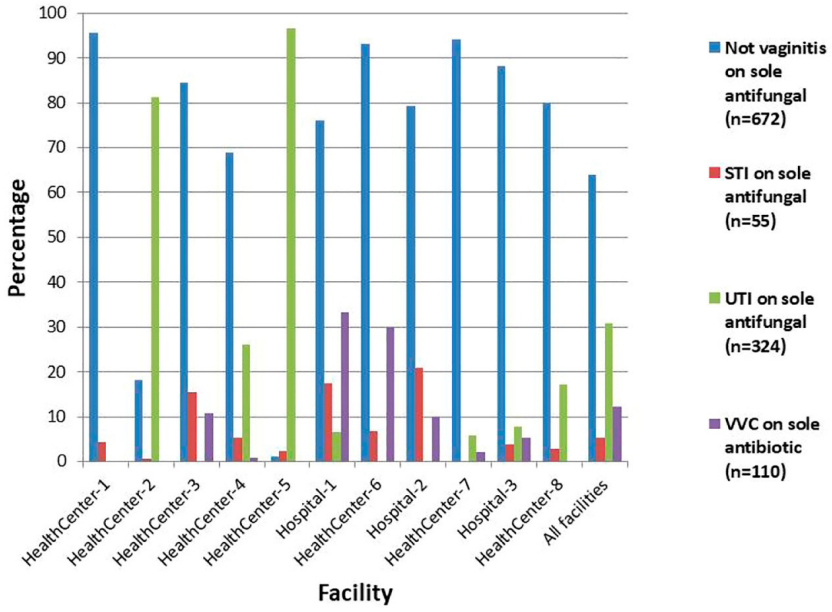

Figure 4. Proportion of lower genital tract symptoms patients with diagnosis-treatment mismatch by facility, year 2016.

STI: sexually transmitted infections; UTI: urinary tract infections; VVC: vulvovaginal candidiasis.

Discussion

In our large-scale assessment of routine clinical management of non-pregnant Kenyan women with LGTS, we found that about two-thirds of women, mostly young, were clinically categorized as cases of vaginitis. The proportion was significantly different across the health facilities and showed monthly variations. Observance of the guidelines' recommendations in the management of vaginal discharge syndrome was wanting and variant across the healthcare facilities, irrespective of facility category. A vaginal examination was barely performed widely while the specific non-syndromic diagnosis of VVC was applied frequently. Moreover, adherence to the syndromic treatment of vaginitis was low and correlated with the patient's age, while unnecessary antifungal prescription was common.

We were unique by the way we assessed vaginitis, conforming to the syndromic guidelines and expected practice. Previous studies from Kenya have not quantified vaginitis; instead, they determined the frequencies of the specific entities of the syndrome based on microbiological testing.[23,24] Using vaginitis-relevant symptoms as a surrogate, the rate of vaginitis from some past local studies concurs with our findings;[10,23] but in younger populations lower rates are noted.[25] Published work from elsewhere gives varied rates of vaginitis[3,5,26–29]; the dissimilarities are likely due to differences in study populations, settings, and diagnostic approaches.

The Kenyan RTI guidelines recommend a syndromic approach for the management of LGTS and vaginitis, in which there is no place for an etiological diagnosis. It is all the more remarkable that over two-thirds of the health facilities very often reported clinical VVC as a diagnosis. Particularly because symptoms of VVC are not very specific, self-diagnosis can result in overtreatment of up to two-thirds of women.[28] The combination of certain symptoms with signs on vaginal examination improves the accuracy in VVC diagnosis although studies give varied levels (33–89%) of sensitivity; however, combining clinical symptoms and signs gives a high positive predictive value, 90%, which may justify sole antifungal treatment.[28,30,31] Notably in our study, vaginal examination frequency overall was poor and mainly happened in only one-third of the health facilities; a pelvic examination may not always be feasible due patient reluctance or health worker factors.[32,33] Moreover, we detected very low prescriber compliance with the treatment guideline and wide inter-facility disparities. Still, a non-vaginitis diagnosis did not unconditionally exclude antifungal treatment either: inappropriate antifungal use was high. The phenomenon of patient's age influencing prescriber compliance, and variations in practice by time in the year were surprising. We speculate that these were occasioned by workplace context effects,[34] erratic supplies, and staffing changes.

Discrepancies in practice at health facilities that should be similarly staffed, equipped, and applying the same guidelines calls for further evaluation. Local studies have indeed noted the presence of insufficiencies in supplies and drugs, deficits in staff training and supervision, staffing shortages, and staff truancy.[35–37] The success of treatment algorithms relies on well-trained and compliant staff, as well as the provision of supportive structures for implementation. Even programs that seem successful at inauguration do later on encounter new challenges during scale-up, as evidenced by the Voluntary Medical Male Circumcision programs.[38] Besides, low adherence to guidelines has been identified elsewhere, including in settings expected to have less or none of the issues discussed above.[5] Therefore, institution of monitoring and evaluation of the guidelines' implementation is vital. For the management of RTIs, this can be accomplished through antimicrobial stewardship (AMS) programs.[39,40] We have revealed clear areas for improvement that should be subjected to further investigation to understand the determinants that hinder or facilitate adherence to the prescribed guideline for management of LGTS in women.

Our study was not without weaknesses. Being a retrospective review of records, we encountered missing records, especially on vaginal examination and treatment, clustered to some facilities. One health facility skewed the data in numbers and practice, and in some facilities, clinic notes were not available as these records are in books kept by patients. The impact of such missing data was however small due to the study's large data set: nonetheless, data from more than 4,000 patients could be evaluated. Secondly, it would have been valuable to compute recurrences of vaginitis and VVC; this however was not possible to measure because each facility allocated patient numbers independently yet patients likely visited multiple health facilities within the year. A prospective study design coordinated across the health facilities would be best for measuring recurrences.

Conclusion

Two-thirds of women presenting with LGTS had vaginitis syndrome, over one-third of whom received a tentative diagnosis of VVC, while the vaginal examination was infrequently performed. Implementation of the applicable syndromic treatment guidelines for vaginal discharge syndrome at the health facilities appeared to be inadequate, which should prompt the establishment of local AMS programs. Studies designed to conduct focus group discussions or in-depth interviews with healthcare workers are needed to identify the actual barriers associated with the low adherence to the guidelines.

Acknowledgments

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CHAPTER 3: EVALUATION AND OPTIMIZATION OF THE SYNDROMIC MANAGEMENT OF FEMALE GENITAL TRACT INFECTIONS IN NAIROBI, KENYA

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Abstract

Background: Genital tract infections pose a public health concern. In many low-middle-income countries, symptom-based algorithms guide treatment decisions. Advantages notwithstanding, this strategy has important limitations. We aimed to determine the infections causing lower genital tract symptoms in women, evaluated the Kenyan syndromic treatment algorithm for vaginal discharge, and proposed an improved algorithm.

Methods: This cross-sectional study included symptomatic non-pregnant adult women presenting with lower genital tract symptoms at seven outpatient health facilities in Nairobi. Clinical, socio-demographic information and vaginal swabs microbiological tests were obtained. Multivariate logistic regression analyses were performed to find predictive factors for the genital infections and used to develop an alternative vaginal discharge treatment algorithm (using 60% of the dataset). The other 40% of data was used to assess the performance of each algorithm compared to laboratory diagnosis.

Results: Of 813 women, 66% had an infection (vulvovaginal candidiasis 40%, bacterial vaginosis 17%, *Neisseria gonorrhoea* 14%, multiple infections 23%); 56% of women reported ≥ 3 lower genital tract symptoms episodes in the preceding 12 months. Vulvovaginal itch predicted vulvovaginal candidiasis (odds ratio (OR) 2.20, 95% CI 1.40–3.46); foul-smelling vaginal discharge predicted bacterial vaginosis (OR 3.63, 95% CI 2.17–6.07), and sexually transmitted infection (*Neisseria gonorrhoea, Trichomonas vaginalis*, *Chlamydia trachomatis*, *Mycoplasma genitalium*) (OR 1.64, 95% CI 1.06–2.55). Additionally, lower abdominal pain (OR 1.73, 95% CI 1.07–2.79) predicted sexually transmitted infection. Inappropriate treatment was 117% and 75% by the current and alternative algorithms respectively. Treatment specificity for bacterial vaginosis/*Trichomonas vaginalis* was 27% and 82% by the current and alternative algorithms, respectively. Performance by other parameters was poor to moderate and comparable between the two algorithms.

Conclusion: Single and multiple genital infections are common among women presenting with lower genital tract symptoms at outpatient clinics in Nairobi. The conventional vaginal discharge treatment algorithm performed poorly, while the alternative algorithm achieved only modest improvement. For optimal care of vaginal discharge syndrome, we recommend the inclusion of point-of-care diagnostics in the flowcharts.

Introduction

Female genital tract infections constitute a significant public health problem with high disease burden [1,2,3], which is even higher among pregnant women and those living with HIV [4, 5]. Common symptoms associated with lower genital tract infections (LGTI) include vaginal discharge, dysuria, lower abdominal pain, dyspareunia, and pruritus. Vaginal discharge is commonest among these, being present in up to 75% of women with LGTI [6, 7]. However, these symptoms are not specific for LGTI, for example, in sub-Saharan African studies 27–49% of women with vaginal discharge did not have an infection [8, 9]. Presence of vaginal discharge does not necessarily imply a manifestation of a pathological condition; it could be present as a normal physiological phenomenon.

The commonly detected LGTI include vulvovaginal candidiasis (VVC), bacterial vaginosis (BV) and sexually transmitted infections (STI) caused by *Trichomonas vaginalis* (TV), *Neisseria gonorrhoea* (NG), *Chlamydia trachomatis* (CT), and *Mycoplasma genitalium* (MG) [9]. The infections are responsible for significant morbidity in women negatively affecting quality of life, increasing the risk of HIV transmission, and possibly causing adverse gynaecological and obstetrical outcomes [10,11,12].

In many low- and middle-income countries, syndromic algorithms based on genital tract symptoms guide treatment decisions for genital tract infections. The Kenyan guidelines for reproductive tract infections 2018 [13] recommends a syndromic approach using algorithms based on the World Health Organization (WHO) guidelines [14]. For patients presenting with vaginal discharge and vulvovaginal itch, the algorithm for management of vaginal discharge syndrome is applied [13] (Fig. 1).

The use of laboratory tests to diagnose LGTI allows species identification and tailored treatment. Yet, these tests are not widely applied in many resource-limited countries, but are reserved for women whose symptoms persist after the syndromic treatment. The syndromic approach has the advantage of providing treatment to patients immediately at the initial visit and without laboratory-associated delays and costs [15]. Additionally, rapid initiation of treatment reduces further transmission of infections and increases treatment coverage. However, the downside of this approach is under-treatment or delayed treatment of infections due to misdiagnosis or a missed diagnosis, as well as overtreatment and therefore the unnecessary use of antimicrobials contributing to the development of antimicrobial resistance [15].

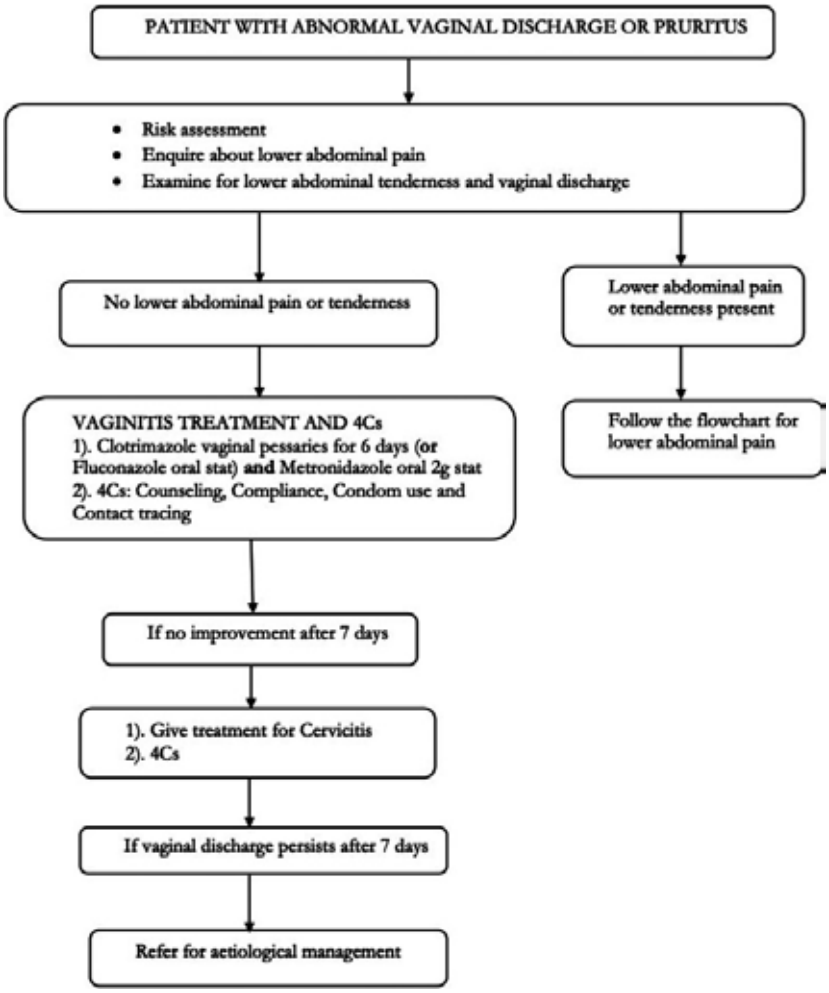

Fig. 1. Algorithm used in Kenya for management of Vaginal Discharge Syndrome [13]

Legend:
A risk assessment is performed, enquiry made on lower abdominal pain, and examination for lower abdominal tenderness carried out. Patients without lower abdominal pain or lower abdominal tenderness are given treatment for vaginitis which includes antifungal medication for VVC and metronidazole for BV and TV. Those with lower abdominal pain or lower abdominal tenderness are managed using the lower abdominal pain (LAP) flowchart. Patients with unresolved symptoms after 7 days are given treatment for cervicitis. Laboratory testing is recommended on the 3rd visit, fourteen days later, for those with persistent vaginal discharge

Studies have shown that the syndromic approach is inadequate in the management of STI [16,17,18,19]; however, there is scarcity of data from Kenya interrogating the performance of the syndromic approach in the management of vaginal discharge

syndrome/vaginitis. Hence, the question is whether the present guideline for management of vaginitis is performing well and if there is a need to improve it. The present study therefore aimed to evaluate and improve the performance of the vaginal discharge syndromic treatment algorithm currently in use in Kenya for the management of women presenting with lower genital tract symptoms (LGTS). For this purpose, we (1) analysed the LGTI causing the LGTS; (2) determined the social, demographic, behavioural, and clinical characteristics associated with the LGTI; (3) assessed the performance of the currently used vaginal discharge syndrome algorithm; (4) developed and evaluated an alternative algorithm.

Methods

Study aim, design and setting

This was a cross-sectional study to evaluate and improve the performance of the vaginal discharge syndromic treatment algorithm currently used in Kenya. The study was part of a larger study on VVC and recurrent VVC (RVVC), conducted between October 2018 and March 2020, among adult women presenting with LGTS at seven outpatient health facilities in Nairobi City County (NCC), Kenya. These health facilities serve a non-exclusive/ordinary population, and attend to large volumes of patients. During the study period, the management of LGTS followed a syndromic approach as described in the Kenyan guidelines for reproductive tract infections 2018 [13].

Inclusion and exclusion criteria

Included were women aged 18–50 years, presenting with LGTS, who gave informed consent. Participants were excluded if they were pregnant, menopausal, had genital malignancy, or tested positive for HIV or glycosuria, as determined in the larger study on VVC and RVVC. Diabetes mellitus, HIV and pregnancy are established factors associated with increased risk for various LGTI; to be more representative of the general population, we therefore excluded women with these conditions.

Participant recruitment and data collection

Health care workers at the participating outpatient clinics identified patients with LGTS during medical history taking and informed them of the study; interested patients were referred to the research room within the health facility prior to physical examination. At the research room, the study nurse gave study information to potential participants using the informed consent document, after which individuals willing to join the study provided informed consent. The following information was then collected using a standardized questionnaire: socio-demographic data (age,

marital status, education, occupation, and ethnicity/tribe), sexual behaviour, vaginal practices (douching, use of inserts), medical history including the occurrence of previous episodes of LGTS, and use of medications. Thereafter a urine sample was obtained for pregnancy and dipstick testing; pregnant participants were excluded from further participation and referred to the health facility's antenatal clinic. Measurements of temperature, weight, height, pulse rate, and blood pressure were obtained. A clinical officer then conducted a physical examination directed by symptoms, including vulvovaginal examination, and obtained vaginal swabs specimens via a vaginal speculum. Subsequently, the clinical officer offered treatment using the syndromic treatment guidelines (Fig. 1) [13]. Next, HIV counselling and testing was done and the results released in real time to the participant. Participants were asked to make a study follow-up visit for possible adaptation of the given treatment, based on the laboratory test results.

Laboratory testing

Urine was used to test for pregnancy by detection of Human Chorionic Gonadotropin, and for glycosuria using dipstick test. HIV-1 counselling and testing was performed according to the Kenya National HIV testing guideline, at the clinic in real-time with rapid-kit-testing using blood from finger pricking [20]. Vaginal smear specimens were tested for candidiasis by microscopic examination and culture on Sabouraud dextrose agar media; BV by the Nugent score; and for CT, NG, MG, and TV by multiplex Real Time polymerase chain reaction (PCR) test (Sacace Biotechnologies, Como Italy).

Outcomes measures

The outcome measures were: (1) the aetiology of LGTS (2) the association between patient characteristics (social, demographic, behavioural and clinical) and the aetiology of LGTS (3) the performance of the current vaginal discharge syndrome algorithm with respect to recommending appropriate/correct treatment for the LGTI (4) the development and performance of an alternative algorithm incorporating logistic regression-derived variables from outcome 2.

Definitions

Vaginal discharge with or without presence of foul smell, vulvar or vaginal itch/pruritus, vulvovaginal soreness or burning sensation, lower abdominal pain, dysuria, and dyspareunia were all defined as LGTS.

We distinguished VVC, BV, TV, CT, NG, and MG as cause of LGTI. STI were the infections caused by TV, CT, NG, and MG. VVC was defined as at least one positive

test from either direct microscopic examination or culture on Sabouraud dextrose agar. BV was defined as a Nugent score of 7 or above. CT, NG, MG, and TV were diagnosed by a positive PCR test. If all microbiological tests were negative, the LGTS were regarded to have a non-infectious cause.

Recurrence of LGTS was defined as 3 or more episodes of LGTS in the preceding 12 months, including the episode at the study visit; in women with confirmed VVC, this was defined as RVVC.

We defined the performance of a treatment algorithm (current or alternative algorithm) as the ability of the algorithm to recommend appropriate treatment according to the laboratory-based diagnosis.

For evaluation of the algorithms' performance, we classified the algorithm-based treatment recommendations as correct, inappropriate or missed. A treatment was defined as correct when the algorithm-recommended treatment was consistent with the microbiological diagnosis. For BV and TV, receiving metronidazole when either one was present was considered correct; for VVC the correct treatment was vaginal or oral antifungal. Treatment was regarded as inappropriate if a patient received irrelevant treatment with reference to the laboratory test results. There was missed treatment if the correct treatment would not be recommended despite a laboratory-confirmed infection. We defined correct treatment for any of CT, NG, and MG as referral to the lower abdominal pain (LAP) flowchart; by this we classified all VVC or BV-TV referred to the LAP by the current flowchart as missed treatment.

A patient could be classified into the correct treatment, and/or inappropriate treatment, and/or missed treatment categories at the same time.

Data analysis

Descriptive analyses consisted of frequencies (including percentages), mean (including standard deviation), and/or ranges as appropriate, to describe the study population and potential predictors. As an initial step to relate independent variables to the aetiology of LTGS, we calculated odds ratios (OR) and determined statistical significance using Chi-Square statistics. To further assess possible predictors of an aetiology/infection, multivariate binary logistic regression analyses were performed. We started with the purest groups, the mono-infections; patients with multiple infections were therefore included in more than one regression analysis.

The data were then randomly divided into a training and validation dataset in a ratio of 60:40 respectively [21]. A backward conditional logistic multivariate regression method was used on the training (60%) dataset. All statistically significant variables from the bivariate analysis were included in the model. With the most discriminative and plausible variables from this multivariate analysis an alternative algorithm was developed, while considering the relative frequency of each infection and predictor. The remaining 40% of the data were used to assess the algorithms' performance. For each patient in the validation dataset, correct, inappropriate, and/or missed treatment were assessed as defined above, and summarized for each treatment option in the current or alternative algorithm. In the current algorithm, treatment options were for VVC and BV-TV, and LAP referral. The alternative algorithm had the treatment options for VVC, BV-TV, LAP referral, and no infection. A McNemar test was performed to compare the classification of treatment groups, between the current and alternative algorithms.

The algorithm performance results obtained from the mono-infections did not warrant us to proceed to the next step i.e. to include the most common combinations of infections in the regression analysis. Hence the potential association between different types of infections was not assessed in this study.

We further determined the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the current and alternative algorithms for treatment allocation. Calculations were made using the laboratory test results as a reference or 'gold standard'. Sensitivity represented the probability that a patient with a certain infection or syndrome was correctly assigned by the algorithm to a treatment of that infection or syndrome. Specificity represented the percentage of patients without an infection who were assigned as not having to receive treatment by the algorithm. PPV was the probability that a patient who was allocated to an infection- or syndrome-specific treatment did in fact have that infection or syndrome; NPV as the probability that a patient who was not allocated to an infection-specific or syndrome-specific treatment would indeed not have that condition.

All analyses were performed using IBM SPSS Statistical Software (version 25). A p-value of < 0.05 was considered statistically significant.

Results

Participants' characteristics

Of the 856 women who provided written informed consent for the parent study, 8 (1%) were excluded due incomplete data, and 35 (4%) due to pregnancy (n = 24) and HIV positivity (n = 11), leaving a total of 813 participants for this analysis. The mean age of the participants was 29.5 years (standard deviation 7.1, range 18 to 50 years); 55% were married, and 34% were housewives or unemployed. Contraceptive (excluding condoms) use in the preceding 12 months was in 65%, and condom use during the preceding three months in 26% of participants, and 85% reported only 1 sexual partner in the preceding 3 months. (Table 1).

Table 1. Socio-demographic, clinical and sexual-behavioural characteristics of women presenting with LGTS at NCC outpatient clinics, Kenya

Characteristic		Total n (%)[#]	Infection n (%)	No infection n (%)	Odds Ratio[¥] (95% CI)	p-value
Overall		813 (100)	540 (66.4)	273 (33.6)		
Age	18–25 years	306 (38.4)	214 (69.9)	92 (30.1)	ref*	
	26–35 years	325 (40.8)	211 (64.9)	114 (35.1)	0.80 (0.57–1.11)	0.18
	> 35 years	166 (20.8)	105 (63.3)	61 (36.7)	0.74 (0.50–1.10)	0.14
Marital status	Single	271 (33.4)	186 (68.6)	85 (31.4)	ref*	
	Married	445 (54.8)	290 (65.2)	155 (34.8)	0.86 (0.62–1.18)	0.34
	Separated/ divorced/widow	96 (11.8)	64 (66.7)	32 (33.3)	0.91 (0.56–1.50)	0.72
Occupation	Unemployed, housewife	279 (34.4)	183 (65.6)	96 (34.4)	ref*	
	Professional worker	101 (12.4)	76 (75.2)	25 (24.8)	1.60 (0.95–2.67)	0.07
	Self employed	260 (32.0)	159 (61.2)	101 (38.8)	0.83 (0.58–1.17	0.29)
	Student	64 (7.9)	49 (76.6)	15 (23.4)	1.71 (0.91–3.21)	0.09
	Other	108 (13.3)	73 (67.6)	35 (32.4)	1.09 (0.68–1.76)	0.71
Educational level	None/primary	213 (26.2)	138 (64.8)	75 (35.2)	ref*	
	Secondary	363 (44.7)	245 (67.5)	118 (32.5)	1.13 (0.79–1.61)	0.51
	Tertiary	236 (29.1)	157 (66.5)	79 (33.5)	1.08 (0.73–1.60)	0.70

Table 1. Continued

Characteristic		Total n (%)[#]	Infection n (%)	No infection n (%)	Odds Ratio[¥] (95% CI)	p-value
Symptoms (LGTS)	Discharge curdy/curdled	640 (79.6)	433 (67.7)	207 (32.3)	0.81 (0.57–1.15)	0.24
	Discharge foul-smelling	230 (28.3)	174 (75.7)	56 (24.3)	0.54 (0.39–0.77)	**<0.001**
	Vulvovaginal itch or pruritus	613 (75.4)	419 (68.4)	194 (31.6)	0.71 (0.51–0.99	**0.04**
	Lower abdominal pain	233 (28.7)	142 (60.9)	91 (39.1)	0.71 (0.52–0.98)	**0.04**
	Vulvovaginal soreness	232 (28.5)	170 (73.3)	62 (26.7)	1.56 (0.46–0.90)	**0.01**
	Dysuria	363 (44.6)	231 (64.6)	132 (36.4)	1.25 (0.94–1.68)	0.13
	Dyspareunia	333 (41.0)	224 (67.3)	109 (32.7)	0.94 (0.70–1.26)	0.67
	Recurrent LGTS[@]	458 (56.3)	276 (60.3)	91 (25.6)	1.91 (1.41–2.59)	**<0.001**
Clinical signs	Abdominal tenderness	29 (3.6)	19 (65.5)	10 (34.5)	1.04 (0.48–2.27)	0.92
	Genital excoriations/ ulcers	58 (7.2)	40 (69.0)	18 (31.0)	0.89 (0.50–1.59)	0.70
	Genital erythema/ redness	135 (16.7)	92 (68.1)	43 (31.9)	0.92 (0.62–1.37)	0.68
	Genital vesicles	15 (1.9)	12 (80.0)	3 (20.0)	0.49 (0.14–1.76)	0.27
	Genital oedema	38 (4.7)	25 (65.8)	13 (34.2)	1.04 (0.52–2.07)	0.91
	Genital growths/warts	20 (2.5)	15 (75.0)	5 (25.0)	0.66 (0.24–1.83)	0.42
Contraceptive use in past 12 months	Contraceptive use ∝[1]	528 (64.9)	348 (65.9)	180 (34.1)	0.18 (0.70 – 0.37)	0.67
	Hormonal contraceptive ∝[2]	267 (32.8)	177 (66.3)	90 (33.7)	0.99 (0.72–1.35)	0.96
Antibiotic use in past 4 weeks		192 (23.6)	129 (67.2)	63 (32.8)	0.96 (0.68–1.35)	0.80
Antifungal use in past 4 weeks		83 (10.2)	51 (61.4)	32 (38.6)	1.27 (0.80–2.03)	0.31
Vaginal practices[@] present		269 (33.1)	190 (70.6)	79 (29.4)	1.33 (0.97–1.83)	0.07

Table 1. Continued

Characteristic		Total n (%)#	Infection n (%)	No infection n (%)	Odds Ratio¥ (95% CI)	p-value
Parity	0	203 (25.2)	134 (66.0)	69 (34.0)	ref*	
	1–2	441 (54.3)	290 (65.8)	151 (34.2)	0.59 (0.42–0.83)	**< 0.001**
	3 or more	166 (20.5)	114 (68.7)	52 (31.3)	1.13 (0.73–1.75)	0.59
Condom use past 3 months		208 (25.6)	134 (64.4)	74 (35.6)	0.89 (0.64–1.24)	0.48
Sex partners last 3 months	0 sex partners	82 (10.1)	50 (61.0)	32 (39.0)	ref*	
	1 sex partner	694 (85.4)	464 (66.9)	230 (33.1)	1.29 (0.81–2.07)	0.29
	2 or more sex partners	37 (4.5)	26 (70.3)	11 (29.7)	1.51 (0.66–3.48)	0.33
Last sexual contact	0 to 7 days	356 (46.3)	240 (67.4)	116 (32.6)	ref*	
	8 to 14 days	129 (15.9)	94 (72.9)	35 (27.1)	1.30 (0.83–2.03)	0.25
	More than 14 days	284 (34.9)	182 (64.1)	102 (35.9)	0.86 (0.62–1.20)	0.38

LGTS: Lower genital tract symptoms, NCC: Nairobi City County, CI: confidence interval

#The first column in this table uses column percentages to show the distribution of participants over the different variable categories; for the other columns row percentages are used

¥Odds for infection

*For variables with multiple categories, the first category was used as reference for the odds ratio

@Recurrent LGTS: 3 or more LGTS episodes in preceding 12 months, including the episode during the study visit

∝1All contraceptives excluding condoms - hormonal, tubal ligation, intrauterine device, natural/herbal

∝2Hormonal contraceptives - oral pills, Norplant, injectables

⊗Vaginal practices - douching, use of vaginal inserts

All the participants reported having vaginal discharge. The next most common symptom was vulvovaginal itch (75%), followed by dysuria (45%) and dyspareunia (41%). Experience of three or more LGTS episodes in the preceding 12 months was reported by 56% of the participants. Clinical signs were infrequent, ranging from 2% (genital vesicles) to 17% (vulvovaginal erythema). Recurrence of LGTS and the symptoms of foul-smelling vaginal discharge, vulvovaginal itch, lower abdominal pain, and vulvovaginal soreness were more prevalent in women with a laboratory confirmed infection compared to those without an infection. (Table 1) The distribution of participant characteristics by specific aetiologies are presented in Supplementary Tables S1 – S8.

Table 2. Laboratory confirmed infections in women presenting with LGTS at outpatient clinics in NCC, Kenya (n = 813)

Infection type	Overall* n (%) 813 (100)	Single infection n (%)	Dual infections n (%)	≥ 3 infections n (%)	Combinations in the dual infections					
					MG	NG	CT	TV	BV	VVC
Vulvovaginal Candidiasis (VVC)	325 (40.0)	207 (63.7)	84 (25.8)	34 (10.5)	7	19	17	14	27	207
Bacterial vaginosis (BV)	137 (17.1)	57 (41.6)	52 (38.0)	28 (20.4)	4	6	10	5	57	
Trichomonas vaginalis (TV)	76 (9.8)	27 (35.5)	27 (35.5)	22 (29.0)	0	7	1	27		
Chlamydia trachomatis (CT)	97 (12.5)	21 (21.6)	37 (38.1)	39 (40.2)	2	7	21			
Neisseria gonorrhoea (NG)	111 (14.3)	39 (35.1)	39 (35.1)	33 (29.7)	0	39				
Mycoplasma genitalium (MG)	43 (5.6)	6 (14.0)	13 (30.2)	24 (55.8)	6					
Total	540 (66.4)	357 (66.1)	126 (23.3)	57 (10.6)	13	39	37	27	52	84

*The first column in this table uses column percentages to show the overall distribution of the various infections; for the other columns row percentages are used. Denominator for each infection is less by the respective missed tests. Missing laboratory tests per aetiology were: BV 12, TV 39, NG 39, CT 39, MG 39

LGTS: Lower genital tract symptoms

NCC: Nairobi City County

Laboratory confirmed aetiologies for LGTS

Of the 813 participants, 540 (66%) had at least one infection. The prevalence of the specific infections was: VVC 40% with 52% of these being RVVC, BV 17%, NG 14%, CT 13%, TV 10%, and MG 6%. Overall, there were 183 (23%) participants with two or more infections, of whom 126 (69%) had dual infections while the rest had multiple (three or more infections), mostly among the STI. (Table 2)

Predictors for the aetiologies

The multivariate logistic regression analysis showed that only vulvovaginal itch was associated with the diagnosis VVC (OR 2.20, 95% CI 1.40–3.46). A foul-smelling vaginal discharge was the predictor for BV (OR 3.63, 95% CI 2.17–6.07), while dysuria (OR 0.46, 95% CI 0.27–0.80) and dyspareunia (OR 0.46, 95% CI 0.26–0.82) were negatively predictive of BV. The predictors of any STI were a foul-smelling vaginal discharge (OR 1.64, 95% CI 1.06–2.55), and lower abdominal pain (OR 1.73, 95% CI 1.07–2.79); while recurrent LGTS episodes was negatively predictive of STI (OR 0.45, 95% CI 0.29–0.68). For individual STI, TV was predicted by having a foul-smelling vaginal discharge (OR 2.49, 95% CI 1.29–4.82) and lower abdominal pain (OR 3.02, 95% CI 1.23–7.42); a low level of education was negatively predictive of NG (OR 0.50, 95% CI 0.26–0.95); recurrent LGTS episodes was negatively predictive of CT (OR 0.44, 95% CI 0.25–0.81) and NG (OR 0.46, 95% CI 0.27–0.76); and condom use in the previous 3 months was negatively predictive of MG (OR 0.39, 95% CI 0.17–0.91). Lastly recurrent LGTS episodes was predictive of absence of an infection (OR 2.00, 95% C1.32-3.13); while having foul-smelling vaginal discharge (OR 0.46, 95% CI 0.29–0.74), and lower abdominal pain (OR 0.62, 95% CI 0.39–0.97) were negatively predictive of absence of infection. (Table 3 and Supplementary tables S9 -S16).

Development of an alternative algorithm

Applying the predictors from the multivariate logistic regression analyses, we developed a flowchart (alternative algorithm) categorizing patients for treatment. Starting with the most prevalent infection, vulvovaginal itch was applied to identify patients for VVC treatment, next foul-smelling vaginal discharge was applied to identify patients for BV-TV treatment, and finally lower abdominal pain was used to identify patients with CT, NG, MG for referral to LAP flowchart. Then patients with no vulvovaginal itch, foul-smelling discharge and lower abdominal pain were categorized for no treatment (Fig. 2).

Table 3. Statistical associations between participant characteristics and aetiologies in multivariate logistic regression (n = 507)

Aetiology	Characteristic	Number of participants n* (%)		OR (95% CI)	p-value
		With the aetiology n (%)	Without the aetiology n (%)		
VVC	Total	204 (100.0)	303 (100)		
	Itch or pruritus	172 (84.3)	215 (71.0)	2.20 (1.40–3.46)	< 0.001
BV	Total	94 (100%)	406 (100)		
	Foul smell	48 (51.1)	106 (26.1)	3.63 (2.17–6.07)	< 0.001
	Dysuria	28 (29.8)	206 (50.7)	0.46 (0.27–0.80)	0.01
	Dyspareunia	25 (26.6)	185 (45.6)	0.46 (0,26-0.82)	0.01
Any STI	Total	136 (100.0)	347 (100.0)		
	Foul smell discharge	45 (33.1)	104 (30.0)	1.64 (1.06–2.55)	0.03
	LAP	106 (77.9)	242 (69.7)	1.73 (1.07–2.79)	0.03
	Recurrent LGTS@	59 (43.4)	211 (60.8)	0.45 (0.29–0.68)	< 0.001
TV	Total	53 (100.0)	430 (100.0)		
	Foul smell discharge	27 (50.9)	122 (28.4)	2.49 (1.29–4.82)	0.01
	LAP	44 (83.0)	304 (70.7)	3.02 (1.23–7.42)	0.02
CT	Total	62 (100.0)	421 (100.0)		
	Recurrent LGTS@	26 (41.9)	244 (58.0)	0.44 (0.25–0.81)	0.01
NG	Total	79 (100.0)	404 (100.0)		
	≤ Primary education	15 (19.0)	109 (27.0)	0.50 (0.26–0.95)	0.03
	Recurrent LGTS@	33 (41.8)	237 (58.7)	0.46 (0.27–0.76)	< 0.001
MG	Total	28 (100.0)	455 (100.0)		
	Condom use†	12 (42.9)	122 (26.8)	0.39 (0.17–0.91)	0.03
None	Total	165 (100.0)	342 (100.0)		
	Foul smell discharge	34 (20.6)	121 (35.4)	0.46 (0.29–0.74)	< 0.001
	LAP	55 (33.3)	88 (25.7)	0.62 (0.39–0.97)	0.04
	Recurrent LGTS@	108 (65.5)	177 (51.8)	2.00 (1.32–3.13)	< 0.001

VVC - Vulvovaginal candidiasis; BV - Bacterial vaginosis; STI – Sexually transmitted infection; TV - Trichomonas vaginalis; NG - Neisseria gonorrhoea; CT - Chlamydia trachomatis; MG - Mycoplasma genitalium
LAP – Lower abdominal pain; LGTS: Lower genital tract symptoms; OR: Odds ratio; CI: Confidence interval
*Denominator for each infection is less by the respective missed tests
@Recurrent LGTS: 3 or more LGTS episodes in preceding 12 months, including the episode during the study visit
†Condom use: Condom use in preceding 3 months

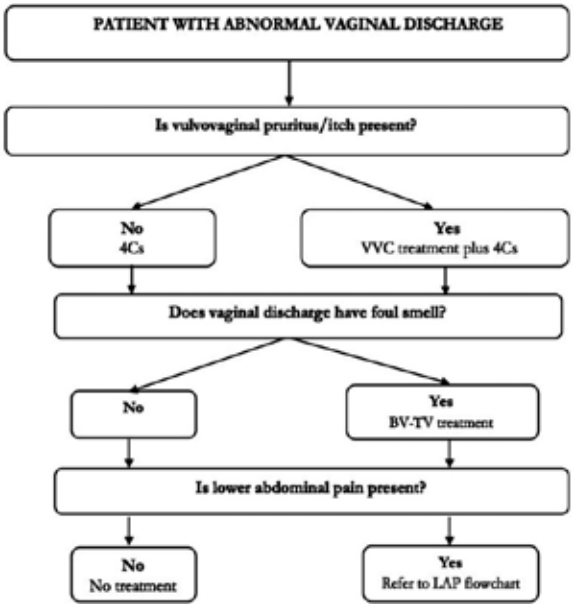

Fig. 2. Alternative algorithm for management of vaginal discharge syndrome in Kenya

Legend:
VVC - Vulvovaginal candidiasis; BV - Bacterial vaginosis; TV - Trichomonas vaginalis
VVC treatment = Antifungal (intravaginal or oral)
BV-TV treatment = Metronidazole;
LAP: Lower abdominal pain
4Cs: Counseling, Compliance, Condom use and Contact tracing

Performance of the current and alternative algorithms in the diagnosis and treatment of LGTI

Classification of participants by treatment category

From the 306 validation participants the treatment categories by laboratory testing were 121 (40%) VVC, 66 (22%) BV-TV, and 64 (21%) LAP. By the current syndromic algorithm 68% (n = 209) of the 306 validation participants were classified into vaginitis treatment category (VVC plus BV- TV), while the rest (32%, n = 97) were classified into the LAP referral category. The current algorithm's ability to classify individuals into no treatment was – by definition – nil. The alternative algorithm classified participants into the treatment categories as follows: – 74% (n = 226) VVC, 23% (n = 71) BV-TV, and 28% (n = 86) LAP referral; in addition, 11% were classified into no treatment category. McNemar test showed that the treatment classification accuracy by the current and alternative algorithms for the categories VVC, BV-TV and No treatment differed significantly; $p = 0.04$, $p = 0.02$ and $p < 0.001$ respectively.

The classification for LAP treatment did not differ between the two algorithms, p = 0.5. (Table 4 and Supplementary table S17)

Table 4. Treatment allocation for LGTI/syndrome, by the current and alternative algorithms (n = 306)

Treatment category (n)	Correct treatment n (% of those with the infection)		Missed treatment n (% of those with the infection)		Inappropriate treatment n (% of those without the infection)		χ^2, p-value (McNemar)
	Current algorithm	Alternative algorithm	Current algorithm	Alternative algorithm	Current algorithm	Alternative algorithm	
VVC (121)	89 (73.6)	102 (84.3)	32 (26.4)	19 (15.7)	120 (64.9)	124 (67.0)	4.11, 0.04
BV-TV (66)	45 (68.2)	31 (47.0)	21 (31.8)	35 (53.0)	164 (68.3)	40 (16.7)	5.63, 0.02
LAP (64)	22 (34.4)	20 (31.3)	42 (65.6)	44 (68.8)	75 (31.0)	66 (27.3)	0.5, 0.5
No treatment (108)	0 (0.0) *	15 (13.9) *	108 (100) #	93 (86.1) #	198 (100) ¥	93 (47.0) ¥	13.07, p < 0.001

McNemar analysis: Comparisons are per correct treatment and missed treatment; inappropriate treatment was (per definition) not included in the analysis
LGTI: Lower genital tract infections
VVC: Vulvovaginal candidiasis
BV-TV: Bacterial Vaginosis-Trichomonas vaginalis
LAP: Lower abdominal pain; includes any of Neisseria gonorrhoea, Chlamydia trachomatis, Mycoplasma genitalium
*Participants without an infection that were correctly classified to receive no treatment
Participants (with an infection) who needed treatment but were incorrectly classified into No treatment
¥ Participants (with no infection) who didn't require treatment but received it, of those not requiring treatment

Algorithms' accuracy in overall treatment allocation

By the current algorithm, the overall rate of correct treatment was 51% (n = 156), inappropriate treatment was 117% (n = 359), while missed treatment was 31% (n = 95). The rates by the alternative algorithm were 50% (n = 153) correct treatment, 75% (n = 230) inappropriate treatment, and 32% (n = 98) missed treatment. (Fig. 3)

Algorithms' accuracy in specific treatment category allocation

By the current algorithm, 74%, 68%, 34% and 0% of participants with VVC, BV-TV, LAP, and No infection respectively, were correctly treated, while by the alternative algorithm 84%, 47%, 31%, and 14% of participants with VVC, BV-TV, LAP and no infection respectively would get correct treatment. Inappropriate treatment rates by the current algorithm were 65%, 68% and 31% for VVC, BV-TV and LAP respectively, and 100% for the 'no infection' group; while by the alternative algorithm inappropriate

treatment was 67%, 17%, and 27% for VVC, BV-TV and LAP respectively, and 47% for the no infection group. Failure to give the required treatment was lowest for VVC and highest for LAP group, by both algorithms. (Table 4)

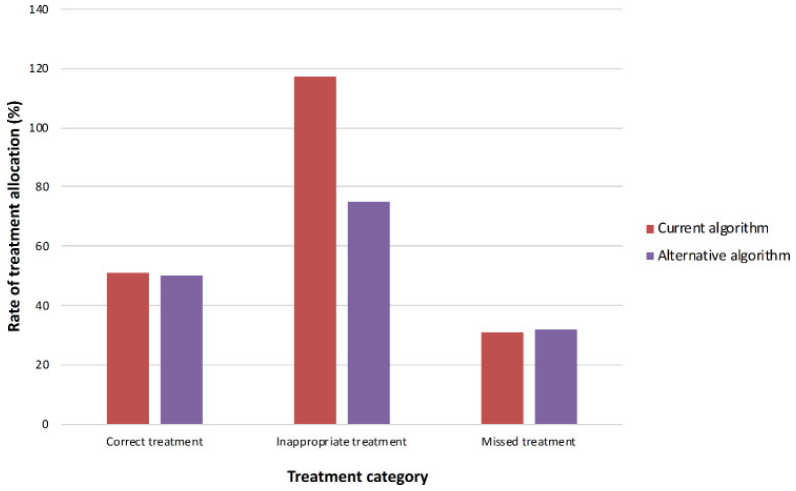

Fig. 3. Overall LGTI treatment allocation rates by the current and alternative algorithms (n = 306)

Legend:

LGTI: Lower genital tract infections

Correct treatment: Treatment consistent with the microbiological diagnosis

Inappropriate treatment: Irrelevant treatment with reference to the laboratory test results

Missed treatment: The necessary treatment not recommended despite laboratory-confirmed infection

Performance scores in treatment allocation

The performance scores in treatment allocation for VVC treatment by both algorithms were similar i.e. high sensitivity (> 73%), moderate NPV (> 66%), and low specificity and PPV (33-45%). For BV-TV treatment, the specificity by the current algorithm was curiously low (32%), but notably high by the alternative algorithm (82%); the NPV for BV-TV treatment was 78% and 85% by the current and alternative algorithms respectively. For LAP referral the performance of both algorithms was similar: - low sensitivity (< 35%), low PPV (< 24%), moderate specificity (about 70%), and a fairly high NPV (80%). With regard to no treatment, the alternative algorithm had a specificity of 91%, a moderate NPV of 66%, but a poor sensitivity of 14%.

Overall, the alternative algorithm performed better than the current algorithm. This was especially notable in BV-TV treatment - accuracy scores of 74% compared to 40%; and in allocation into No treatment, accuracy of 64% versus 0%. (Table 5)

Table 5. Performance scores in LGTI treatment allocation by the current and alternative algorithms

	VVC treatment		BV-TV treatment		LAP treatment*		No Treatment	
	Current algorithm	Alternative algorithm	Current algorithm	Alternative algorithm	Current algorithm	Alternative algorithm	Current algorithm	Alternative algorithm
Statistic	**Value(95% CI)**	**Value(95% CI)**	**Value(95% CI)**	**Value(95% CI)**	**Value(95% CI)**	**Value(95% CI)**	**Value(95% CI)**	**Value(95% CI)**
Sensitivity (%)	73.6 (64.8–81.2)	84.3 (76.6–90.3)	68.2 (55.6–79.1)	47.0 (34.6–59.7)	34.4 (23.0-47.3)	31.3 (20.2–44.1)	-	13.9 (8.0-21.9)
Specificity (%)	35.1 (28.3–42.5)	33.0 (26.3–40.3)	31.7 (25.8–38.0)	81.7 (76.2–86.4)	69.0 (62.8–74.8)	71.1 (64.9–76.7)	-	90.9 (86.0-94.5)
PPV (%)	42.6 (35.8–49.6)	45.1 (38.5–51.9)	21.5 (16.2–27.7)	41.3 (30.1–53.3)	22.7 (14.8–32.3)	22.2 (14.1–32.2)	-	45.5 (28.1–63.7)
NPV (%)	67.0 (56.7–76.2)	76.3 (65.4–85.1)	78.4 (68.8–86.1)	84.9 (79.6–89.2)	79.9 (73.8–85.1)	79.6 (73.6–84.8)	-	65.9 (60.0-71.5)
Accuracy (%)	50.3 (44.6–56.1)	53.3 (47.5–59.0)	39.5 (34.0-45.3)	74.2 (68.9–79.0)	61.8 (56.1–67.2)	62.8 (57.1–68.2)	-	63.7 (58.1–69.1)

LGTI: Lower genital tract infections, VVC: Vulvovaginal candidiasis, BV-TV: Bacterial Vaginosis-Trichomonas vaginalis, LAP: Lower abdominal pain; includes any of Neisseria gonorrhoea, Chlamydia trachomatis, and Mycoplasma genitalium

VVC treatment = Antifungal (intravaginal or oral)

BV-TV treatment = Metronidazole

*LAP treatment = Referral to LAP syndromic treatment algorithm

PPV - Positive Predictive Value

NPV - Negative Predictive Value

Discussion

Two-thirds of the women presenting with LGTS at outpatient clinics in Nairobi had at least one confirmed LGTI, more than one third of the infected had multiple infections, and a majority reported at least 3 episodes in a year. VVC was the most frequent infection (2 of 5 women) while BV prevalence was remarkably low. Symptoms of vaginitis were predominant in these mostly young women, but clinical signs were scanty. Contraceptive use was high; condom use was low, in line with a predominance of reported monogamous relationships. The vaginal discharge syndrome algorithm used in Kenya proved to be insufficient for the management of genital infections, but our proposed alternative achieved only modest improvement.

The frequencies of specific genital infections in our study vary from rates detected previously at similar clinics in Nairobi in which VVC was 6% higher and TV was double, but NG and CT rates were lower [22]. With vaginal discharge and itch being the commonest clinical presentations and coupled with recurrent symptoms, we speculate that a high usage of vaginitis (VVC and BV-TV) treatment with delayed opportunity for STI treatment over the years may explain these variations. Our detection of an infection in two-thirds of patients is similar to proportions in studies from elsewhere in Africa [8, 9], but somewhat lower than studies from India (80%) [7]. For the specific female genital infections, our findings do not concur with other studies from Africa where BV was predominant [2, 8, 9, 17]. These variations are likely due to study population differences. Indeed, we noted associations between patient characteristics and the infections. Influence by study population characteristics such as sexual risk behaviour, level of education, age, condom use, prior use of antimicrobials, and perhaps genetics have been cited as determinants of aetiology in other studies [3, 8, 9, 17, 23].

About one-third of the patients in our study tested negative to the six common LGTI despite being symptomatic. We think that the probability of false negative test results due to prior antimicrobial use is small because we used very sensitive testing methods. We employed PCR for detection of the four STIs; this technique would identify even antimicrobial-suppressed bacteria and TV. For Candida infection we employed 3 techniques i.e., KOH, gram stain and culture, hence the possibility of false negative cases was low. For bacterial vaginosis we used the Nugent score, which is the gold standard.

Although the vaginal discharge syndrome tool has the advantage of providing treatment to patients at an opportune time and without the laboratory-testing-

associated delays and costs, we identified concerning discrepancies between the syndromic predictions and actual infections. Hence, we sought to improve the algorithm's accuracy by determining patient characteristics more predictive of the infections. The symptoms of vulvovaginal itch for VVC and repulsive vaginal discharge for BV-TV were crucial in delineating the vaginitis syndrome to guide specific treatment for VVC and BV-TV, so as to reduce the over-use of metronidazole given the disparate burden of VVC and BV-TV. It is however worth noting that although important for detection of STI, LAP was an infrequent symptom hence contributing to the low sensitivity and PPV by both algorithms.

Our study showed no association between contraceptive use (including hormonal) and STI in general or with specific STI. Although controversial, studies in the past have pointed toward higher likelihood of some STI in clients using hormonal contraceptives. Indeed, a recent systematic review and meta-analysis on studies investigating the influence of hormonal contraceptives on STI reports mixed findings that included no effect, a protective effect, and increased risk [24].

The significant association between symptom recurrence and absence of infection was unexpected, especially because we employed DNA detection for most microbes. We speculate that, in addition to the effects of prior antimicrobial use in almost one quarter our participants, vaginal pathobionts (not tested in our study) could partly explain this; additionally women may not be able to distinguish between physiologic and abnormal leucorrhoea, hence overreport vaginal discharge as has been shown elsewhere [25]. Future studies are necessary, to elucidate this.

A syndromic-only approach can be misleading as a diagnosis and treatment tool. Indeed, we demonstrate here that the vaginal discharge syndrome algorithm used in Kenya is poor at detecting or excluding infections. With the algorithm's low specificity and PPV, about two-thirds of patients in our study received unnecessary metronidazole and antifungal treatment, while a similar proportion of patients requiring treatment for bacterial STI did not receive it. Both algorithms had low sensitivity and poor PPV scores for STI. Our findings are in line with other studies which have revealed the inadequacies of the syndromic flowcharts in diagnosis and treatment of female genital infections [18, 19, 26]. Our substitute algorithm had advantages over the current algorithm. By avoiding blanket treatment of VVC, BV and TV, our algorithm performed better for BV-TV treatment by lowering the unnecessary use of metronidazole, and somewhat for VVC treatment too. Our substitute algorithm also recognized women without infection leading to less overtreatment.

Vaginal discharge-based syndromic approaches have been shown in the past, and confirmed in this study, to miss common bacterial STI. This however should not motivate for inclusion of bacterial STI treatment to these algorithms. Such a move would result in unnecessary use of antibiotics in three-quarters of STI-free patients, posing the risk of development of antimicrobial resistance. We rather advocate that the savings from such unnecessary antibiotic prescriptions instead be channelled to point-of-care (POC) testing costs for patients triaged to have STI by the syndromic algorithm. A broad interrogation of the syndromic approach, beyond accuracy in treatment allocation, is needed to determine the full value of our proposal.

Being symptom-dependent, the syndromic flowcharts are poor at detecting mixed infections, yet we found this to be common, particularly with STI whose mode of transmission and clinical presentation are shared. Several studies have shown coupling of some infections, especially TV with BV and with bacterial STI [27,28,29]; improved/future algorithms should thus take this into consideration. Moreover, the symptom-dependent approaches do not recognize the existence of asymptomatic infections, yet studies show that up to 80% of patients with TV or BV are asymptomatic [2, 30]; these patients remain unrecognized and not treated in the symptom-dependent algorithms.

Efforts by others elsewhere to improve the syndromic algorithm's performance have yielded limited improvement. Such attempts included addition of sexual partner risk behavior information, and bedside tests e.g. vaginal swab pH and whiff test [17, 31,32,33]. The problems are that different etiologies share similar clinical characteristics and certain patients lack certain symptoms and signs despite having the disease; additionally, symptoms are largely subjective. For example, vulvovaginal itch is more common in women with VVC, but a large proportion of women with other infections also have it; and foul-smelling vaginal discharge is associated with BV, TV and STI [17, 26, 34, 35]. Hence the algorithm's performance is limited by indistinguishable behavioral factors, and symptoms and signs. The result of this is a suboptimal sensitivity and specificity. Therefore, only with integration of POC into the algorithms is good discriminative power achievable.

POC testing is feasible and accepted by women [36]. Such tests would be crucial in delineating mixed infections, asymptomatic infections or deciphering infections with shared symptom(s). For example, inclusion of POC pH and biochemical testing, for BV and TV respectively, yields notable improvement in diagnostic accuracy [17]. Additionally, several studies show that real-time PCR testing for STI is very promising with high sensitivity and specificity. These rapid and accurate tests are relatively

affordable, making it possible to implement them in resource-limited settings. For such settings, a combination of syndromic triage plus POC testing would be best suited [37,38,39]. However, given the long-standing funding gaps in the public sector in these settings, widespread use of POC is unlikely to be realized in the immediate future. Therefore, while use of the syndromic approach remains the most feasible option, regular review and revision of the algorithms' performance in line with emerging evidence is vital. Relatedly, it is necessary to rethink the present approaches to algorithm evaluation; they are limited to diagnostic and treatment accuracy. We propose that future evaluations of algorithms be comprehensive and include short-term and long-term opportunity costs determinations, and cost-benefit analyses.

A limitation of our study is that a significant number of patients had multiple infections, and our analyses did not look at the influence of multiple infections on the predictors. However, we were able to interrogate the performance of the conventional vaginal discharge syndrome treatment flowchart using a large dataset. Our sizeable dataset additionally allowed us to subject our alternative algorithm to internal validation. Secondly, our study did not access patients who seek care at private healthcare facilities. We however believe that our study population is representative of women in Nairobi. While it is expected that patients of lower socioeconomic status would seek health care mainly from public health facilities, it has been shown that only one-third of patients from a slum in Nairobi seek care at public health services with a majority utilizing private facilities [40]. Notably, 43% of people in informal settlements in Nairobi have health insurance cover compared to a national proportion of 20% [41, 42]. Moreover, key bio-behavioral characteristics of the participants such as age, marital status etc., do not vary between those who use public and private facilities and therefore the prevalence and type of LGTS is not expected to vary.

Conclusion

Most symptomatic women had a genital infection including multiple infections, yet the algorithm in use was largely inadequate in offering the required treatment. A significant proportion of patients therefore were not given the correct treatment, but many also received unnecessary antimicrobials. This is the first time in Kenya that the performance of the syndromic algorithm presently used in the management of vaginitis has been interrogated, and an improved flowchart proposed and validated. Our alternative algorithm provides only modest improvement, especially

in reducing the inappropriate use of metronidazole. For timely and optimum management of genital tract infections in women, we recommend a combination of syndromic triage and POC testing.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Contributions

GSO-M, AJAMdV and JtO conceptualized the study, and contributed to the design of the study; GO-M, MAM, NN and MdK contributed to the acquisition and sorting of the data; GO-M, MdK, AT, MMO, JtO and AJAMdV contributed to the data analysis; GO-M, MdK, AT, MMO, JtO and AJAMdV contributed to interpretation of data; GO-M and MdK wrote the initial draft of this manuscript; and all authors provided input and feedback on succeeding drafts. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee granted ethical approval for this study, including the informed consent documents (P980/12/2016). We also obtained a research license (permit) from the National Commission for Science Technology and Innovation, and authorization from NCC. All participants gave informed consent prior to enrolment and participation in the study. All methods were performed in accordance with the relevant guidelines and regulations.

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Supplementary tables and Figures

Table S1: Socio-demographic and clinical characteristics in vulvovaginal candidiasis (VVC) positive and negative cases

	Candida positive n (%) n = 325	No candida n (%) n = 488	OR (95% CI, p-value)
Demographics			
Age			
18 to 25 years	119 (37.4)	187 (39.0)	ref*
26 to 35 years	141 (44.3)	184 (38.4)	0.83 (0.60-1.14, 0.25)
36 and above	58 (18.2)	108 (22.5)	1.19 (0.80-1.76, 0.40)
Educational level			
None/primary	83 (25.5)	130 (26.7)	ref*
Secondary	143 (44.0)	220 (45.2)	0.98 (0.70-1.39, 0.92)
Tertiary	99 (30.5)	137 (28.1)	0.88 (0.61-1.29, 0.52)
Marital status			
Single	109 (33.5)	162 (33.3)	ref*
Married	186 (57.2)	259 (53.2)	0.94 (0.69-1.27, 0.68)
Separated, divorced or widowed	30(9.2)	66 (13.6)	1.48 (0.90-2.43, 0.12)
Occupation			
Unemployed, housewife	110 (33.8)	169 (34.7)	ref*
Professional worker	45 (13.8)	56 (11.5)	
Self employed	103 (31.7)	157 (32.2)	
Student	28(8.6)	36(7.4)	
Other	39 (12.0)	69 (14.2)	
Ethnicity			
Other	72 (22.4)	126 (26.0)	ref*
Luhya	69 (21.4)	98 (20.2)	0.82 (0.53-1.24, 0.33)
Kikuyu	129 (40.1)	192 (39.6)	0.85 (0.59-1.23, 0.39)
Kamba	52 (16.1)	69 (14.2)	0.76 (0.48-1.20, 0.24)
Symptoms			
Discharge curdy/curdled	269 (83.0)	371 (77.3)	1.44 (1.00-2.06, **0.05**)
Discharge foul smell	91 (28.0)	139 (28.5)	0.98 (0.72-1.33, 0.88)
Vulvar itch or pruritus	274 (84.3)	339 (69.5)	2.36 (1.66-3.37, **0.00**)
Lower abdominal pain	83 (25.5)	150 (30.7)	0.77 (0.56-1.06, 0.11)
Soreness	110 (33.8)	122 (25.0)	1.54 (1.13-2.09, **0.01**)
Erythema redness	58 (17.9)	77 (16.0)	1.15 (0.79-1.67, 0.47)
Dysuria	133 (40.9)	230 (47.1)	0.78 (0.59-1.03, 0.08)
Dyspareunia	147 (45.2)	186 (38.1)	1.34 (1.01-1.78, **0.04**)
Recurrent LGTS previous 12 months	168 (51.7)	290 (59.4)	0.73 (0.55-0.97, **0.03)**
Signs			
Abdominal tenderness	10(3.1)	19(3.9)	0.78 (0.36-1.71, 0.54)
Excoriations, ulcers or skin lesions	23(7.1)	35(7.3)	0.98 (0.57-1.69, 0.93)
Vesicles	8(2.5)	7(1.5)	1.72 (0.62-4.79, 0.30)

Table S1. Continued

	Candida positive n (%) n = 325	No candida n (%) n = 488	OR (95% CI, p-value)
Oedema	18(5.6)	20(4.1)	1.36 (0.71-2.61, 0.36)
Growth warts	12(3.7)	8(1.7)	2.28 (0.92-5.64, 0.07)
Use of contraceptives	214 (65.8)	314 (64.3)	1.07 (0.796-1.435, 0.66)
Contraceptives natural/herbal	21(6.5)	29(5.9)	1.09 (0.61-1.95, 0.76)
Contraceptives hormonal	100 (30.8)	167 (34.2)	0.85 (0.63-1.15, 0.31)
Contraceptives IUCD	47 (14.5)	49 (10.0)	1.52 (0.99-2.32, 0.06)
Contraceptives tubal ligation	3(0.9)	3(0.6)	1.51 (0.30-7.51, 0.62)
Vaginal practices	109 (33.5)	160 (32.8)	1.03 (0.77-1.39, 0.83)
Parity			
0	91 (28.1)	112 (23.0)	ref*
1-2	158 (48.8)	283 (58.2)	1.46 (1.04-2.04, **0.03**)
3 or more	75 (23.1)	91 (18.7)	0.99 (0.65-1.49, 0.95)
Sexual behaviour			
Condom use last 3 months	78 (24.0)	130 (26.6)	1.15 (0.83-1.59, 0.40)
Number of sexual partners previous 3 months			
0 partners	29(8.9)	53 (10.9)	ref*
1 partner	283 (87.1)	411 (84.2)	0.80 (0.49-1.28, 0.35)
2 or more partners	13(4.0)	24(4.9)	1.01 (0.45-2.28, 0.98)
Number of sexual partners previous 12 months			
0 partners	10(3.1)	26(5.3)	ref*
1 partner	262 (80.6)	378 (77.5)	0.56 (0.26-1.17, 0.12)
2 or more partners	53 (16.3)	84 (17.2)	0.61 (0.27-1.37, 0.23)
Days since last sexual contact			
0 to 7 days	139 (45.0)	217 (47.2)	ref*
8 to 14 days	59 (19.1)	70 (15.2)	0.76 (0.51-1.14, 0.19)
More than 14 days	111 (35.9)	173 (37.6)	1.00 (0.73-1.37, 0.99)
Allergies	22(6.8)	32(6.6)	1.04 (0.59-1.82, 0.91)
BMI			
Under/normal weight	155 (52.4)	232 (50.1)	ref*
Overweight	100 (33.8)	156 (33.7)	1.04 (0.75-1.44, 0.80)
Obesity	41 (13.9)	75 (16.2)	1.22 (0.79-1.88, 0.36)
Medication use previous 4 weeks			
Antibiotics	89 (27.4)	103 (21.1)	1.41 (1.02-1.95, **0.04**)
Antifungals	30(9.2)	53 (10.9)	0.84 (0.52-1.34, 0.45)
Steroids	11(3.4)	16(3.3)	1.03 (0.47-2.26, 0.93)
None	184 (55.6)	290 (59.4)	0.89 (0.67-1.18, 0.43)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. e.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S2: Socio-demographic and clinical characteristics in bacterial vaginosis (BV) positive and negative cases

	BV positive n (%) n = 137	No BV n (%) n = 664	OR (95% CI, p-value)
Demographics			
Age			
18 to 25 years	61 (46.2)	239 (36.6)	ref*
26 to 35 years	47 (35.6)	275 (42.1)	1.49 (0.98-2.27, 0.06)
36 and above	24 (18.2)	139 (21.3)	1.48 (0.88-2.48. 0.14)
Educational level			
None/primary	35 (25.5)	174 (26.2)	ref*
Secondary	62 (45.3)	297 (44.8)	0.96 (0.61-1.52, 0.87)
Tertiary	40 (29.2)	192 (29.0)	0.97 (0.59-1.59, 0.89)
Marital status			
Single	59 (43.1)	210 (31.7)	ref*
Married	56 (40.9)	379 (57.2)	1.90 (1.27-2.84, **0.00**)
Separated, divorced or widowed	22 (16.1)	74 (11.2)	0.95 (0.54-1.65, 0.84)
Occupation			
Unemployed, housewife	42 (30.7)	233 (35.1)	ref*
Professional worker	20 (14.6)	77 (11.6)	
Self employed	37 (27.0)	221 (33.3)	
Student	15 (10.9)	48(7.2)	
Other	23 (16.8)	84 (12.7)	
Ethnicity			
Other	28 (20.6)	169 (25.6)	ref*
Luhya	39 (28.7)	126 (19.1)	0.54 (0.31-0.92, **0.02**)
Kikuyu	60 (44.1)	255 (38.6)	0.70 (0.43-1.15, 0.16)
Kamba	9(6.6)	110 (16.7)	2.03 (0.92-4.46, 0.08)
Symptoms			
Discharge curdy/curdled	108 (79.4)	523 (79.6)	0.99 (0.63-1.56, 0.90)
Discharge foul smell	67 (48.9)	162 (24.4)	2.97 (2.03-4.33, **0.00**)
Vulvar itch or pruritus	92 (67.2)	512 (77.1)	0.61 (0.41-0.91, **0.01**)
Lower abdominal pain	36 (26.3)	193 (29.1)	0.87 (0.57-1.32, 0.51)
Soreness	37 (27.0)	191 (28.8)	0.92 (0.61-1.39, 0.68)
Erythema redness	18 (13.1)	114 (17.4)	0.72 (0.42-1.23, 0.23)
Dysuria	46 (33.6)	311 (46.8)	0.57 (0.39-0.84, **0.00**)
Dyspareunia	43 (31.4)	282 (42.5)	0.62 (0.42-0.92, **0.02**)
Recurrent LGTS previous 12 months	65 (47.4)	384 (57.8)	0.66 (0.45-0.95, **0.03)**
Signs			
Abdominal tenderness	6(4.4)	23(3.5)	1.28 (0.51-3.20, 0.60)
Excoriations, ulcers or skin lesions	11(8.0)	43(6.5)	1.25 (0.63-2.48, 0.53)
Vesicles	3(2.2)	12(1.8)	1.20 (0.34-4.32, 0.78)
Oedema	2(1.5)	35(5.3)	0.26 (0.06-1.11, 0.05)

Table S2. Continued

	BV positive n (%) n = 137	No BV n (%) n = 664	OR (95% CI, p-value)
Growth warts	5(3.6)	15(2.3)	1.62 (0.58-4.54, 0.35)
Use of contraceptives	89 (65.0)	430 (64.8)	1.01 (0.69-1.48, 0.96)
Contraceptives natural/herbal	8(5.8)	42(6.3)	0.92 (0.42-2.00, 0.83)
Contraceptives hormonal	48 (35.0)	215 (32.4)	1.13 (0.77-1.66, 0.55)
Contraceptives IUCD	18 (13.1)	75 (11.3)	1.19 (0.69-2.06, 0.54)
Contraceptives tubal ligation	0(0.0)	6(0.9)	1.21 (1.17-1.25, 0.26)
Vaginal practices	49 (35.8)	216 (32.5)	1.16 (0.79-1.70, 0.46)
Parity			
0	36 (26.5)	163 (24.6)	ref*
1-2	76 (55.9)	360 (54.4)	1.05 (0.68-1.62, 0.84)
3 or more	24 (17.6)	139 (21.0)	1.28 (0.73-2.25, 0.39)
Sexual behaviour			
Condom use last 3 months	32 (23.4)	174 (26.2)	0.86 (0.56-1.32, 0.49)
Condom use during last sexual contact	26 (19.0)	130 (19.6)	0.96 (0.60-1.54, 0.87)
Number of sexual partners previous 3 months			
0 partners	13(9.5)	68 (10.2)	ref*
1 partner	112 (81.8)	573 (86.3)	0.98 (0.52-1.83, 0.95)
2 or more partners	12(8.8)	23(3.5)	0.37 (0.15-0.92, **0.03**)
Number of sexual partners previous 12 months			
0 partners	1(0.7)	34(5.1)	ref*
1 partner	93 (67.9)	537 (80.9)	0.17 (0.02-1.26, **0.05**)
2 or more partners	43 (31.4)	93 (14.0)	0.06 (0.01-0.48. **0.00**)
Days since last sexual contact			
0 to 7 days	64 (47.4)	285 (45.8)	ref*
8 to 14 days	23 (17.0)	103 (16.6)	1.01 (0.59-1.70, 0.98)
More than 14 days	48 (35.6)	234 (37.6)	1.10 (0.73-1.65, 0.67)
Allergies	10(7.3)	42(6.3)	1.17 (0.57-2.39, 0.67)
BMI			
Under/normal weight	74 (56.5)	304 (49.4)	ref*
Overweight	39 (29.8)	214 (34.7)	1.34 (0.87-2.04, 0.18)
Obesity	18 (13.7)	98 (15.9)	1.33 (0.76-2.33, 0.33)
Medication use previous 4 weeks			
Antibiotics	17 (12.4)	169 (25.5)	0.42 (0.24-0.71, **0.00**)
Antifungals	12(8.8)	68 (10.2)	0.84 (0.44-1.60, 0.60)
Steroids	7(5.1)	20(3.0)	1.73 (0.72-4.19, 0.22)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. e.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S3: Socio-demographic and clinical characteristics in any STI (TV, MG, CT, NG) positive and negative cases

	Any STI positive n (%) n = 249	No STI n (%) n = 525	OR (95% CI, p-value)
Demographics			
Age			
18 to 25 years	112 (45.7)	178 (34.7)	ref*
26 to 35 years	85 (34.7)	223 (43.5)	1.65 (1.17-2.33, **0.00**)
36 and above	48 (19.6)	112 (21.8)	1.47 (0.97-2.22, 0.07)
Educational level			
None/primary	66 (26.5)	138 (26.3)	ref*
Secondary	121 (48.6)	228 (43.5)	0.90 (0.62-1.30, 0.58)
Tertiary	62 (24.9)	158 (30.2)	1.22 (0.81-1.85, 0.35)
Marital status			
Single	83 (33.3)	171 (32.6)	ref*
Married	133 (53.4)	291 (55.5)	1.06 (0.76-1.48, 0.72)
Separated, divorced or widowed	33 (13.3)	62 (11.8)	0.91 (0.56-1.50, 0.72)
Occupation			
Unemployed, housewife	89 (35.7)	180 (34.4)	ref*
Professional worker	31 (12.4)	62 (11.8)	
Self employed	62 (24.9)	185 (35.3)	
Student	26 (10.4)	34(6.5)	
Other	41 (16.5)	63 (12.0)	
Ethnicity			
Other	59 (24.0)	130 (24.9)	ref*
Luhya	76 (30.9)	87 (16.7)	0.52 (0.34-0.80, **0.00**)
Kikuyu	86 (35.0)	217 (41.6)	1.15 (0.77-1.70, 0.50)
Kamba	25 (10.2)	88 (16.9)	1.60 (0.93-2.74, 0.09)
Symptoms			
Discharge curdy/curdled	189 (76.8)	421 (81.0)	0.78 (0.54-1.13, 0.19)
Discharge foul smell	87 (34.9)	133 (25.3)	1.58 (1.14-2.19, **0.01**)
Vulvar itch or pruritus	187 (75.1)	401 (76.4)	0.93 (0.66-1.33, 0.70)
Lower abdominal pain	61 (24.5)	160 (30.5)	0.74 (0.53-1.04, 0.09)
Soreness	69 (27.7)	150 (28.6)	0.96 (0.69-1.34, 0.80)
Erythema redness	41 (16.7)	88 (16.9)	0.99 (0.66-1.48, 0.95)
Dysuria	120 (48.2)	227 (43.2)	1.22 (0.90-1.65, 0.20)
Dyspareunia	96 (38.6)	221 (42.1)	0.86 (0.63-1.18, 0.35)
Recurrent LGTS previous 12 months	113 (45.4)	318 (60.6)	0.54 (0.39-0.74, **0.00)**
Signs			
Abdominal tenderness	10(4.0)	17(3.2)	1.25 (0.56-2.78, 0.58)
Excoriations, ulcers or skin lesions	12(4.9)	44(8.4)	0.56 (0.29-1.08, 0.08)
Vesicles	7(2.8)	7(1.3)	2.16 (0.75-6.21, 0.15)
Oedema	11(4.5)	27(5.2)	0.86 (0.42-1.76, 0.68)
Growth warts	4(1.6)	15(2.9)	0.56 (0.18-1.70, 0.30)

Table S3. Continued

	Any STI positive n (%) n = 249	No STI n (%) n = 525	OR (95% CI, p-value)
Use of contraceptives	162 (65.1)	341 (65.0)	1.01 (0.73-1.38, 0.98
Contraceptives natural/herbal	17(6.8)	31(5.9)	1.17 (0.63-2.15, 0.62)
Contraceptives hormonal	85 (34.1)	169 (32.2)	1.09 (0.79-1.50, 0.59)
Contraceptives IUCD	25 (10.0)	68 (13.0)	0.75 (0.46-1.22, 0.24)
Contraceptives tubal ligation	1(0.4)	5(1.0)	0.42 (0.05-3.61, 0.41)
Vaginal practices	93 (37.3)	168 (32.0)	1.27 (0.92-1.74, 0.14)
Parity			
0	52 (20.9)	137 (26.2)	ref*
1-2	147 (59.0)	276 (52.8)	0.71 (0.49-1.04, 0.08)
3 or more	50 (20.1)	110 (21.0)	0.84 (0.53-1.33, 0.44)
Sexual behaviour			
Condom use last 3 months	73 (29.3)	123 (23.4)	1.36 (0.97-1.90, 0.08)
Condom use during last sexual contact	52 (20.9)	98 (18.7)	1.15 (0.79-1.68, 0.47)
Number of sexual partners previous 3 months			
0 partners	21(8.4)	58 (11.0)	ref*
1 partner	214 (85.9)	450 (85.7)	0.76 (0.45-1.29, 0.31)
2 or more partners	14(5.6)	17(3.2)	0.44 (0.19-1.05, 0.06)
Number of sexual partners previous 12 months			
0 partners	10(4.0)	23(4.4)	ref*
1 partner	186 (74.7)	428 (81.5)	1.00 (0.47-2.14, 1.00)
2 or more partners	53 (21.3)	74 (14.1)	0.61 (0.27-1.38, 0.23)
Days since last sexual contact			
0 to 7 days	112 (47.5)	228 (46.1)	ref*
8 to 14 days	46 (19.5)	74 (14.9)	0.79 (0.51-1.22, 0.29)
More than 14 days	78 (33.1)	193 (39.0)	1.22 (0.86-1.72, 0.27)
Allergies	21(8.4)	32(6.1)	1.42 (0.80-2.52, 0.23)
BMI			
Under/normal weight	122 (52.6)	243 (49.8)	ref*
Overweight	79 (34.1)	163 (33.4)	1.04 (0.73-1.46, 0.84)
Obesity	31 (13.4)	82 (16.8)	1.33 (0.83-2.12, 0.23)
Medication use previous 4 weeks			
Antibiotics	54 (21.7)	128 (24.4)	0.86 (0.60-1.23, 0.41)
Antifungals	22(8.8)	54 (10.3)	0.85 (0.50-1.42, 0.53)
Steroids	8(3.2)	18(3.4)	0.94 (0.40-2.18 (0.88)
None	144 (57.8)	309 (58.9)	0.96 (0.71-1.30, 0.79)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. E.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S4: Socio-demographic and clinical characteristics in Trichomonas vaginalis (TV) positive and negative cases

	TV positive n (%) n = 76	**No TV n (%) n = 698**	**OR (95% CI, p-value)**
Demographics			
Age			
18 to 25 years	22 (29.3)	268 (39.2)	ref*
26 to 35 years	32 (42.7)	276 (40.4)	0.71 (0.40-1.25, 0.23)
36 and above	21 (28.0)	139 (20.4)	0.54 (0.29-1.02, 0.06)
Educational level			
None/primary	30 (39.5)	174 (25.0)	ref*
Secondary	31 (40.8)	318 (45.6)	1.77 (1.04-3.02, **0.04**)
Tertiary	15 (19.7)	205 (29.4)	2.36 (1.23-4.52, **0.01**)
Marital status			
Single	20 (26.3)	234 (33.6)	ref*
Married	39 (51.3)	385 (55.2)	0.84 (0.48-1.48, 0.55)
Separated, divorced or widowed	17 (22.4)	78 (11.2)	0.39 (0.20-0.79, **0.01**)
Occupation			
Unemployed, housewife	30 (39.5)	239 (34.3)	ref*
Professional worker	6(7.9)	87 (12.5)	
Self employed	20 (26.3)	227 (32.6)	
Student	4(5.3)	56(8.0)	
Other	16 (21.1)	88 (12.6)	
Ethnicity			
Other	20 (26.7)	169 (24.4)	ref*
Luhya	27 (36.0)	136 (19.6)	0.60 (0.32-1.11, 0.10)
Kikuyu	23 (30.7)	280 (40.4)	1.44 (0.77-2.70, 0.25)
Kamba	5(6.7)	108 (15.6)	2.56 (0.93-7.01, 0.06)
Symptoms			
Discharge curdy/curdled	65 (85.5)	545 (79.0)	1.57 (0.81-3.06, 0.18)
Discharge foul smell	39 (51.3)	181 (25.9)	3.01 (1.86-4.87, **0.00**)
Vulvar itch or pruritus	61 (80.3)	527 (75.5)	1.32 (0.73-2.38, 0.36)
Lower abdominal pain	15 (19.7)	206 (29.5)	0.59 (0.33-1.06, 0.07)
Soreness	19 (25.0)	200 (28.7)	0.83 (0.48-1.43, 0.50)
Dysuria	38 (50.0)	309 (44.3)	1.26 (0.78-2.02, 0.34)
Dyspareunia	28 (36.8)	289 (41.4)	0.83 (0.51-1.35, 0.44)
Recurrent LGTS previous 12 months	34 (44.7)	397 (56.9)	0.61 (0.38-0.99, **0.04)**
Signs			
Abdominal tenderness	2(2.6)	25(3.6)	0.73 (0.17-3.13, 0.67)
Excoriations, ulcers or skin lesions	4(5.3)	52(7.5)	0.68 (0.24-1.95, 0.47)
Erythema/redness	19 (25.0)	110 (15.9)	1.76 (1.01-3.08, **0.04**)
Vesicles	5(6.6)	9(1.3)	5.34 (1.74-16.38, **0.00**)
Oedema	8 (10.5)	30(4.3)	2.60 (1.15-5.89, **0.02**)

Table S4. Continued

	TV positive n (%) n = 76	No TV n (%) n = 698	OR (95% CI, p-value)
Growth warts	1(1.3)	18(2.6)	0.50 (0.07-3.79. 0.49)
Use of contraceptives	50 (65.8)	453 (64.9)	1.04 (0.63-1.71, 0.88)
Contraceptives natural/herbal	6(7.9)	42(6.0)	1.34 (0.55-3.26, 0.52)
Contraceptives hormonal	27 (35.5)	227 (32.5)	1.14 (0.70-1.88, 0.60)
Contraceptives IUCD	10 (13.2)	83 (11.9)	1.12 (0.56-2.27, 0.75)
Contraceptives tubal ligation	1(1.3)	5(0.7)	1.85 (0.21-16.03, 0.57)
Vaginal practices	33 (43.4)	228 (32.7)	1.58 (0.98-2.56, 0.06)
Parity			
0	10 (13.2)	179 (25.7)	ref*
1-2	41 (53.9)	382 (54.9)	5.21 (0.26-1.06, 0.07)
3 or more	25 (32.9)	135 (19.4)	0.30 (0.14-0.65, **0.00**)
Sexual behaviour			
Condom use last 3 months	19 (25.0)	177 (25.4)	0.98 (0.57-1.69, 0.95)
Condom use during last sexual contact	14 (18.4)	136 (19.5)	0.93 (0.51-1.72, 0.82)
Number of sexual partners previous 3 months			
0 partners	10 (13.2)	69(9.9)	ref*
1 partner	62 (81.6)	602 (86.2)	1.41 (0.69-2.87, 0.35)
2 or more partners	4(5.3)	27(3.9)	0.98 (0.28-3.39, 0.97)
Number of sexual partners previous 12 months	6(7.9)	27(3.9)	ref*
0 partners	55 (72.4)	559 (80.1)	2.26 (0.89-5.71, 0.08)
1 partner	15 (19.7)	112 (16.0)	1.66 (0.59-4.68, 0.33)
2 or more partners			
Days since last sexual contact			
0 to 7 days	31 (43.1)	309 (46.9)	ref*
8 to 14 days	17 (23.6)	103 (15.6)	0.61 (0.32-1.14, 0.12)
More than 14 days	24 (33.3)	247 (37.5)	1.03 (0.59-1.81, 0.91)
Allergies	10 (13.2)	43(6.2)	2.31 (1.11-4.80, **0.02**)
BMI			
Under/normal weight	39 (53.4)	326 (50.4)	ref*
Overweight	23 (31.5)	219 (33.8)	1.14 (0.66-1.96, 0.64)
Obesity	11 (15.1)	102 (15.8)	1.11 (0.55-2.25, 0.77)
Medication use previous 4 weeks			
Antibiotics	16 (21.1)	166 (23.8)	0.86 (0.48-1.52, 0.59)
Antifungals	5(6.6)	71 (10.2)	0.62 (0.24-1.59, 0.32)
Steroids	1(1.3)	25(3.6)	0.36 (0.05-2.69, 0.30)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. e.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S5: Socio-demographic and clinical characteristics in Neisseria Gonorrhoea (NG) positive and negative cases

	NG positive n (%) n = 111	No NG n (%) n = 663	OR (95% CI, p-value)
Demographics			
Age			
18 to 25 years	52 (47.7)	238 (36.7)	ref*
26 to 35 years	38 (34.9)	270 (41.6)	1.55 (0.99-2.44, 0.06)
36 and above	19 (17.4)	141 (21.7)	1.62 (0.92-2.85, 0.09)
Educational level			
None/primary	24 (21.6)	180 (27.2)	ref*
Secondary	62 (55.9)	287 (43.4)	0.62 (0.37-1.02, 0.06)
Tertiary	25 (22.5)	195 (29.5)	1.04 (0.57-1.89, 0.90)
Marital status			
Single	39 (35.1)	215 (32.5)	ref*
Married	57 (51.4)	367 (55.4)	1.17 (0.75-1.82, 0.49)
Separated, divorced or widowed	15 (13.5)	80 (12.1)	0.97 (0.51-1.85, 0.92)
Occupation			
Unemployed, housewife	41 (36.9)	228 (34.4)	ref*
Professional worker	12 (10.8)	81 (12.2)	
Self employed	27 (24.3)	220 (33.2)	
Student	13 (11.7)	47(7.1)	
Other	18 (16.2)	86 (13.0)	
Ethnicity			
Other	29 (26.4)	160 (24.3)	ref*
Luhya	34 (30.9)	129 (19.6)	0.69 (0.40-1.19, 0.18)
Kikuyu	36 (32.7)	267 (40.6)	1.34 (0.79-2.28, 0.27)
Kamba	11 (10.0)	102 (15.5)	1.68 (0.80-3.51, 0.16)
Symptoms			
Discharge curdy/curdled	80 (74.1)	530 (80.5)	0.69 (0.43-1.11, 0.12)
Discharge foul smell	30 (27.0)	190 (28.7)	0.92 (0.59-1.45, 0.72)
Vulvar itch or pruritus	83 (74.8)	505 (76.2)	0.93 (0.58-1.48, 0.75)
Lower abdominal pain	29 (26.1)	192 (29.0)	0.87 (0.55-1.37, 0.54)
Soreness	35 (31.5)	184 (27.8)	1.20 (0.78-1.85, 0.41)
Dysuria	55 (49.5)	292 (44.0)	1.25 (0.83-1.87, 0.28)
Dyspareunia	47 (42.3)	270 (40.7)	1.07 (0.71-1.61, 0.75)
Recurrent LGTS previous 12 months	46 (41.4)	385 (58.1)	0.51 (0.34-0.76, **0.00)**
Signs			
Abdominal tenderness	5(4.5)	22(3.3)	1.37 (0.51-3.71, 0.53)
Excoriations, ulcers or skin lesions	4(4.6)	51(7.7)	0.58 (0.23-1.49, 0.25)
Erythema/redness	14 (13.0)	115 (17.4)	0.71 (0.39-1.28, 0.25)
Vesicles	1(0.9)	13(2.0)	0.47 (0.06-3.59, 0.45)
Oedema	4(3.7)	34(5.2)	0.71 (0.25-2.04, 0.52)
Growth warts	1(0.9)	18(2.7)	0.33 (0.04-2.52, 0.26)

Table S5. Continued

	NG positive n (%) n = 111	No NG n (%) n = 663	OR (95% CI, p-value)
Use of contraceptives	70 (63.1)	433 (65.3)	0.91 (0.60-1.38, 0.65)
Contraceptives natural/herbal	8(7.2)	40(6.0)	1.21 (0.55-2.66, 0.64)
Contraceptives hormonal	37 (33.3)	217 (32.7)	1.03 (0.67-1.58. 0.90)
Contraceptives IUCD	9(8.1)	84 (12.7)	0.61 (0.30-1.25, 0.17)
Contraceptives tubal ligation	0(0.0)	6(0.9)	0.86 (0.83-0.88, 0.31)
Vaginal practices	44 (39.6)	217 (32.7)	1.35 (0.89-2.04, 0.15)
Parity			
0	25 (22.5)	164 (24.8)	ref*
1-2	67 (60.4)	356 (53.9)	0.81 (0.49-1.33, 0.40)
3 or more	19 (17.1)	141 (21.3)	1.13 (0.60-2.14, 0.70)
Sexual behaviour			
Condom use last 3 months	35 (31.5)	161 (24.3)	1.44 (0.93-2.23, 0.10)
Condom use during last sexual contact	30 (27.0)	120 (18.1)	1.68 (1.06-2.66, **0.03**)
Number of sexual partners previous 3 months			
0 partners	10(9.0)	69 (10.4)	ref*
1 partner	97 (87.4)	567 (85.5)	0.85 (0.42-1.70, 0.64)
2 or more partners	4(3.6)	27(4.1)	0.98 (0.28-3.39, 0.97)
Number of sexual partners previous 12 months			
0 partners	6(5.4)	27(4.1)	ref*
1 partner	82 (73.9)	532 (80.2)	1.44 (0.58-3.60, 0.43)
2 or more partners	23 (20.7)	104 (15.7)	1.01 (0.37-2.71, 0.99)
Days since last sexual contact			
0 to 7 days	47 (45.6)	293 (46.7)	ref*
8 to 14 days	20 (19.4)	100 (15.9)	0.80 (0.45-1.42, 0.45)
More than 14 days	36 (35.0)	235 (37.4)	1.05 (0.66-1.67, 0.85)
Allergies	8(7.2)	45(6.8)	1.07 (0.49-2.33, 0.87)
BMI			
Under/normal weight	53 (52.5)	312 (50.4)	ref*
Overweight	34 (33.7)	208 (33.6)	1.04 (0.65-1.65, 0.87)
Obesity	14 (13.9)	99 (16.0)	1.20 (0.64-2.26, 0.57)
Medication use previous 4 weeks			
Antibiotics	27 (24.3)	155 (23.4)	1.05 (0.66-1.69, 0.83)
Antifungals	12 (10.8)	64(9.7)	1.13 (0.59-2.18, 0.70)
Steroids	2(1.8)	24(3.6)	0.49 (0.11-2.10, 0.33)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. e.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S6: Socio-demographic and clinical characteristics in Chlamydia trachomatis (CT) positive and negative cases

	CT positive N (%) n = 97	No CT N (%) n = 677	OR (95% CI, p-value)
Demographics			
Age			
18 to 25 years	60 (62.5)	230 (34.7)	ref*
26 to 35 years	23 (24.0)	285 (43.1)	3.23 (1.94-5.39, **0.00**)
36 and above	13 (13.5)	147 (22.2)	2.95 (1.56-5.56, **0.00**)
Educational level			
None/primary	21 (21.6)	183 (27.1)	ref*
Secondary	48 (49.5)	301 (44.5)	0.72 (0.42-1.24, 0.26)
Tertiary	28 (28.9)	192 (28.4)	0.79 (0.43-1.44, 0.43)
Marital status			
Single	40 (41.2)	214 (31.7)	ref*
Married	49 (50.5)	375 (55.5)	1.43 (0.91-2.24, 0.12)
Separated, divorced or widowed	8(8.2)	87 (12.9)	2.03 (0.91-4.52, 0.08)
Occupation			
Unemployed, housewife	36 (37.1)	233 (34.5)	ref*
Professional worker	15 (15.5)	78 (11.5)	
Self employed	16 (16.5)	231 (34.1)	
Student	15 (15.5)	45(6.7)	
Other	15 (15.5)	89 (13.2)	
Ethnicity			
Other	18 (18.8)	171 (25.4)	ref*
Luhya	32 (33.3)	131 (19.5)	0.43 (0.23-0.80, **0.01**)
Kikuyu	36 (37.5)	267 (39.7)	0.78 (0.43-1.42, 0.42)
Kamba	10 (10.4)	103 (15.3)	1.08 (0.48-2.44, 0.85)
Symptoms			
Discharge curdy/curdled	75 (77.3)	535 (80.0)	0.85 (0.51-1.42, 0.55)
Discharge foul smell	31 (32.0)	189 (27.9)	1.21 (0.77-1.92, 0.41)
Vulvar itch or pruritus	73 (75.3)	515 (76.1)	0.96 (0.58-1.57, 0.86)
Lower abdominal pain	24 (24.7)	197 (29.1)	0.80 (0.49-1.31, 0.37)
Soreness	31 (32.0)	188 (27.8)	1.22 (0.77-1.93, 0.39)
Dysuria	49 (50.5)	298 (44.0)	1.30 (0.85-1.99, 0.23)
Dyspareunia	38 (39.2)	279 (41.2)	0.92 (0.59-1.42, 0.70)
Recurrent LGTS previous 12 months	44 (45.4)	387 (57.2)	0.62 (0.40-0.95, **0.03**)
Signs			
Abdominal tenderness	3(3.1)	24(3.5)	0.87 (0.26-2.94, 0.82)
Excoriations, ulcers or skin lesions	5(5.2)	51(7.6)	0.66 (0.26-1.70, 0.39)
Erythema redness	12 (12.4)	117 (17.4)	0.67 (0.35-1.26, 0.21)
Vesicles	1(1.0)	13(1.9)	0.53 (0.07-4.08, 0.53)
Oedema	3(3.1)	35(5.2)	0.58 (0.18-1.92, 0.37)
Growth warts	3(3.1)	16(2.4)	1.31 (0.37-4.57, 0.68)

Table S6. Continued

	CT positive N (%) n = 97	No CT N (%) n = 677	OR (95% CI, p-value)
Use of contraceptives	65 (67.0)	438 (64.7)	1.11 (0.71-1.74, 0.66)
Contraceptives natural/herbal	4(4.1)	44(6.5)	0.62 (0.22-1.76, 0.36)
Contraceptives hormonal	35 (36.1)	219 (32.3)	1.18 (0.76-1.84, 0.46)
Contraceptives IUCD	9(9.3)	84 (12.4)	0.72 (0.35-1.49, 0.38)
Contraceptives tubal ligation	0(0.0)	6(0.9)	0.87 (0.85-0.90, 0.35)
Vaginal practices	30 (30.9)	231 (34.1)	0.87 (0.55-1.37, 0.53)
Parity			
0	26 (26.8)	163 (24.1)	ref*
1-2	58 (59.8)	365 (54.1)	1.00 (0.61-1.65, 0.99)
3 or more	13 (13.4)	147 (21.8)	1.80 (0.89-3.64, 0.10)
Sexual behaviour			
Condom use last 3 months	30 (30.9)	166 (24.5)	1.38 (0.87-2.19, 0.18)
Condom use during last sexual contact	19 (19.6)	131 (19.4)	1.02 (0.59-1.74, 0.96)
Number of sexual partners previous 3 months			
0 partners	7(7.2)	72 (10.6)	ref*
1 partner	83 (85.6)	581 (85.8)	0.68 (0.30-1.53, 0.35)
2 or more partners	7(7.2)	24(3.5)	0.33 (0.11-1.05-0.05)
Number of sexual partners previous 12 months			
0 partners	1(1.0)	32(4.7)	ref*
1 partner	69 (71.1)	545 (80.5)	0.25 (0.03-1.84, 0.14)
2 or more partners	27 (27.8)	100 (14.8)	0.12 (0.02-0.89 (**0.01**)
Days since last sexual contact			
0 to 7 days	40 (42.6)	300 (47.1)	ref*
8 to 14 days	20 (21.3)	100 (15.7)	0.67 (0.37-1.19, 0.17)
More than 14 days	34 (36.2)	237 (37.2)	0.93 (0.57-1.51, 0.77)
Allergies	9(9.3)	44(6.5)	1.47 (0.69-3.12, 0.31)
BMI			
Under/normal weight	51 (56.0)	314 (49.9)	ref*
Overweight	31 (34.1)	211 (33.5)	1.11 (0.69-1.79, 0.68)
Obesity	9(9.9)	104 (16.5)	1.88 (0.89-3.94, 0.09)
Medication use previous 4 weeks			
Antibiotics	14 (14.4)	168 (24.8)	0.51 (0.28-0.92, **0.02**)
Antifungals	8(8.2)	68 (10.0)	0.81 (0.37-1.73, 0.58)
None	63 (64.9)	390 (57.6)	1.36 (0.88-2.13, 0.17)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. e.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S7: Socio-demographic and clinical characteristics in Mycoplasma genitalium (MG) positive and negative cases

	MG positive N (%) n = 43	No MG N (%) n = 731	OR (95% CI, p-value)
Demographics			
Age			
18 to 25 years	26 (60.5)	264 (36.9)	ref*
26 to 35 years	11 (25.6)	297 (41.5)	2.66 (1.29-5.49, **0.01**)
36 and above	6 (14.0)	154 (21.5)	2.53 (1.02-6.28, **0.04**)
Educational level			
None/primary	11 (25.6)	193 (26.4)	ref*
Secondary	15 (34.9)	334 (45.8)	1.27 (0.57-2.82, 0.56)
Tertiary	17 (39.5)	203 (27.8)	0.68 (0.31-1.49, 0.33)
Marital status			
Single	19 (44.2)	235 (32.2)	ref*
Married	22 (51.2)	402 (55.1)	1.48 (0.78-2.79, 0.23)
Separated, divorced or widowed	2(4.7)	93 (12.7)	3.76 (0.86-16.5, 0.06)
Occupation			
Unemployed, housewife	14 (32.6)	255 (34.9)	ref*
Professional worker	8 (18.6)	85 (11.6)	
Self employed	9 (20.9)	238 (32.6)	
Student	8 (18.6)	52(7.2)	
Other	4(9.3)	100 (13.7)	
Ethnicity			
Other	13 (31.0)	176 (24.2)	ref*
Luhya	11 (26.2)	152 (20.9)	1.02 (0.44-2.35, 0.96)
Kikuyu	10 (23.8)	293 (40.4)	2.16 (0.93-5.04, 0.07)
Kamba	8 (19.0)	105 (14.5)	0.97 (0.39-2.42, 0.95)
Symptoms			
Discharge curdy/curdled	31 (72.1)	579 (80.1)	0.64 (0.32-1.28, 0.21)
Discharge foul smell	10 (23.3)	210 (28.7)	0.75 (0.36-1.55, 0.44)
Vulvar itch or pruritus	34 (79.1)	554 (75.8)	1.21 (0.57-2.57, 0.62)
Lower abdominal pain	11 (25.6)	210 (28.7)	0.85 (0.42-1.72, 0.66)
Soreness	11 (25.6)	208 (28.5)	0.86 (0.43-1.75, 0.68)
Dysuria	27 (62.8)	320 (43.8)	2.17 (1.15-4.09, **0.02**)
Dyspareunia	21 (48.8)	296 (40.5)	1.40 (0.76-2.60, 0.28)
Recurrent LGTS previous 12 months	27 (62.8)	404 (55.3)	1.37 (0.72-2.63, 0.33)
Signs			
Abdominal tenderness	1(2.3)	26(3.6)	0.65 (0.09-4.87, 0.67)
Excoriations, ulcers or skin lesions	3(7.0)	53(7.3)	0.95 (0.29-3.18, 0.94)
Erythema redness	8 (18.6)	121 (16.7)	1.14 (0.52-2.52, 0.74)
Vesicles	1(2.3)	13(1.8)	1.30 (0.17-10.21, 0.80)
Oedema	2(4.7)	36(5.0)	0.93 (0.22-4.01, 0.93)

Table S7. Continued

	MG positive N (%) n = 43	No MG N (%) n = 731	OR (95% CI, p-value)
Growth warts	0(0.0)	19(2.6)	0.94 (0.93-0.96, 0.28)
Use of contraceptives	31 (72.1)	472 (64.6)	1.42 (0.72-2.81), 0.32)
Contraceptives natural/herbal	3(7.0)	45(6.2)	1.14 (0.34-3.84, 0.83)
Contraceptives hormonal	15 (34.9)	239 (32.7)	1.10 (0.58-2.10, 0.77)
Contraceptives IUCD	2(4.7)	91 (12.4)	0.34 (0.08-1.44, 0.13)
Contraceptives tubal ligation	0(0.0)	6 (0.8)	0.94 (0.93-0.96, 0.55)
Vaginal practices	20 (46.5)	241 (33.0)	1.77 (0.95-3.28, 0.07)
Parity			
0	12 (27.9)	177 (24.3)	ref*
1-2	27 (62.8)	396 (54.3)	0.99 (0.49-2.01, 0.99)
3 or more	4(9.3)	156 (21.4)	2.64 (0.84-8.37, 0.09)
Sexual behaviour			
Condom use last 3 months	19 (44.2)	177 (24.2)	0.40 (0.22-0.75, **0.00**)
Condom use during last sexual contact	10 (23.3)	140 (19.2)	1.28 (0.62-2.66, 0.51)
Number of sexual partners previous 3 months			
0 partners	2(4.7)	77 (10.5)	ref*
1 partner	39 (90.7)	625 (85.5)	0.42 (0.10-1.76, 0.22)
2 or more partners	2(4.7)	29(4.0)	0.38 (0.05-2.80, 0.32)
Number of sexual partners previous 12 months			
0 partners	0(0.0)	33(4.5)	ref*
1 partner	31 (72.1)	583 (79.8)	1.05 (1.03-1.07, 0.19)
2 or more partners	12 (27.9)	115 (15.7)	1.10 (1.04-1.17, 0.07)
Days since last sexual contact			
0 to 7 days	19 (46.3)	321 (46.5)	ref*
8 to 14 days	6 (14.6)	114 (16.5)	1.13 (0.44-2.89, 0.81)
More than 14 days	16 (39.0)	255 (37.0)	0.94 (0.48-1.87, 0.87)
Allergies	3(7.0)	50(6.8)	1.02 (0.31-3.42, 0.97)
BMI			
Under/normal weight	22 (53.7)	343 (50.5)	ref*
Overweight	10 (24.4)	232 (34.3)	1.49 (0.69-3.20, 0.31)
Obesity	9 (22.0)	104 (15.3)	0.74 (0.33-1.66, 0.47)
Medication use previous 4 weeks			
Antibiotics	9 (20.9)	173 (23.7)	0.85 (0.40-1.82, 0.68)
Antifungals	5 (11.6)	71(9.7)	1.22 (0.47-3.21, 0.68)
Steroids	3(7.0)	23(3.1)	2.31 (0.67-8.02, 0.18)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. e.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S8: Prevalence of symptoms for no infection and any infection cases

	No infection N (%) n = 273	Infection N (%) n = 540	OR (95% CI, p-value)
Demographics			
Age			
18 to 25 years	92 (34.5)	214 (40.4)	ref*
26 to 35 years	114 (42.7)	211 (39.8)	0.80 (0.57-1.11, 0.18)
36 and above	61 (22.8)	105 (19.8)	0.74 (0.50-1.10, 0.14)
Educational level			
None/primary	75 (27.6)	138 (25.6)	ref*
Secondary	118 (43.4)	245 (45.4)	1.13 (0.79-1.61, 0.51)
Tertiary	79 (29.0)	157 (29.1)	1.08 (0.73-1.60, 0.70)
Marital status			
Single	85 (31.3)	186 (34.4)	ref*
Married	155 (57.0)	290 (53.7)	0.86 (0.62-1.18, 0.34)
Separated, divorced or widowed	32 (11.8)	64 (11.9)	0.91 (0.56-1.50, 0.72)
Occupation			
Unemployed, housewife	96 (35.3)	183 (33.9)	ref*
Professional worker	25(9.2)	76 (14.1)	
Self employed	101 (37.1)	159 (29.4)	
Student	15(5.5)	49(9.1)	
Other	35 (12.9)	73 (13.5)	
Ethnicity			
Other	76 (27.9)	122 (22.8)	ref*
Luhya	43 (15.8)	124 (23.2)	1.80 (1.15-2.82, **0.01**)
Kikuyu	104 (38.2)	217 (40.6)	1.30 (0.90-1.88, 0.16)
Kamba	49 (18.0)	72 (13.5)	0.92 (0.58-1.45, 0.71)
Symptoms			
Discharge curdy/curdled	207 (77.2)	433 (80.8)	0.81 (0.57-1.15, 0.24)
Discharge foul smell	56 (20.5)	174 (32.2)	0.54 (0.39-0.77, **0.00**)
Vulvar itch or pruritus	194 (71.1)	419 (77.6)	0.71 (0.51-0.99, **0.04**)
Lower abdominal pain	91 (33.3)	142 (26.3)	1.40 (1.02-1.92, **0.04)**
Soreness	62 (22.7)	170 (31.5)	0.64 (0.46-0.90, **0.01**)
Dysuria	132 (48.4)	231 (42.8)	1.25 (0.94-1.68, 0.13)
Dyspareunia	109 (39.9)	224 (41.5)	0.94 (0.70-1.26, 0.67)
Recurrent LGTS previous 12 months	182 (66.7)	276 (51.1)	1.92 (1.41-2.56, **0.00)**
Signs			
Abdominal tenderness	10(3.7)	19(3.5)	1.04 (0.48-2.27, 0.92)
Excoriations, ulcers or skin lesions	18(6.7)	40(7.4)	0.89 (0.50-1.59, 0.70)
Erythema redness	43 (16.0)	92 (17.1)	0.92 (0.62-1.37, 0.68)
Vesicles	3(1.1)	12(2.2)	0.49 (0.14-1.76, 0.27)
Oedema	13(4.8)	25(4.7)	1.04 (0.52-2.07, 0.91)
Growth warts	5 (1.9)	15 (2.8)	0.66 (0.24-1.83, 0.42)

Table S8. Continued

	No infection N (%) n = 273	Infection N (%) n = 540	OR (95% CI, p-value)
Use of contraceptives	180 (65.9)	348 (64.4)	1.07 (0.79-1.45, 0.67)
Contraceptives natural/herbal	16 (5.9)	34 (6.3)	0.93 (0.50-1.71, 0.81)
Contraceptives hormonal	90 (33.0)	177 (32.8)	1.01 (0.74-1.38, 0.96)
Contraceptives IUCD	28 (10.3)	68 (12.6)	0.79 (0.50-1.27, 0.33)
Contraceptives tubal ligation	2 (0.7)	4 (0.7)	0.99 (0.18-5.43, 0.99)
Vaginal practices	79 (28.9)	190 (35.2)	0.75 (0.55-1.03, 0.07)
Parity			
0	69 (25.4)	134 (24.9)	ref*
1-2	151 (55.5)	290 (53.9)	0.99 (0.70-1.40, 0.95)
3 or more	52 (19.1)	114 (21.2)	1.13 (0.73-1.75, 0.59)
Sexual behaviour			
Condom use last 3 months	74 (27.1)	14 (24.8)	1.13 (0.81-1.57, 0.48)
Condom use during last sexual contact	56 (20.5)	101 (18.7)	1.12 (0.78-1.62, 0.54)
Number of sexual partners previous 3 months			
0 partners	32 (11.7)	50(9.3)	ref*
1 partner	230 (84.2)	464 (85.9)	1.29 (0.81-2.07, 0.29)
2 or more partners	11(4.0)	26(4.8)	1.51 (0.66-3.48, 0.33)
Number of sexual partners previous 12 months			
0 partners	19(7.0)	17(3.1)	ref*
1 partner	219 (80.2)	421 (78.0)	2.15 (1.10-4.22, **0.02**)
2 or more partners	35 (12.8)	102 (18.9)	3.26 (1.53-6.96, **0.00**)
Days since last sexual contact			
0 to 7 days	117 (45.8)	240 (46.5)	ref*
8 to 14 days	35 (13.8)	94 (18.2)	1.30 (0.83-2.03, 0.25)
More than 14 days	102 (40.3)	182 (35.3)	0.86 (0.62-1.20, 0.38)
Allergies	18(6.6)	36(6.7)	0.99 (0.55-1.78, 0.97)
BMI			
Under/normal weight	123 (47.3)	264 (52.9)	ref*
Overweight	92 (35.4)	164 (32.9)	0.83 (0.60-1.16, 0.27)
Obesity	45 (17.3)	71 (14.2)	0.74 (0.48-1.13, 0.16)
Medication use previous 4 weeks			
Antibiotics	63 (23.1)	129 (23.9)	0.96 (0.68-1.35, 0.80)
Antifungals	32 (11.7)	51(9.4)	1.27 (0.80-2.03, 0.31)
Steroids	8(2.9)	19(3.5)	0.83 (0.36-1.92, 0.66)

*The OR is calculated for the symptom category 'yes' compared to 'no', unless stated otherwise. e.g. in the case of a symptom with more than two categories, then the first category was used as a reference.

Table S9: Multivariate binary logistic regression (last step) vulvovaginal candidiasis

	B	S.E.	Wald	OR (95% CI, p-value)
Itch or pruritus	0.79	0.23	11.71	2.20 (1.40-3.46, 0.00)
Constant	0.22	0.10	4.76	

Table S10: Multivariate binary logistic regression (last step) Bacterial vaginosis

	B	S.E.	Wald	OR (95% CI, p-value)
Foul smell	1.29	0.26	24.05	3.63 (2.17-6.07, 0.00)
Itch or pruritus	-0.49	0.28	2.94	0.61 (0.35-1.07, 0.09)
Dysuria	-0.77	0.28	7.62	0.46 (0.27-0.80, 0.01)
Dyspareunia	-0.78	0.30	6.90	0.46 (0,26-0.82, 0.01)
Oedema	1.25	0.79	2.51	
Number of sexual partners previous 12 months*				
0 partners	-2.20	1.06	4.31	0.11 (0.01-0.88, 0.04)
1 partner	-3.09	1.08	8.26	0.05 (0.01-0.37, 0.00)
Marital status				
Single	0.46	0.30	2.34	1.59 (0.88-2.87, 0.13)
Married	-0.34	0.38	0.80	0.71 (0.33-1.50, 0.37)
Ethnicity				
Other	0,76	1,28	0,35	2.13 (0.17-26.19, 0.56)
Luhya	0,38	1,28	0,09	1.47 (0.12-18.09, 0.77)
Kikuyu	0,70	1,28	0,30	2.02 (0.17-24.75, 0.58)
Kamba	1,71	1,34	1,63	5.52 (0.40-75.93, 0.20)
Use of antifungals	0.01	0.42	0.00	1.01 (0.44-2.31, 0.99)
Constant	3.14	1.68	3.49	

*Despite being significant, the number of sexual partners was not used in the alternative algorithm since both 0 and 1 partners are preventive of BV, no clinical difference can be made

Table S11: Multivariate binary logistic regression (last step) Any STI

	B	S.E.	Wald	OR (95% CI, p-value)
Foul smell	0.50	0.22	4.89	1.64 (1.06-2.55, 0.03)
Lower abdominal pain	0.55	0.24	5.00	1.73 (1.07-2.79, 0.03)
Excoriations, ulcers or genital skin lesions	0.91	0.47	3.88	2.50 (1.00-6.23, 0.05)
Use of contraceptives	0.82	0.39	4.46	2.27 (1.06-4.88, 0.03)
Condom use last sexual contact	-0.09	0.27	0.11	0.92 (0.54-1.54, 0.74)
Age				
18-25 years	-0.92	0.87	1.13	0.40 (0.07-2.17, 0.29)
26-35 years	-0.16	0.86	0.03	0.86 (0.16-4.60, 0.86)
36 and above	-0.42	0.88	0.23	0.66 (0.12-3.65, 0.63)
Educational level				
None/primary	-0.40	0.27	2.08	0.67 (0.39-1.15, 0.15)
Secondary	0.00	0.34	0.00	1.00 (0.51-1.96, 1.00)
Occupation				
Unemployed, housewife	-0.19	0.36	0.27	0.83 (0.41-1.69, 0.60)
Professional worker	0.34	0.28	1.41	1.40 (0.80-2.43, 0.23)
Self employed	-0.80	0.45	3.11	0.45 (0.19-1.09, 0.08)
Student	-0.51	0.33	2.36	0.60 (0.31-1.15, 0.12)
Recurrent LGTS episodes previous 12 months	0.81	0.21	14.33	0.45 (0.29-0.68, **0.00)**
Constant	19.72	55837,74	0.00	

Table S12: Multivariate binary logistic regression (last step) Trichomonas vaginalis

	B	S.E.	Wald	OR (95% CI, p-value)
Foul smell	0.91	0.34	7.33	2.49 (1.29-4.82, 0.01)
Lower abdominal pain	1.10	0.46	5.78	3.02 (1.23-7.42, 0.02)
Vesicles*	-3.92	0.88	19.75	0.02 (0.00-0.11, 0.00)
Marital status				
Single	0.90	0.48	3.44	2.45 (0.95-6.34, 0.06)
Married	-0.40	0.53	0.58	0.67 (0.24-1.89, 0.45)
Use of antifungals in the previous 4 weeks	-1.32	0.82	2.56	0.27 (0.05-1.35, 0.11)
Constant	57.35	37921.27	0.00	

*Vesicles was not used in the alternative algorithm since only 15 women in the entire dataset had vesicles, too low number for conclusion on predictive value

Table S13: Multivariate binary logistic regression (last step) Chlamydia trachomatis

	B	S.E.	Wald	OR (95% CI, p-value)
Ethnicity				
Other	2.45	1.50	2.67	11.62 (0.61-220.53, 0.10)
Luhya	1.60	1.49	1.17	4.98(0.27-91.61, 0.28)
Kikuyu	1.72	1.47	1.36	5.57 (0.31-100.24, 0.24)
Kamba	2.57	1.56	2.71	13.04 (0.62-275.59, 0.10)
Use of antibiotics previous 4 weeks	-0.79	0.42	3.50	0.45(0.20-1.04, 0.06)
Recurrent LGTS episodes previous 12 months	0.81	0.30	7.14	0.44 (0.25-0.81, **0.01)**
Constant	56.59	30932.55	0.00	

Table S14: Multivariate binary logistic regression (last step) Neisseria Gonorrhoea

	B	S.E.	Wald	OR (95% CI, p-value)
Soreness	0,43	0,28	2,49	1.54 (0.90-2.65, 0.11)
Excoriations, ulcers or genital skin lesions	2,28	1,55	2,15	9.78 (0.46-205.53, 0.14)
Condom use	0,48	0,28	2,99	1.61 (0.94-2.76, 0.08)
IUCD use	-0,78	0,49	2,48	0.46 (0.17-1.21, 0.12)
Educational level				
None or primary	-0,70	0,33	4,54	0.50 (0.26-0.95, 0.03)
Secondary	-0,03	0,38	0,01	0.97 (0.46-2.07, 0.94)
Recurrent LGTS episodes previous 12 months	0,78	0,26	9,03	0.46 (0.27-0.76, **0.00)**
Constant	-0,91	1,66	0,30	

Table S15: Multivariate logistic regression (last step) Mycoplasma genitalium

	B	S.E.	Wald	OR (95% CI, p-value)
Lower abdominal pain	-0.20	0.47	0.18	0.82 (0.33-2.05, 0.67)
Soreness	-0.86	0.53	2.60	0.42 (0.15-1.20, 0.11)
Condom use previous 3 months	-0.94	0.43	4.73	0.39 (0.17-0.91, 0.03)
Constant	75.97	23302.63	0.00	

Table S16: Multivariate logistic regression (last step) no infection

	B	S.E.	Wald	OR (95% CI, p-value)
Foul smell	-0.78	0.24	10.32	0.46 (0.29-0.74, 0.00)
Itch or pruritus	-0.47	0.25	3.50	0.62 (0.38-1.02, 0.06)
Lower abdominal pain	-0.48	0.23	4.39	0.62 (0.39-0.97, 0.04)
Vulvar soreness	-0.44	0.25	3.17	0.65 (0.40-1.05, 0.08)
Dysuria	0.37	0.22	2.96	1.45 (0.95-2.22, 0.09)
Vesicles	1.61	1.53	1.11	5.01 (0.25-100.24, 0.29)
Condom use	0.44	0.24	3.29	1.55 (0.97-2.48, 0.07)
Number of sexual partners previous 12 months*				
0 partners	1.01	0.44	5.24	2.73 (1.16-6.46, 0.02)
1 partner	1.32	0.51	6.75	3.75 (1.38-10.14, 0.01)
Educational level				
None or primary	0.31	0.26	1.44	1.36 (0.82-2.24, 0.23)
Secondary	-0.12	0.31	0.16	0.88 (0.48-1.62, 0.69)
Occupation				
Unemployed, housewife	0.48	0.38	1.57	1.62 (0.76-3.42, 0.21)
Professional worker	-0.51	0.26	3.90	0.60 (0.36-1.00, 0.05)
Self employed	0.37	0.45	0.66	1.44 (0.60-3.50, 0.42)
Student	0.21	0.33	0.41	1.24 (0.64-2.38, 0.52)
Use of antibiotics previous 4 weeks	-0.50	0.25	3.94	0.61 (0.37-0.99, 0.05)
Recurrent LGTS episodes previous 12 months	-0.70	0.22	10.46	2.00 (1.32-3.13, 0.00)
Constant	20.69	27645.71	0.00	

*Despite being significant, the number of sexual partners was not used in the alternative algorithm since both 0 and 1 partners are preventive of BV, no clinical difference can be made

Table S17: McNemar test - computation comparing treatment allocation for LGTI/syndrome, by the current and alternative algorithms (n=306)

Treatment category	Alternative algorithm	Current algorithm			χ^2, P-value
VVC (n=121)		Correct	Incorrect	Total	
	Correct	78	24	102	4.11, 0.04
	Incorrect	11	8	19	
	Total	89	32	121	
BV-TV (n=66)		Correct	Incorrect	Total	
	Correct	23	8	31	5.63, 0.02
	Incorrect	22	13	35	
	Total	45	21	66	
LAP (64)		Correct	Incorrect	Total	
	Correct	20	0	20	0.5, 0.5
	Incorrect	2	42	44	
	Total	22	42	64	
No treatment (n=108)		Correct	Incorrect	Total	
	Correct	0	15	15	13.07, p<0.001
	Incorrect	0	93	93	
	Total	0	108	108	

Correct: those with the condition and classified to treatment for it.
Incorrect: those with the condition but classified as not having the condition hence missed treatment for it.
Inappropriate treatment was – per definition – not included in the analysis
LGTI: Lower genital tract infections
VVC: Vulvovaginal candidiasis
BV-TV: Bacterial vaginosis-Trichomonas vaginalis
LAP: Lower abdominal pain; includes any of Neisseria gonorrhoea, Chlamydia trachomatis, Mycoplasma genitalium

CHAPTER 4: ACCEPTABILITY AND FEASIBILITY OF REPEATED MUCOSAL SPECIMEN COLLECTION IN CLINICAL TRIAL PARTICIPANTS IN KENYA

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Abstract

Background: Mucosal specimens are essential to evaluate compartmentalized immune responses to HIV vaccine candidates and other mucosally targeted investigational products. We studied the acceptability and feasibility of repeated mucosal sampling in East African clinical trial participants at low risk of HIV and other sexually transmitted infections.

Methods and Findings: The Kenya AIDS Vaccine Initiative (KAVI) enrolled participants into three Phase 1 trials of preventive HIV candidate vaccines in 2011–2012 at two clinical research centers in Nairobi. After informed consent to a mucosal sub-study, participants were asked to undergo collection of mucosal secretions (saliva, oral fluids, semen, cervico-vaginal and rectal), but could opt out of any collection at any visit. Specimens were collected at baseline and two additional time points. A tolerability questionnaire was administered at the final sub-study visit. Of 105 trial participants, 27 of 34 women (79%) and 62 of 71 men (87%) enrolled in the mucosal sub-study. Nearly all sub-study participants gave saliva and oral fluids at all visits. Semen was collected from about half the participating men (47–48%) at all visits. Cervico-vaginal secretions were collected by Softcup from about two thirds of women (63%) at baseline, increasing to 78% at the following visits, with similar numbers for cervical secretion collection by Merocel sponge; about half of women (52%) gave cervico-vaginal samples at all visits. Rectal secretions were collected with Merocel sponge from about a quarter of both men and women (24%) at all 3 visits, with 16% of men and 19% of women giving rectal samples at all visits.

Conclusions: Repeated mucosal sampling in clinical trial participants in Kenya is feasible, with a good proportion of participants consenting to most sampling methods with the exception of rectal samples. Experienced staff members of both sexes and trained counselors with standardized messaging may improve acceptance of rectal sampling.

Introduction

In sub-Saharan Africa and other low- and middle-income countries that bear the brunt of the pandemic, the dominant route of transmission of HIV-1 is across the genital mucosa during sexual intercourse. Immune responses, both humoral and cellular, have been identified at mucosal surfaces and may be protective [1], [2]. As new vaccine candidates with the potential of inducing a compartmentalized mucosal response have become available (e.g., Sendai virus vector, now in trial in East Africa; ClinicalTrials.gov Identifier NCT01705990), the ability to induce protective responses in the mucosa is a key opportunity to control or even halt HIV infection in its early stages [3].

Mucosal sampling in HIV preventive trials is becoming more common, but much of the work is being conducted in North America or Europe [4]–[8]. Sub-Saharan Africa, the region with the highest HIV prevalence and incidence, will be a site of future efficacy trials and would be the region to benefit the most from an efficacious HIV vaccine. Additionally, there is a high likelihood of population-specific differences in mucosal immune responses, due to genetic factors and effects from endemic infections and environmental factors. It is therefore important to build capacity to collect, process, and analyze mucosal specimens at sub-Saharan African clinical trial centers. A large body of mucosal work in HIV-exposed but uninfected populations has already been done in Nairobi [1], [2], [9].

The International AIDS Vaccine Initiative (IAVI) and the Kenya AIDS Vaccine Initiative Institute of Clinical Research (KAVI-ICR) of the University of Nairobi have been working together to adapt existing mucosal sample collection and analysis methods and test new ones in preparation for HIV vaccine clinical trials. The current study was attached to three IAVI-sponsored Phase 1 HIV vaccine trials in Nairobi as an optional sub-study to assess acceptability of repeated mucosal sampling and the nature of vaccine-induced mucosal HIV-1-specific immune responses. This paper reviews the acceptability of a wide range of mucosal sampling methods including rectal, oral, cervico-vaginal secretions and semen, taken at three time points within the main vaccine trial schedule.

Methods

This mucosal sub-study recruited participants who had enrolled in one of three IAVI-sponsored HIV preventive vaccine trials conducted either at the main KAVI

clinical research center at the Kenyatta National Hospital (KNH) complex or at KAVI's research unit in Kangemi, on the outskirts of Nairobi. The mucosal and vaccine trial protocols were approved by the Kenyatta National Hospital-University of Nairobi Ethical Review Committee. After written informed consent and enrollment into the main vaccine trial, participants underwent the informed consent process and signed a separate consent form for the mucosal study. Participants who did not initially consent to the mucosal sub-study could enroll at any time before the final study visit.

Study Participants

Eligibility criteria for the three vaccine trials included being healthy, at low risk for HIV and between the ages of 18 and 50 (18–40 years for one trial, B002). All participants were advised to use condoms. In addition, a long-lasting non-barrier method of contraception, such as Depo-Provera, Norplant, intra-uterine device (IUD) or tubal ligation was required of all female participants of child-bearing potential (oral contraceptives were not allowed). Female participants with an IUD were excluded from cervico-vaginal sampling due to a risk of the IUD being dislodged by the Softcup [10].

Vaccine Trials and Study Schedule

Participants were drawn from the following Phase 1 vaccine trials: IAVI B002 (ClinicalTrials.gov Identifier NCT01264445), B003 (NCT01215149), and B004 (NCT01496989), conducted at sites in Eastern Africa, South Africa, and the USA, with the mucosal sub-study conducted at the Kenyan sites only. In Kenya, trials B002 and B003 were conducted in 2011–2012 at KAVI-KNH and KAVI-Kangemi, respectively, and B004 was conducted in 2012–2013 at KAVI-Kangemi. Mucosal specimens were collected at three time points for each trial, with the timing dependent on trial design: in B002 and B003, sampling was at baseline, one month after the final vaccination and at the next vaccine trial visit; in B004 sampling was at baseline, one month after the prime and one month after the boost.

Study Procedures

Participants were free to opt out of any collection at any time or to provide samples they had previously refused. Reasons for refusing any sample collection were recorded at each visit. A questionnaire was administered at the final mucosal study visit, asking participants the main reason they agreed to provide mucosal specimens, what mucosal specimens they would agree to in future studies, and any general suggestions for making the procedures more tolerable. Questions were open-ended. If responses fit with a pre-determined list of responses, answers were

coded accordingly. If responses did not fit with one of the pre-set answers, they were recorded verbatim. Up to two reasons for refusal were collected at each visit for each sample type not given.

Saliva was collected by placing a Salimetrics Oral Swab (Salimetrics LLC, State College, PA, USA) against the parotid duct for 5 minutes. Oral fluid (transudate) was collected by allowing fluids to pool in the mouth then passed into a Falcon tube [11]. Participants were instructed not to eat or drink anything but water for 2 hours prior to saliva and oral fluid collection.

In female participants, the Instead Softcup (Evofem Inc., San Diego, CA, USA), was inserted by the clinician and kept in place for 5 minutes (10 minutes for B004) to collect cervico-vaginal secretions. The cervix was then accessed with a disposable speculum and two pre-moistened Merocel sponges (Medtronic, Minneapolis, MN, USA) were placed against the cervical mucosa for 5 minutes each, serially. The Softcup and Merocel sponge have been used to collect cervico-vaginal secretions in other research studies [12], [13]. Cervico-vaginal collection was not performed during menstruation. If possible, samples were taken approximately 2 days after bleeding ended, except baseline samples, which were considered missed if the participant was menstruating on the day of vaccination. Male participants provided semen specimens, by masturbation, into a universal container.

Rectal secretions were collected from both male and female participants by accessing the rectal mucosa through a disposable clinician-inserted proctoscope. Rectal secretions were collected using two pre-moistened Merocel sponges placed against the rectal mucosa for 5 minutes each, serially.

B002 and B003 participants were reimbursed a set amount at the end of each visit, regardless of the actual collections performed. In B004, the reimbursement structure was changed so that participants were given a set amount per sample type in order to reimburse participants for the significant additional time involved in providing all specimen types as opposed to just one.

Humoral responses were assessed by anti-HIV specific IgG and IgA ELISAs on frozen samples. Results will be published separately.

Statistical Methods

Participants' overall acceptance of a mucosal sampling method was calculated as the proportion of participants who provided any specimen for that sampling

method during the study. 95% confidence intervals for the observed proportions were estimated using exact (Clopper-Pearson) binomial method in PASS 2008 (NCSS, Kaysville, UT).

Due to limited sample size, the statistical comparisons were primarily exploratory and were conducted for evaluation of any observed site, trial or gender differences in acceptability of mucosal sampling methods. Comparisons of categorical and continuous factors were conducted using the Fisher's exact test and Wilcoxon rank-sum test, respectively. A two-sided p-value of less than 0.05 was considered to be statistically significant. Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Participant Characteristics at Enrollment

Male participants in the vaccine trials outnumbered female participants 2 : 1 overall and this disproportion was also reflected in the mucosal sub-study. 27/34 (79%) females and 62/71 (87%) males consented and enrolled in the mucosal sub-study ($p=0.11$, Table 1). Participant ages ranged from 18 to 46 years. B002 participants were significantly younger ($p<0.0001$) than participants in the other two trials due to the stricter age criteria specified in the protocol. Most female participants in all three trials were on an injectable hormonal contraceptive (Depo-Provera), although a few used Norplant or had a tubal ligation. Three female participants had an IUD and were therefore excluded from cervico-vaginal collection.

Table 1. Participant characteristics at enrollment.

Vaccine Trial	B002		B003		B004		Total	
	Female	Male	Female	Male	Female	Male	Female	Male
# Participants Enrolled in Vaccine Trial	11	29	13	27	10	15	34	71
# Participants Enrolled in Mucosal Substudy (%)	9 (82)	28 (97)	8 (62)	20 (74)	10 (100)	14 (93)	27 (79)	62 (87)
Median Age Enrolled in Mucosal Substudy (range)	23 (18–37)	23 (18–33)	27 (22–43)	25 (19–41)	28 (25–46)	28 (21–45)	27 (18–46)	24 (18–45)

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Of 16 vaccine trial participants that were not enrolled in the mucosal study, seven participants cited discomfort or fear of the sampling methods, two refused without giving a specific reason, for two participants the consent form was not yet available in the local language, and one participant each reported not wanting more procedures, lack of time or parental advice against joining. One participant was excluded for vertebral and pelvic bone deformities and one participant was not enrolled because vaccinations were discontinued following an adverse event not related to vaccination. Participants who declined participation in the mucosal study

did not differ significantly in gender and age characteristics from those who participated.

Acceptability of Sample Collection

Saliva and oral fluids, the least invasive samples, were collected from all participants in the mucosal sub-study at nearly all visits, while rectal sample collection was the least likely to be accepted (Figure 1). Based on overall acceptance and corresponding 95% confidence intervals, female participants agreed to both types of cervico-vaginal sampling more readily than rectal sampling (Table 2). Similarly, male participants were more likely to provide semen than rectal samples.

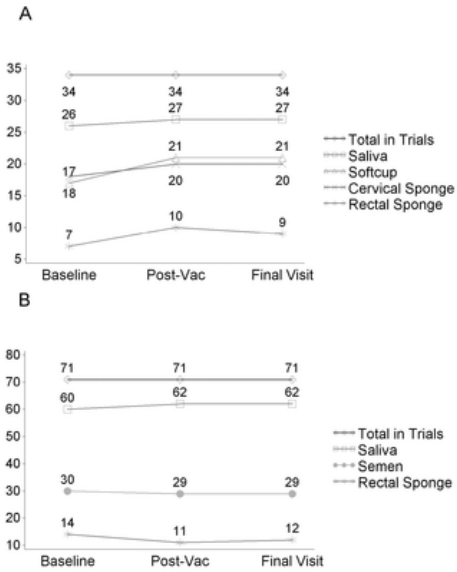

Figure 1. Total number of participants undergoing mucosal collection by gender.

A. Total number of female participants in the three trials combined and the number providing each type of specimen at the three time points.

B. Total combined number of male participants and the number providing specimens at each time point.

Table 2. Number (%) of participants providing a total of 0, 1, 2 or 3 specimens in mucosal substudy and overall acceptance of mucosal sampling by gender*.

	Female (N = 27)					Male (N = 62)				
# Specimens	0	1	2	3	Overall acceptance (95%CI)†	0	1	2	3	Overall acceptance (95%CI)†
Total Saliva	0 (0)	0(0)	1 (4)	26 (96)	100 (87–100)	0 (0)	0 (0)	2 (3)	60 (97)	100 (94–100)
Total Semen	N/A	N/A	N/A	N/A	N/A	30 (48)	3 (5)	2 (3)	27 (44)	52 (39–65)
Total Softcup	4 (15)	1 (4)	8 (30)	14 (52)	85 (66–96)	N/A	N/A	N/A	N/A	N/A
Total Cervical Sponge	4 (15)	2 (7)	7 (26)	14 (52)	85 (66–96)	N/A	N/A	N/A	N/A	N/A
Total Rectal Sponge	16 (59)	1 (4)	5 (19)	5 (19)	41 (22–61)	46 (74)	5 (8)	1 (2)	10 (16)	26 (16–39)

*No drop-outs over time were observed in the study.
†Calculated as the percentage of participants who provided any specimen for that sampling method during the study; CI: Confidence Interval.
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Cervico-vaginal sampling by Softcup and Merocel sponge was well-accepted and tolerated by the majority of women. Of 27 female participants, 18 (67%) had cervico-vaginal samples collected with Merocel sponge at baseline and 17 (63%) also had cervico-vaginal samples collected with Softcup (Table 3). Consent for cervico-vaginal sampling remained consistent across the two follow-up visits. Most missed samples were attributable to menstruation or IUD; only three participants refused cervico-vaginal sampling because of physical or emotional discomfort (data not shown). Participants in B004 were given the choice of self-inserting the Softcup or having a clinician place the device. All participants chose to have a clinician insert the Softcup.

Table 3. Number of participants providing specimens at each visit.

	# Participants Giving Samples Baseline (%)		# Participants Giving Samples Second Visit (%)		# Participants Giving Samples Final Visit (%)	
	Female	Male	Female	Male	Female	Male
B002 Saliva	8 (89)	26 (93)	9 (100)	28 (100)	9 (100)	28 (100)
B002 Semen	N/A	9 (32)	N/A	8 (29)	N/A	7 (25)
B002 Softcup	6 (67)	N/A	7 (78)	N/A	6 (67)	N/A
B002 Cervical Sponge	6 (67)	N/A	7 (78)	N/A	6 (67)	N/A
B002 Rectal Sponge	2 (22)	3 (11)	2 (22)	3 (11)	1 (11)	3 (11)
B003 Saliva	8 (100)	20 (100)	8 (100)	20 (100)	8 (100)	20 (100)
B003 Semen	N/A	12 (60)	N/A	12 (60)	N/A	13 (65)
B003 Softcup	3* (38)	N/A	6 (75)	N/A	7 (88)	N/A
B003 Cervical Sponge	4* (50)	N/A	5 (63)	N/A	6 (75)	N/A
B003 Rectal Sponge	2 (25)	6 (30)	1 (13)	2 (10)	1 (13)	2 (10)
B004 Saliva	10 (100)	14 (100)	10 (100)	14 (100)	10 (100)	14 (100)
B004 Semen	N/A	9 (64)	N/A	9 (64)	N/A	9 (64)
B004 Softcup	8 (80)	N/A	8 (80)	N/A	8 (80)	N/A
B004 Cervical Sponge	8 (80)	N/A	8 (80)	N/A	8 (80)	N/A
B004 Rectal Sponge	3 (30)	5 (36)	7 (70)	6 (43)	7 (70)	7 (50)
Total Saliva	26 (96)	60 (97)	27 (100)	62 (100)	27 (100)	62 (100)
Total Semen	N/A	30 (48)	N/A	29 (47)	N/A	29 (47)
Total Softcup	17 (63)	N/A	21 (78)	N/A	21 (78)	N/A
Total Cervical Sponge	18 (67)	N/A	20 (74)	N/A	20 (74)	N/A
Total Rectal Sponge	7 (26)	14 (23)	10 (37)	11 (18)	9 (33)	12 (19)

*Two women missed baseline cervico-vaginal samples due to menstruation.

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Overall, semen was provided by 30 out of 62 (48%) male participants across all studies at baseline, with a similar percentage (29/62, 47%) at the second and third visits (Table 3). There was a significant difference in the proportion of male participants providing any semen specimen in B002, conducted at the KNH clinic (32%) and in B003 and B004, conducted at the Kangemi clinic (68%, p=0.01). Among participants who did not provide semen specimens, approximately half cited embarrassment/emotional discomfort as the main reason. Another 8 (23%) were specifically uncomfortable about masturbating at the clinic (Table 4).

Table 4. Main reason specimen not collected (reported at first refusal) – number of participants (% of refusals).

Specimens	Semen	Softcup	Cervical Sponge	Rectal Sponge
Too invasive				6 (8)
Physical discomfort or pain		2 (15)	1 (8)	19 (26)
Embarrassment/emotional discomfort	17 (49)	2 (15)	2 (15)	34 (46)
Partner/family disapproval	1 (3)			
Uncomfortable masturbating in the clinic	8 (23)			
Concern about inability to provide required specimen on demand	1 (3)			
Procedures too time consuming	1 (3)			1 (1)
Menstruating		4 (31)	4 (31)	1 (1)
Clinician decision that collection is contraindicated		3 (23)	2 (15)	
Discomfort with the clinician				1 (1)
Difficulty masturbating	4 (11)			
Religious reasons	2 (6)			5 (7)
Being in a hurry	1 (3)			1 (1)
Pregnancy			1 (8)	1 (1)
Site decision		1 (8)	1 (8)	2 (3)
Being scared about the procedure			1 (8)	
Not wanting rectal exam				2 (3)
Procedure being unnatural				1 (1)
Not reported		1 (8)	1 (8)	

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Rectal secretions were collected with Merocel sponge from 21/89 (24%) male and female participants at each of the three visits, however the male to female ratio differed slightly with each visit (Table 3). Overall, there was no significant difference in the proportion of participants providing any rectal sponge specimens between females (41%) and males (26%) (Table 2; p=0.21). The proportion of participants who provided any rectal specimen was similar for B002 and B003 (16% combined), but significantly larger for B004 participants (56%) (data not shown; p=0.0002). A slight downward trend over time in B002 and B003 was reversed in B004, with more participants agreeing to rectal sampling at the second and third visits compared to baseline (Table 3).

When asked at the final mucosal study visit the reason for agreeing to provide specimens, contribution to HIV research was the primary reason given (50–64% for various specimen types), finding out more about one's health ranked second (20–32%), belief that the samples were a requirement ranked third (4–12%), belief that the study would help them access more health care ranked fourth (4–5%), and "easiness of giving" was specified as another reason for providing specimens (3–9%, with the highest rate being for saliva). When asked for suggestions to improve the mucosal sampling experience, 32 out of 89 (36.0%) participants said they had no problems or issues with the sampling methods in this study and 28/89 (31.5%) had no comments. Suggestions to improve semen sampling included quieter rooms, provision of pornographic materials (not used in this study due to Kenyan anti-pornography law) or allowing partners to assist the participant. Rectal sampling was another area of

focus, with suggestions to use a smaller proctoscope or to find a method without the need for a proctoscope. Suggestions are summarized in Table S1.

Discussion

This study aimed to assess the acceptability of various mucosal sampling methods in healthy, adult, HIV-uninfected Kenyan clinical trial participants over the course of three visits. While the study was done within the context of HIV vaccine trials, the results apply to any type of study requiring mucosal sampling. The acceptability, as measured by proportion of samples collected, varied by sample type. Saliva was easily accessed and given by all participants. Cervico-vaginal and semen sample collection rates were not as high as saliva but many participants consented to the procedures and those that did remained consistent across visits. Rectal sampling was the least acceptable, with significant variance between study sites.

Cervico-vaginal sample collection had high acceptability and tolerability, but many samples were missed because of menstruation at baseline, or because the participants had IUDs. Although the IUD exclusion was connected to the Softcup, the protocol was written in such a way that IUDs excluded participants from all cervico-vaginal collection. Otherwise, the rate of cervico-vaginal sponge collection may have been higher. The three women who refused cervico-vaginal sampling cited physical and emotional discomfort as their reasons.

The Softcup is a well-accepted device for collection of menstrual fluid by self-insertion [14] and has been used to obtain self-collected cervico-vaginal specimens in clinical trials [15]. It was therefore chosen for this study to collect undiluted samples with the hypothesis that a self-inserted method would improve acceptability. Yet when participants were given the option of self-insertion, all chose clinician insertion of the Softcup. Perhaps non-familiarity with the Softcup device in this population contributed to this phenomenon; a survey by Rositch et al reported 82% acceptability of self-sampling for pap-smear screening among women in Nairobi [16]. Larger studies of women from different cultures to further understand their preferred methods for collection of genital mucosal samples are needed. The aversion to self-inserting the Softcup in this population would seem to abrogate its perceived benefit. It was thought that using a dry Merocel sponge might disrupt the mucosal epithelium, however the pre-wet sponge results in a diluted sample. The use of dry sponges and other cervico-vaginal collection methods should be further explored.

Semen samples were more acceptable at the Kangemi center than at the KNH center. A possible contributor to the difference was that private rooms were more readily available at Kangemi than KNH at the time of the study. Additionally, all the clinic personnel at KNH were female as opposed to Kangemi, which had one male nurse and a male clinical officer. Some participants declined or were unsuccessful in providing a semen sample the same day as other samples but did so without any problems on a return visit when fewer specimens were collected. This suggests that multiple complex specimen collections at a single visit may be less feasible.

Collection of rectal secretion with Merocel sponge was the most challenging. Differences between the studies could be attributable to a number of factors. One possible explanation is that the staff at one site were relatively inexperienced with rectal sampling initially, but became more adept by the time the next study was conducted. It is conceivable that word spread amongst the participants in the last study that the procedure was tolerable. Additionally, the staff's increased familiarity and comfort with rectal sampling may have come through in the counseling process, increasing the participants' willingness to consent.

Another contributing factor may have been the different reimbursement schemes. B002 and B003 participants received the same reimbursement whether they gave rectal samples or not. B004 participants received additional reimbursement for each sample type they agreed to. Although rectal specimen collection was significantly greater in B004 compared to B002 and B003, no one, including B004 participants, cited money as the reason they gave any particular sample. More than half reported that they gave samples for altruistic reasons. Without a comparison group at the same site at the same time, it is difficult to draw any conclusions about the impact of the different reimbursement schemes.

It is discouraging that despite an in-depth information session(s) and consent process, 4–12% of the participants, depending on sample type, gave the sample because they thought it was required. In the clinical trials that this mucosal sub-study was nested, blood draws were a requirement; it is therefore possible that participants may have assumed that the same requirement applied to the mucosal samples, especially since specimen sampling for the two studies coincided. It is also likely that participants understood they had a choice about which samples to provide when they first entered the study, but may have thought that once they agreed to something it was required for the remaining visits. We recommend that consent forms include a section where the participant can indicate which sampling methods they consent to. Since the consent form is signed at the beginning of the

study, we also recommend verbal confirmation of which samples the participant is agreeing to at each visit. This should be documented in writing in clinic notes or other source documents. KAVI is currently conducting a clinical trial with these added precautions. We plan to compare participants' reasons for agreeing to provide specimens across these studies and see if this additional step has improved understanding of the study requirements.

Study Limitations

The demographic data collected in the three vaccine trials were limited to age, gender, and race/ethnicity. As a result, other information such as marital status, education, parity for women and socio-economic differences could not be examined. The study participants were selected for their low risk of HIV infection, and their knowledge, attitudes and flexibility may differ from people who are at higher risk, particularly sex workers.

The mucosal sampling time points in each trial were dependent on the vaccination schedules, which varied between protocols. The length of the gap between the second and third visits did not appear to have affected compliance.

The informed consent process was similar between the two sites, but scripts were not used to explain the procedures. Individual differences between counselors could have affected the likelihood of enrollment. Without a strictly standardized method of explaining the procedures, it is possible the consent process had an impact on the initial acceptability of the various sample types. This is an area for further development and research.

Conclusions

Repeated mucosal sampling including saliva, oral fluids, semen, cervico-vaginal and rectal specimens in healthy, adult, HIV-uninfected clinical trial participants in Kenya is feasible. Participants consented to most specimen collection methods with the exception of rectal sampling. Given the high HIV incidence demonstrated in MSM populations in Africa [17], rectal mucosal sampling should not be dismissed because of its challenges. Experienced staff members that include both men and women, well-trained counselors and standardized language during the informed consent process may improve acceptability of rectal and other sampling.

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Author Contributions

Conceived and designed the experiments: GOM HP GM BF PJB DL JL KC BB PF JG OA. Performed the experiments: GOM GM BF OA. Analyzed the data: BB HP PJB DL. Contributed reagents/materials/analysis tools: BF PJB JG. Wrote the paper: GOM HP GM BF PJB DL JL KC BB PF JG OA.

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Supporting Information

Table S1. Participant suggestions for improving mucosal sampling experience, n=89

Suggestions	Number*
Semen	
Provide pornographic materials or allow partner	6
Allow semen collection at home	3
Provide quieter room for collection	3
Provide larger container to avoid spillage	1
Rectal	
Use a smaller proctoscope	4
Find a way to sample without the proctoscope	3
Find a self-collected method of rectal sampling	2
Associates rectal sampling with homosexuality	2
Has no suggestion, just not comfortable with any rectal sampling	1
Other Comments	
Give more time for consent process	1
Have the same doctor follow up at all study visits	1
Just do saliva collection; the others are too involving	1
Use straw for oral fluid collection	1
Remind participants when a mucosal collection visit is due (participant misplaced visit calendar)	1
No problem with methods used in this study	32
No Comment	28

*Out of 89 participants, 88 gave up to one suggestion. One participant gave two suggestions.

CHAPTER 5: ESTABLISHMENT AND IMPLEMENTATION OF A REGIONAL MUCOSAL TRAINING PROGRAM TO FACILITATE MULTI-CENTER COLLABORATION IN BASIC AND CLINICAL RESEARCH IN EASTERN AFRICA

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Abstract

Background: The recent outbreaks of novel endemic and pandemic diseases have highlighted the importance of collaborative networks in rapid response to emerging pathogens. Over the last two decades International AIDS Vaccine Initiative (IAVI), with the support of United States Agency for International Development (USAID) and other international donors, has invested in research capacity and infrastructure in Africa. A significant portion of this support has facilitated establishing regional centers of excellence for African scientists to develop and lead a collaborative research agenda, implemented within the IAVI-led Accelerate the Development of Vaccines and New Technologies to Combat the AIDS Epidemic (ADVANCE) program. One such regional center is the University of Nairobi's Kenya AIDS Vaccine Initiative-Institute of Clinical Research (KAVI-ICR).

Objective: We designed and implemented a development program to foster inter-institutional South-South technology transfer within Africa, and address a capacity gap in mucosal research.

Methods: KAVI-ICR and IAVI developed standardized mucosal sample collection, processing and technical assay methods; these were subsequently applied into several observational studies, and Phase I HIV vaccines, Varicella zoster virus vaccine, and broadly neutralizing antibodies clinical trials at KAVI-ICR. Thereafter, KAVI-ICR facilitated the technology transfer of the methods, by training staff at regional establishments in Africa.

Results: Twelve standardized methodologies were developed for the collection, processing and storage of 10 mucosal sample types. Subsequently, eight regional research centers received training for a variety of clinical and laboratory methodologies; the centers later applied the techniques in follow-up collaborative research. Additionally, the training fostered collaboration while allowing the development of local networks of research groups.

Conclusion: By such South-South initiatives, supported by international donors, the development of regional capacity and expertise is realizable. The established expertise can be leveraged when needed, and builds the capability for African scientists to engage at an international level, actively participating in driving internationally relevant research.

Background

It is important that there are global networks of excellence that can rapidly facilitate basic and translational research in response to new and emerging pathogens. The recent Covid-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has highlighted the need for a collaborative response with different groups working together and sharing advances in 'real time'.

Countries in continental Africa feel a disproportionate effect of emerging and endemic infectious diseases. It is of vital importance that centers of excellence, in academic and scientific research, are developed in Africa, and the regional transfer of technology and knowledge supported. It is imperative to support and equip investigators, institutions, and associated stakeholders in the countries most affected by the diseases to allow them a leading role and active participation in research programs.

Over the last two decades there has been sustained, and increasing, investment in the development of basic and clinical research capacity in Africa.[1] There is need to encourage more inter-institutional South-South technology transfer, within Africa, during collaborative clinical research. This type of support allows local scientists to initiate and drive cutting-edge research in sub-Saharan Africa (SSA).

The majority of South-South Collaborations (SSC) for technology transfer are large and intergovernmental, mainly involving Latin American nations, SSA, India, and China; with countries from SSA largely being recipients.[2] In the health sector, most SSC within SSA have been supported by international charities or have occurred during emergency situations, as seen during the Ebola disease outbreak in 2014-2015.[3] Mutual respect and benefit to all stakeholders is vital in all forms of the collaboration. Although there have been great achievements made through North-South collaborations, these may be accompanied with power imbalances.[2,4] There exists ample capacity and underutilized talent and expertise in Africa that must be harnessed to foster SSC, and efforts towards this are underway, for example through the African Network for Drugs and Diagnostics Innovation (ANDI),[5] the European and Developing Countries Clinical Trials Partnership (EDCTP)-supported networks of excellence (NoEs),[6] the Sub-Saharan African Network for TB/HIV Research Excellence (SANTHE),[1] and the International AIDS Vaccine Initiative (IAVI) network of independent partner research centers in SSA.[7]

IAVI, with support from the United States Agency for International Development (USAID) and other international partners, have over the last 20 years developed a

network of independent partner research centers in SSA and India to facilitate the design and testing of vaccines against HIV-1. This 'network' of independent groups has leveraged this capacity to perform research and clinical trials into a number of other endemic diseases within the region, such as tuberculosis and Ebola, as well as catalyzing roll-out of HIV treatment and prevention for at-risk groups.[8]

Since the discovery of HIV-1, there has been a concerted effort to develop therapeutic and/or preventative interventions against the virus, including the development of an efficacious vaccine. While there have been a number of different approaches utilized in the design of vaccines, both in their composition and the vaccine regimen, the primary focus was initially based on assessing immune responses in peripheral blood to 'triage' vaccine candidates and determine potential vaccine efficacy in early phase studies. The vast majority of the global HIV-1 transmission occurs at the genital mucosa (cervico-vaginally, rectally, or through the penis foreskin, glans or urethra) during sexual intercourse.[9] It is in these mucosal compartments where the initial infection gains an early foothold and where a preventative vaccine or microbicide is likely to be required to act. We demonstrated a strict genetic bottleneck in the number of viruses that are transmitted and establish infection.[10] After several days, to weeks, the infecting virus replicates to such an extent that it passes through the draining lymph nodes[11] into the peripheral circulation and other distal mucosal compartments, establishing infection and where it is likely to form a reservoir.

Many HIV vaccine and microbicide clinical trials are multisite and based in Africa and other low-middle income and resource-limited countries; which bear the burden of the HIV-1 pandemic and where HIV therapy, vaccines or other preventative interventions are most needed. In addition to the traditional approach of assessing systemic immune responses and depending on the vaccine mode of action, investigating the participant's mucosal immune responses in clinical trials may be essential. Over the past few years, an increasing number of studies in SSA outside of South Africa have included mucosal sampling in such trials. Past studies have demonstrated the presence of HIV-relevant immune responses at mucosal sites.[12] Moreover, the field of vaginal microbiome (VMB) has lately attracted significant interest due to the role of VMB in the transmission of genital infections, including HIV. Although VMB studies have been scarce in Africa, this in now changing especially with the recognized role of VMB in the transmission of HIV.[13–16]

Feasibility studies from Kenya showed that study participants accepted several mucosal sampling techniques from the genital and non-genital mucosal sites.[12,17]

For comparable data across studies and research centers, mucosal samples should be obtained in a standardized and reproducible manner. Unlike blood sampling, mucosal sampling methods have not been widely standardized, with many groups using different sampling equipment and methods, compromising data comparability and reproducibility across studies and study centers.

To foster inter-institutional South-South technology transfer, Kenya AIDS Vaccine Initiative-Institute of Clinical Research (KAVI-ICR) developed a mucosal training program to help standardize sampling and processing methods across the region and address the capacity gap in mucosal sampling techniques and immunology in Eastern Africa. While this was focused on developing and testing HIV-1 vaccines, the approaches developed can be used in a range of fields and diseases.

Methods

Ethics and consent

KAVI-ICR and the trainees' research centers obtained local ethical approval for the respective research protocols. The research centers were KEMRI/Walter Reed Project (KEMRI/WRP)-Kericho Kenya, Center for Family Health Research (CFHR) Kigali Rwanda (previously known as Rwanda Zambia HIV Research Group-Projet San Francisco), UVRI-IAVI HIV Program Entebbe Uganda (UVRI-IAVI), KEMRI-Center of Geographical Medicine Research Coast (KEMRI-CGMRC) Kilifi Kenya, MRC/UVRI & LSHTM Uganda Research Unit (former MRC/UVRI Uganda Research Unit on AIDS Entebbe), Wits RHI, University of the Witwatersrand (WRHI) South Africa, Partners in Health Research and Development (PHRD), Kiambu Kenya, and Infectious Diseases Institute (IDI) Kasangati Uganda.

Kenyatta National Hospital-University of Nairobi Ethics and Research Committee granted approval for the studies at KAVI-ICR with the following ethical approval numbers: - P60/03/2008, P208/06/2010, P304/09/2010, P520/10/2012, and P353/05/2016, and P831/10/2019. KEMRI-CGMRC, KEMRI/WRP Kericho and PHRD received training at KAVI-ICR using two KAVI-ICR studies, approval numbers P60/03/2008 and P353/05/2016. Uganda Virus Research Institute Research and Ethics Committee granted approval for the studies at UVRI-IAVI (approval number GC/127/15/12/486), MRC/UVRI & LSHTM (approval number GC/127/12/07/33), and IDI (approval number GC/127/20/01/759). Rwanda National Ethics Committee approved the studies at CFHR (approval numbers 154/RNEC/2011, 373/RNEC/2012, and 820/RNEC/2019), and *Wits* Human Research Ethics Committee approved the

study at WRHI (approval number 200101B). All participants in these studies gave informed consent prior to collection of the mucosal samples.

Development of the mucosal procedures: KAVI-ICR worked with IAVI to develop Standard Operating Procedures (SOPs) for the collection, transport, processing, storage and downstream immunological assays of gastrointestinal, oral, genital, ano-rectal and upper respiratory tract mucosal specimens as described elsewhere.[12,17,18] Various sampling methods and devices were used. The procedures were applied into a range of studies to establish the feasibility of mucosal sampling and processing methods in an African setting,[17,19] compared to the experience of this sample collection in Europe and the USA.[20–22] Mucosal specimens included cells and secretions from the gastrointestinal, oral, genital, ano-rectal, and upper respiratory mucosal surfaces collected by various devices (Figure 1). All study participants provided informed consent and were given the opportunity to opt out of any mucosal sampling procedure. Reasons for refusal and other acceptability/tolerability data were collected. Depending on acceptability/tolerability and assay results, collection methods were 'dropped' or SOPs modified as needed. The resulting methods were subsequently applied into three Phase 1 HIV vaccine clinical trials at KAVI-ICR, all utilizing a common adenovirus 35 (Ad35) vector with varying vaccine regimens and prime/boost strategies, generating immunological data that were comparable across the trials.[17,23]

Mucosal training: After proof of concept studies at KAVI-ICR, a training program on mucosal specimen collection and processing was developed, in order to train other regional centers in the standardized collection and processing protocols.[24] Several centers within IAVI partner research centers were targeted, but training was also provided in response to requests from centers initiating unrelated mucosal studies. In their request for training, each center specified the type of mucosal sampling technique they required training in. Training was either provided on-site at KAVI-ICR or at the trainees' research centers based on the trainees' preference, and under locally approved protocols allowing the collection of mucosal samples. Following the restrictions occasioned by the covid-19 pandemic in the year 2020, we introduced an online mucosal training option backed with video demonstrations.

For training that occurred on-site at the trainees' research centers outside Kenya, it was first established that it was permissible for the trainers (medically certified in Kenya) to obtain clinical samples from study participants in that country. Prior to each training course the trainees' center was advised on the required equipment and supplies, and KAVI-ICR/IAVI facilitated access to supplies and potential

suppliers. As the consent process for mucosal sampling can be challenging, training was also provided in advance to 'transfer' the 'soft' skills acquired by KAVI-ICR staff during the development of their participant information sheets, informed consent documents and the consent process. Each center identified potential participants/ volunteers for mucosal sampling in advance and provided preliminary information, including informing the volunteers of their involvement in the incumbent training and sample collection by visiting foreign trainers.

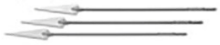
Mucosal specimen type	Device used
Rectal and cervical secretions	Merocel sponge 
Rectal and cervical cells	Digene cytobrush 
Rectal biopsies/cells	Sarratt rectal pinch forceps 
Cervicovaginal secretions	Instead Softcup 
Semen	Self-collection container 
Cervical secretions	Cervical aspirator 
Parotid gland secretions	Salimetrics oral swab 
Saliva drool	Straw 
Nasopharyngeal secretions	Flocked swab 
Nasal secretions	Synthetic absorptive matrix (SAM) strip 
Gut biopsies	Gut forceps 
Rectal secretions	OriCol rectal device 

Figure 1. Mucosal specimen types and devices usd at KAVI-ICR

The studies used for the training were several and independent/unconnected, hence had varied inclusion/exclusion criteria for participants, recruitment methods and other study procedures. Common across the studies was that the participants were adults aged at least 18 years; and that the study procedures included mucosal sampling and processing. There were eight studies at KAVI-ICR; four of these were used to develop the mucosal procedures plus the training, the other four (including clinical trials) applied the developed mucosal procedures.

The training team consisted of at least one research physician, one research nurse, and a medical laboratory technologist. The training curriculum covered both clinical and laboratory procedures, and was designed to last for two and a half to five days depending on the number of trainees and the techniques to be covered.

SOPs training and introduction to consent for mucosal sampling: After familiarization with the local clinic and laboratory facilities, training commenced with didactic sessions during which KAVI-ICR staff shared their experience with mucosal studies and the lessons learnt, highlighting potential issues regarding obtaining informed consent for mucosal sampling. This was followed with a step-by-step discussion of the relevant SOPs, including a showcase of equipment.[24] Trainees were then split into two groups for practical demonstrations, one of laboratory staff and the other of clinical staff.

Training in standardized mucosal sampling for clinicians: For the clinical demonstrations, the trainers and trainees were introduced to each potential volunteer and informed consent obtained prior to any mucosal sampling. KAVI-ICR clinicians (trainers) then performed a practical demonstration of each technique to the trainees. Subsequently, each trainee performed the techniques; initially assisted by the trainers, and then without assistance but under direct supervision. For the online training, the trainers used dummies instead of volunteers in the video demonstrations.

Samples were collected and processed as previously described.[12,17] Briefly, Synthetic Absorptive Matrix (SAM) strips, swabs, merocel sponges, or the OriCol device were momentarily placed against respective mucosal surfaces (nasopharyngeal, oral, cervical, and rectal) to collect mucosal secretions. A cervical aspirator was inserted into the cervical canal and secretions aspirated. The Instead Softcup was placed in the vagina to collect secretions then removed, by a clinician or the participant depending on participant's preference. The merocel sponges and swabs with collected secretions were each placed into a spin-X tube containing extraction buffer, and then placed on 'wet' ice until processing. The Instead Softcup with

cervicovaginal secretions was placed in a 50ml falcon tube and kept on 'wet' ice. Saliva/oral fluid (transudate) was obtained by allowing a volunteer to drool through a drinking straw, while parotid gland secretions were collected using a salimetrics oral swab placed against the buccal mucosal surface of the parotid gland. Semen was collected into a sterile plastic container containing transport media. Endocervical and rectal mucosal cells were collected by placing a Digene cytobrush on the endocervical or rectal mucosa then rotating it against the mucosal surface to obtain mucosal cells. Rectal biopsies were collected by pinching off the rectal mucosa using Sarrratt rectal pinch biopsy forceps. Cytobrushes with collected cells and rectal biopsies were placed in 15ml falcon tubes containing transport media at 4^0C until processing. All samples were transported to the laboratory and processed within two hours of collection.

Access to cervicovaginal and rectal mucosal surfaces was achieved using a sterile and disposable, vaginal speculum and a rectal proctoscope respectively.

Mucosal sample processing and storage training for laboratory personnel: Training covered processing and storage of all sample types, and several routinely used downstream assays and analyses as described previously.[12,18] Mucosal secretions were eluted from the swab/sponge by centrifugation then analyzed for antibodies using Enzyme Linked Immunosorbent Assays (ELISA). Cellular samples harvested from the Digene cytobrushes, and where applicable single cell suspensions from Collagenase II digested biopsies, were stimulated and analyzed using standardized multiparametric flow cytometry with intracellular cytokine staining. Flow cytometry data were analyzed using Flow-Jo Software (version 10.0.8; Tree Star, Ashland, Oregon) after acquisition.[18] Flow-Jo was provided to colleagues in Africa through the Flow-Jo Africa program which provides free licenses for researchers on the entire African continent who are enrolled with an African University and permanently located in Africa. Subsequent to training, there was follow-up with the regional centers to support their establishment of the assays, including continued sharing of SOPs. Whenever required, we arranged regular contacts *via* email or online calls for discussion of experimental design and troubleshooting where necessary.

Results

Development of the mucosal procedures: Clinical and laboratory SOPs were developed for the collection, processing and storage of:
- Rectal and cervical secretions using Merocel sponge;
- Cervicovaginal secretions using the Instead Softcup;
- Cervical secretions using the cervical aspirator;
- Seminal fluid by self-masturbation;
- Parotid gland secretions using the Salimetrics Oral Swab;
- Whole oral fluid (transudate) via a drinking straw;
- Nasal and nasopharyngeal secretions by the nasal flocked swab;
- Nasal secretions using the SAM strip;
- Rectal secretions using the OriCol rectal device;
- Rectal and cervical mucosal cells using the Digene cytobrush;
- Rectal mucosal cells using Sarrratt pinch forceps; and
- Gut mucosal cells using gut biopsy forceps.

These methodologies were subsequently successfully applied in three epidemiological mucosal studies at KAVI-ICR in the years 2009 to 2013 and thereafter in four collaborative Phase 1 HIV vaccine clinical trials, a Varicella Zoster vaccine trial, basic science studies, and most recently an HIV broadly neutralizing antibodies clinical trial. (Table 1)

Mucosal training: We, since the year 2011, have trained eight research centers from Uganda, Rwanda, South Africa and Kenya. The centers were KEMRI/WRP-Kericho Kenya, CFHR Kigali Rwanda, KEMRI-CGMRC Kilifi Kenya, MRC/UVRI & LSHTM Uganda, WRHI South Africa, PHRD Kiambu Kenya, and IDI Kasangati Uganda. All the centers, except WRHI, received training on the sampling of cervicovaginal mucosal secretions using the merocel sponge and/or Instead Softcup, while one center did not receive training on sampling of rectal secretions. Additionally, CFHR Rwanda received training on the collection of oral and upper respiratory secretions; KEMRI/WRP-Kericho and KEMRI-CGMRC Kilifi received training on rectal and cervical cell sampling using the cytobrush; KEMRI-CGMRC Kilifi further received training on rectal biopsy sampling using pinch forceps. CFHR, IDI, WRHI, PHRD, and UVRI-IAVI received training on rectal sampling using the OriCol rectal device in the year 2021. CFHR, MRC/UVRI & LSHTM, UVRI-IAVI, KEMRI/WRP, KEMRI-CGMRC, and PHRD had physical training; all three centers from Kenya received training at KAVI-ICR, while three centers from outside Kenya received training on-site at the trainees' research centers. In the year 2021 all (four) centers from outside Kenya (CHFR, UVRI-IAVI, IDI, and WRHI) received training online. (Table 2)

Table 1. Mucosal studies, techniques/devices used, and year study conducted

Device/method	Study protocol and year study conducted							
	J (2009-2013)	M (2011-2013)	L (2011-2016)	I (2011-2017)	S001 (2013-2015)	VZV (2017)	L015 (2018-ongoing)	C100 (2021-ongoing)
Merocel sponge	X	X			X	X	X	
Digene cytobrush	X					X		
Masturbation	X	X					X	
Aspirator	X							
Instead Softcup	X	X			X	X	X	X
Salimetrics oral swab	X	X	X		X	X	X	
Saliva drool	X	X	X		X			
Nasal flocked swab			X		X		X	
SAM strip			X					
Rectal Sarrratt pinch forceps	X							
Gut biopsy forceps				X	X			
OriCol rectal device							X	X

Table 2. Details of mucosal training conducted by KAVI-ICR at African research centers.

Research Center, Country	Year of training	Venue of training	IRB approval number of study for training	Mucosal sample type and sampling technique							
				CV-S	C-C	R-S-M	R-C	R-T	S-M	O/N-S	R-S-O
CFHR, Rwanda	2012 2021	CFHR Online	154/RNEC/2011 820/RNEC/2019	X X		X			X	X	X
KEMRI/WRP, Kenya	2011	KAVI-ICR	P60/03/2008	X	X	X	X		X		
UVRI-IAVI, Uganda	2015 2021	UVRI-IAVI Online	GC/127/15/12/486 P831/10/2019	X X		X			X		X X
KEMRI-CGMRC, Kenya	2011	KAVI-ICR	P60/03/2008	X	X	X	X	X	X		
MRC/UVRI & LSHTM, Uganda	2014	MRC/UVRI & LSHTM	GC/127/12/07/33	X					X		
WRHI, South Africa	2021	Online	200101B								X
IDI, Uganda	2021	Online	GC/127/20/01/759	X							X
PHRD, Kenya	2021	KAVI-ICR	P831/10/2019	X							X

A total of 108 research staffs received training with CFHR having the highest number of trainees (n=34, 32%). The trainees' cadres included physicians/clinicians (28%), nurses (33%) and laboratory technologists (39%). (Figure 2)

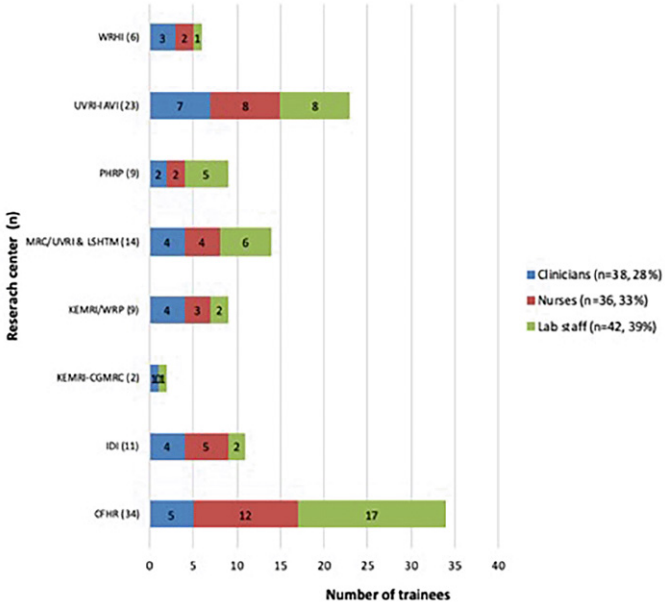


Figure 2. Number and cadre of trainees per research center.

CFHR - Center for Family Health Research, Kigali, Rwanda; IDI - Infectious Diseases Institute (IDI) Kasangati, Uganda; KEMRI/WRP - KEMRI/Walter Reed Project, Kericho, Kenya; KEMRI-CGMRC - KEMRI-Center of Geographical Medicine Research Coast, Kilifi Kenya; MRC/UVRI & LSHTM - MRC/UVRI & LSHTM Uganda Research Unit, Entebbe, Uganda; PHRD - Partners in Health Research and Development, Kiambu, Kenya; UVRI-IAVI - UVRI-IAVI HIV Program Entebbe Uganda; WRHI - Wits Reproductive Health and HIV Institute, South Africa.

All the centers that received training have since applied the skills learnt into a range of mucosal immunological studies. CFHR, Rwanda applied the mucosal skills in a collaborative multi-country observational study (with St. Mary's Hospital, London, UK and KAVI-ICR),[12] and during a clinical trial in collaboration with KAVI-ICR, Kenya, and St. Stephens AIDS Trust, London, UK[12,17,23]; the resulting mucosal immunological assay results were comparable across the participating sites. CFHR Rwanda, IDI Uganda, PHRD Kenya, UVRI-IAVI Uganda, WRHI South Africa, and KAVI-ICR Kenya are all applying the mucosal techniques in an ongoing multisite clinical trial; in this clinical trial KAVI-ICR is the regional center for the mucosal assays.[25]

Furthermore, laboratory personnel from three research centers in India were also trained in the laboratory methods developed by KAVI-ICR. These included staff at the National AIDS Research Institute Pune, YRG Care Chennai, and National Institute for Research in TB, Chennai; the training provided included mucosal samples processing, storage and immunological assays.

Discussion

We present a paper describing how a center of excellence at KAVI-ICR, Nairobi, Kenya, supported by IAVI, USAID and multiple international donors has developed inter-institutional South-South collaborations in Africa through facilitating technology transfer and the sharing of experiences and best practices in mucosal immunological studies. KAVI-ICR spearheaded successful mucosal studies and the transfer of technology to other SSA research centers, making it possible to standardize mucosal sampling and processing across the research centers, obtaining analogous samples that yield comparable data.[12,23]

The development of mucosal studies led to an increase in basic research, and resulted in the development of clinical trials in which assessment of mucosal responses is embedded within the clinical trial protocol.[17,23,25,26] Consequently, in collaboration with IAVI's USAID-funded mucosal immunology program, the IAVI extended network of clinical research centers developed a 'blanket' protocol to allow the collection of peripheral and limited mucosal samples in order to support the transfer of knowledge and training in these standardized methods, and to allow rapid sample collection for collaborative pilot studies to generate preliminary data.[27] While focused on studies that support the development of an HIV-1 vaccine, these methods are available to new collaborators to kick-start collaborative studies and utilize the capacity developed in these state-of-the-art clinical research centers. Indeed, these methods have already been applied in a varicella zoster vaccine study at KAVI-ICR in collaboration with University of Toronto.[26]

Importantly the nature of mucosal immunology and its requirement for fresh assays to be performed on site has facilitated the engagement of local staff at the research centers and the development of increased staff and infrastructure capacity on site. This has allowed local scientists to drive aspects of research to develop their own careers, actively engage with the project teams, and develop lasting collaborations and mentorship with international partners.

For example, researchers at KAVI-ICR are now employing the Widefield microscopy technology to studies assessing the replication of HIV transmitted/founder viruses in cervical and rectal tissues *ex vivo*, mucus mobility studies, and human tissues studies for HIV reservoirs.[28]

Although our program was initially designed for in-person delivery we were, out of necessity, able to successfully execute an online training as well. The four sites

that were trained online have subsequently successfully applied the mucosal techniques in a multisite and multi-country clinical trial,[25] from which we anticipate a yield of comparable data. One of the advantages of online training is the savings on travel and accommodation costs; such savings should be desirable especially in the SSA region where resources are limited. Therefore, online options for SSC in capacity building need to be purposely promoted.[29] For this the SSA institutions can seek support from organizations such as the South-South Galaxy which fosters online SSC through advisory services, capacity advancement, linkage to experts, among others.[30]

Another addition to our original design of the program was the extension of the technology transfer program outside the African continent, supporting training of partners in India. We have thus been able to progress from purely transferring methods in a North-South manner to supporting North-South-South-North collaborations for technology transfer.

Limitations

Our program did not have a formal monitoring and evaluation plan; we instead relied on random follow-up with the trained centers for troubleshooting and to support establishment of assays. However, we only received a few and minor post-training queries and consultations, and there were no requests from the research sites for follow-up repeat/refresher training. The only additional training requests were for new techniques not covered previously *e.g.* use of the OriCol device for the collection of rectal secretions. Hence, we believe that the trainings were effective. Nevertheless, we shall in future include a quality control and evaluation plan in the program. A second limitation was that we did not advertise our courses widely and instead relied on the sites soliciting for the training; this may have limited our reach. Despite this we trained several centers across four countries. Besides, we ultimately were able to successfully implement a tailored training (physical and online) from which we can henceforth confidently expand our reach *via* open calls for trainees.

Conlusions

We successfully established and implemented a South-South mucosal training program in SSA. Our venture for sharing of experiences and best practices, also yielded collaborative research projects across the participating sites. This initiative has now expanded into a state-of-the-art mucosal immunology program, through which KAVI-ICR has expanded its research portfolio.

Lessons from our mucosal capacity building program can be extrapolated to SSC for capacity strengthening and technology transfer in other areas of the clinical research processes spectrum - from research conceptualization, grant writing, research inception and conduct, up to dissemination of research findings.

SSC in research should be encouraged; through this, robust local networks of excellence for expanded collaborative research are realizable. Additionally, pulling together in the principle of horizontality is beneficial for balanced and triangular cooperation in research. Through such initiatives, supported by international donors, the development of regional capacity and expertise is possible. The established expertise can be leveraged when needed, and also builds the capability for African scientists to engage at an international level, actively participating in driving internationally relevant research.

Paper context

Establishment and promotion of research capacity and expertise in Sub-Saharan Africa is essential, for leveraging when needed. This paper describes a regional South-South technology transfer initiative in clinical research, whose advancements are facilitating advanced research and new collaborations in Sub-Saharan Africa. South-South technology transfer in research should be promoted; it builds the capability of African scientists to actively participate in driving internationally relevant research and provides networks for rapid local response to novel emerging pathogens.

Software availability

Flow-Jo was provided to colleagues in Africa through the FlowJo Africa program which provides free licenses for researchers on the entire African continent who are enrolled with an African University and permanently located in Africa. Flow cytometry data can also be analysed using the software provided with individual Flow cytometry machines or using freely available software such as Floreada.io.

Data availability statement

Underlying data

Data underlying the findings of this study are available within the article.

Extended data

Figshare: Extended data - Mucosal sample collection, processing and technical assay methods used in the establishment and implementation of a regional mucosal training program to facilitate multi-center collaboration in research in Eastern Africa. https://doi.org/10.6084/m9.figshare.24116082 (Omosa-Manyonyi GS *et al.,* 2023).

This project contains the following extended data:

Data file 1. Presentation: Mucosal training - Introduction.pdf

Data file 2. Presentation: Mucosal training - Oricol device and Softcup mucosal sampling & processing.pdf

Data file 3. SOP 33 version 4.0 – Collection of Rectal and Cervical Cytobrush and Merocel Specimens and Rectal Biopsy.pdf

Data file 4. SOP 34 version 2.0 - Collection of Semen Sample.pdf

Data file 5. SOP 0075 – Collection, transport, processing and storage of oral fluid (passive drool saliva).pdf

Data file 6. SOP 0076 – Collection, transport, processing and storage of nasal samples using Flocked swabs.pdf

Data file 7. SOP 0078 – Collection, transportation, processing and storage of Vaginal samples using an Instead softcup.pdf

Data file 8. SOP 0080 – Collection, transport, processing and storage of cervico-vaginal samples using a Merocel sponge.pdf

Data file 9. SOP CL 49 - Procedure for processing Semen.pdf

Data file 10. SOP CL 55 - Collection of passive Drool Saliva version 1.1.pdf

Data file 11. SOP CL 56 - Collection of Saliva direct from the Parotid gland Using the Salimterics Oral Swab version 1.1.pdf

Data file 12. SOP CL 60 version 1.0 - Nasal Sample Collection (SAM).pdf

Data file 13. SOP IMM 024 - Collection transport processing and storage rectal secretions using an OriCol sampling device V1.0_250521.pdf

Data file 14. SOP SLM 0016 - Isolation of human mucosal cells from colonic rectal or duodenal tissue.pdf

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CHAPTER 6: GENETIC SUSCEPTIBILITY TO RECURRENT VULVOVAGINAL CANDIDIASIS IN AN AFRICAN POPULATION FROM NAIROBI, KENYA

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Abstract

Vulvovaginal candidiasis (VVC) affects 75% of women worldwide at least once in their lifetime, with up to 9% of women experiencing recurrent episodes (RVVC). Genetic differences may play a role in women developing recurrent VVC infections. Thus, we investigated genetic host factors that may increase the risk of RVVC in women from an African population. We conducted a case–control study in women in Nairobi Kenya, to identify genetic risk factors for RVVC. Our genome-wide association study compared women with RVVC (n = 174) to those with acute VVC (n = 157), and with controls (n = 347). The control group included both symptomatic but uninfected women (n = 246) and asymptomatic/healthy women (n = 101). We identified several genomic variants linked to increased RVVC susceptibility ($P < 10^{-5}$), with the key ones being SNP rs8181503 found near the *MS4A12* gene ($P = 9.28 \times 10^{-7}$, odds ratio (OR) = 0.46), and SNP rs58936172 located near the *TMEM39A* gene ($P = 8.96 \times 10^{-6}$, OR = 2.42). Pathway enrichment analysis revealed enrichment of genetic variants linked to increased risk of RVVC in genes involved in metabolic, disease signalling, and cell adhesion pathways. These included pathways related to gluconeogenesis, fatty acid metabolism, linoleic acid metabolism, pentose phosphate, chemotaxis, and fibroblast growth factor signalling pathways. The genes and pathways identified in our study may help to understand the susceptibility to RVVC in African populations, to improve patient care.

Introduction

Vulvovaginal candidiasis (VVC) affects 75% of women worldwide at least once in their lifetime[1]. Recurrent vulvovaginal candidiasis (RVVC), defined as at least 3 distinct episodes of vulvovaginal candidiasis (VVC) within 12 months[2,3,4], affects up to 9% of women worldwide[5,6]. However, studies from symptomatic women in Africa report higher RVVC rates of almost 25%7,8. In the multifactorial symptomatic vaginal *Candida* infections, host predisposing factors, perturbed microbiota composition, and fungal virulence traits play a role9. Commonly reported predisposing host factors include pregnancy, diabetes, contraceptive use, and broad-spectrum antibiotic treatment10. However, only some women experience recurrent episodes of VVC, and in most cases, the condition is idiopathic. This suggests that genetic differences in genes involved in the antifungal host immune response may influence susceptibility to the infection11.

An individual's genetic makeup plays an important role in disease susceptibility[12,13] and response to treatment14. Understanding the role of genetics in different populations for specific diseases has become increasingly important for improving diagnostics, developing targeted therapies, and enhancing disease prevention strategies. Notably, most human genomics research has focused on populations of non-African ancestry. However, African populations remain underrepresented in research on genetic susceptibility to diseases[15,16,17,18] -despite having the greatest genetic diversity[19,20,21,22,23], the highest infectious disease burden[24] and an increasing load of non-communicable diseases[25]. This situation is however improving through initiatives such as the Human Heredity and Health in Africa (H3Africa) multi-country consortium, which empowers African researchers and fosters collaboration in genomic research—investigating the interplay between human genetics, the environment, and disease susceptibility[26]. Other efforts include the African Genome Variation Project[22], the African BioGenome Project[27], the African pan-genome assembly[21], and the African Genomics Program by Roche[28].

Candidate gene approach studies have identified genetic variations in immune-related genes as potential contributors to increased susceptibility in RVVC. For example, mutations in receptors involved in recognizing *Candida albicans* cell wall components—such as Dectin-129, its adaptor molecule CARD9[30,31], TLR2[32,33], or MBL[34]—can impair fungal detection and the host immune response, and have been linked to a higher risk for RVVC. Polymorphisms in NLRP3 have also been associated with RVVC4,[35], and higher concentrations of IL-1β were detected in vaginal secretions from women carrying the RVVC-associated genotype4,

suggesting that RVVC may be characterized by an enhanced pro-inflammatory immune response[36,37].

The advent of the 'omics' technologies and cohort-based studies has enabled genome-wide association studies (GWAS)[38] to identify candidate loci associated with increased susceptibility to various diseases including (R)VVC. Through the integration of genome-wide genetic analysis and immunological data, sialic acid-binding immunoglobulin-like lectin 15 (*SIGLEC-15*) has been identified as a potential susceptibility gene for RVVC[39]. Another GWAS conducted on a large population-based biobank in Estonia highlighted the role of the vaginal epithelium in recurrent vaginitis, suggesting that epithelial factors may influence host susceptibility to disease[40]. Despite these efforts, there is a scarcity of RVVC-related genomic studies due to challenges related to sample size and the difficulty of validating these findings across diverse populations, including Africans.

Genetic differences and heterogeneity in gene-driven cytokine response to antigen stimulation have been observed between populations of African and European ancestry[41,42,43]. Hence, the findings on genetic susceptibility to RVVC from European cohorts[39,40] need to be complemented by studies in larger and more diverse populations, to better understand RVVC pathogenesis worldwide, and to advise therapeutic and preventive strategies. Therefore, we conducted a GWAS in an African cohort of women with RVVC. In order to better understand the contributing genetic factors that predispose these women to recurrent episodes of *Candida* infection, we compared the genetic landscape of women with RVVC and those with VVC; we also compared RVVC and a control group consisting of 'asymptomatic/healthy women' plus 'symptomatic but uninfected women'. We included the two categories of women in the control group to account for any potential confounding factors, such as the presence of *Candida* commensal colonization, viral infections (e.g. genital herpes), and non-infectious causes of genital symptoms (e.g. atrophic vaginitis, allergic reactions, hormonal effects). The VVC comparator group served to assess for the genetic differences between women with RVVC and those able to limit the VVC episodes.

Methods

Ethical statement

Ethics approval was granted by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee, P980/12/2016. A research license (permit) was

granted by the National Commission for Science Technology and Innovation, and authorization to conduct this study at the health facilities was given by Nairobi City County, Kenya. All methods were performed in accordance with the Declaration of Helsinki, and relevant guidelines and regulations. Informed consent was obtained from all individual participants included in the study.

Study design and populations

We performed a case–control study to determine risk factors for RVVC. This study was part of a larger longitudinal observational study on female lower genital tract infections and RVVC, conducted between October 2018 and March 2023, at seven health facilities in Nairobi, Kenya. The parent study was designed to establish a cohort of 250 women with RVVC, however due to the restrictions occasioned by the COVID-19 pandemic, participant recruitment was terminated prematurely in March 2020.

In this case–control study we performed two different comparisons: 1. RVVC (n = 174) versus Controls (n = 347); and 2. RVVC versus VVC (n = 157). The Controls group consisted of symptomatic but uninfected women (n = 246) plus asymptomatic (healthy) women (n = 101). The symptomatic but uninfected were women with one or more of the following genital tract symptoms (GTS) i.e. vaginal discharge with or without a foul smell, vulvar or vaginal itching/pruritus, vulvovaginal soreness or burning sensation, lower abdominal pain, dysuria, and dyspareunia; and whose vaginal smear sample tested negative for *Candida* species, Bacterial vaginosis (BV), and sexually transmitted infections (STI) (i.e. *Neisseria gonorrhoea* (NG), *Trichomonas vaginalis* (TV), *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG)). Asymptomatic women were those with no GTS. RVVC was defined as having at least three symptomatic episodes of VVC within a 12-month period, with laboratory-confirmed *Candida* species identified in a vaginal smear for at least one the episodes. Participants with fewer than three VVC episodes in 12 months were classified as having acute VVC (VVC group). Participants were consenting non-pregnant women, aged 18–50 years, and who tested negative for both HIV and glycosuria, recruited at outpatient health facilities in Nairobi, Kenya. The study procedures have been previously described in detail8. Briefly, sociodemographic and clinical data, vaginal smear samples for detection of genital infections, and blood (buffy coat) for DNA isolation were obtained from all participants. Genital infections were detected as follows:—candidiasis by microscopic examination and culture on Sabouraud dextrose agar media; BV by the Nugent score; and CT, NG, MG, and TV by multiplex Real Time polymerase chain reaction test (Sacace Biotechnologies, Como, Italy).

Genotyping, quality control, and imputation

DNA isolated from whole blood samples of 678 participants was genotyped using Illumina GSA beadchip GSA MD (Illumina GSA Arrays "Infinium iSelect 24 × 1 HTS Custom Beadchip Kit). Quality control was performed before and after genotype imputation. Briefly, samples with a low call rate (≤ 99%) and variants with a Hardy–Weinberg equilibrium ≤ 0.00001, call rate < 0.99, missingness test (GENO > 0.01), and minor allele frequency (MAF) < 0.001 were excluded from further analyses. Duplicates, and first- and second-degree relatives were identified through multidimensional scaling (MDS) analyses and excluded from further analysis. A total of 265,479 variants passed quality control and were sent for imputation. Imputation was performed using the human reference consortium (HRC) panel[44]. As there is incomplete availability of genome reference data from the African population, for quality control ethnic outliers in our study population were identified based on MDS analyses by comparing the genomes of different populations from the 1000G Project[44,45] with our study population. We performed MDS analyses per analysis, so the number of outliers is different in each of the comparisons i.e. RVVC versus Controls and RVVC versus VVC.

Association analysis

The GWAS was performed at single nucleotide polymorphisms (SNPs) with MAF > 0.05, and genetic susceptibility to RVVC was tested using logistic regression after correcting for five principal components to account for genetic heterogeneity. *P* value distributions were assessed using a Quantile–Quantile (Q–Q) plot to estimate inflation effect on the association results. The impact of identified SNPs on gene expression was then explored using Genotype-Tissue Expression (Gtex) database[46] to reveal expression quantitative traits (eQTL); we next annotated the identified loci with their potential association with other quantitative traits using Open Target database[47].

Results

Participant characteristics

Of the 678 participants, 174 were RVVC, 347 were Controls (246 symptomatic without infection (candidiasis, BV, STI), and 101 asymptomatic (healthy) women), and 157 were VVC. The participants were mainly from 5 ethnic groups, specifically, Kamba, Kikuyu, Kisii, Luhya and Luo, and their median age (IQR) was 27.7 (24.3–33.9) for RVVC, 29 (24–35) for the symptomatic uninfected and the asymptomatic women, and 29 (24.1–33.0) for VVC. Table 1

Table 1. Demographic characteristics of participants by group (RVVC, Controls, and VVC).

Variable	RVVC, n (%)	Controls, n = 347		VVC, n (%)
		Symptomatic with no infection, n (%)	Asymptomatic, n (%)	
Total	174 (25.7)	246 (36.3)	101 (14.9)	157 (23.2)
Age in years				
18–25	56 (32.2)	65 (26.4)	31 (30.7)	49 (31.2)
26–35	80 (46.0)	117 (47.6)	49 (48.5)	76 (48.4)
35 and above	37 (21.3)	58 (23.6)	21 (20.8)	25 (15.9)
Not indicated	1 (0.6)	6 (2.4)	0 (0)	7 (4.5)
Median age (IQR)	27.7 (24.3–33.9)	29 (25–35)	29 (24–34)	29 (24.1–33.0)
Ethnicity				
Kamba	27 (15.5)	43 (17.5)	10 (9.9)	27 (16.6)
Kikuyu	66 (37.9)	95 (38.6)	23 (22.8)	64 (40.8)
Kisii	16 (9.2)	21 (8.5)	8 (7.9)	12 (7.6)
Luhya	33 (19.0)	38 (15.5)	44 (43.6)	38 (24.2)
Luo	17 (9.8)	21 (8.5)	6 (5.9)	4 (2.6)
Other	15 (8.6)	28 (11.4)	10 (9.9)	12 (7.6)

RVVC, recurrent vulvovaginal candidiasis; VVC, vulvovaginal candidiasis; IQR, interquartile range.

MDS analysis confirms the African ancestry of our cohort, but emphasizes the lack of sufficient African reference data

At MDS, 35 duplicates and 19 first- and second-degree relatives were excluded, leaving 624 participants for further analysis. As there is incomplete availability of genome reference data from the African population, we investigated the ancestry of individuals from our study population. To do so, we first conducted MDS analysis using genome reference data available from the 1000G Project45. Genetic data from our cohorts showed a complete overlap with the genomes of the African population (Supplementary Fig. 1A). Next, we compared the genetic data from our cohorts with the African-only reference data available in the 1000G Project. This showed the overlap of subset of individuals from our cohorts with the Luhya (LWK) population of Kenya; but we also observed the presence of some samples that were not overlapping with the available reference data in the 1000G Project (Supplementary Fig. 1B). Lastly, we annotated our study population samples with the names of their different tribes (Supplementary Fig. 1C) and found distinct clusters. This observation points to the lack of sufficient reference genomes to capture fully the genetic heterogeneity of the African population. Nevertheless, MDS analysis comparing the genomes of the cohorts in our study population (RVVC, VVC, Symptomatic without infection, and Asymptomatic) indicated that there is no clustering of individuals based on disease status (Supplementary Fig. 1D).

GWAS of RVVC versus controls and annotation of susceptibility SNPs

To identify genetic variants associated with susceptibility to RVVC, a GWAS was performed in a cohort of 160 Cases (RVVC) and 309 Controls (symptomatic uninfected women, plus asymptomatic women). After imputation and quality-control filtering, 7.18 million variants were analysed using regression analysis. Although no SNPs passed the genome-wide significance threshold of $P < 5 \times 10^{-8}$, 14 independent loci with $P < 10^{-5}$ were suggestive for an association with RVVC (Table 2). Among these loci, a SNP on chromosome 11, rs8181503, showed the strongest association with susceptibility to RVVC ($P = 9.28 \times 10^{-7}$, odds ratio (OR) = 0.46) and is located close to the *MS4A12* gene. Next, we explored the impact of these candidate SNPs on gene expression using Gtex database and revealed expression quantitative traits (eQTL) for five loci. We also annotated the 14 loci with their potential association with other quantitative traits using Open Target database. This led to the confirmation of the impact of one locus, rs6731176 on both RNA and protein levels of the gene *PROC* (Table 2).

Table 2. RVVC versus controls group: SNPs associated with susceptibility to RVVC ($P < 10^{-5}$).

rsID	Chr	Position	*P* value	OR	Closest gene	eQTL	Association with other traits
rs8181503	11	60,264,303	9.28E–07	0.459	MS4A12	MS4A12, MS4A14, MS4A7, MRPL16	Lymphocyte, neutrophil and white blood counts
rs10481602	9	6,623,931	1.90E–06	0.432	GLDC	GLDC, ERMP1	Eosinophils
rs320286	9	104,841,979	3.84E–06	2.300	GRIN3A		TestASV_49 (Parasutterella) abundance
rs12899123	15	79,693,138	4.41E–06	2.006	MINAR1	MINAR1	Monocyte chemoattractant protein-2 levels, Pro-cathepsin H levels
rs7788522	7	11,865,522	5.34E–06	2.062	THSD7A	VWDE	Immature fraction of reticulocytes
rs11995330	8	132,237,096	7.06E–06		ADCY8		
rs2894960	15	96,124,446	7.24E–06	0.496	NR2F2		OTU99_171 (Bacteroides) abundance
rs11120666	1	216,016,605	7.91E–06	0.477	KCTD3		
rs4520208	9	133,386,108	8.13E–06	2.315	ASS1		
rs331429	18	7,806,209	8.33E–06	2.595	PTPRM		
rs8079866	17	8,910,626	8.46E–06	2.288	NTN1		Lymphocyte counts, Serum 25-Hydroxyvitamin D levels

Table 2. Continued

rsID	Chr	Position	*P* value	OR	Closest gene	eQTL	Association with other traits
rs9426356	1	29,776,275	9.03E−06	1.906	PTPRU		CX3CL1 levels, Fibroblast growth factor 23 levels, Interleukin-12 subunit B levels, Leucocyte count
rs79041335	4	156,719,062	9.43E−06	2.423	GUCY1B1		Cathepsin D levels
rs6731176	2	128,071,003	9.85E−06	1.862	ERCC3		PROC levels

RVVC, recurrent vulvovaginal candidiasis; SNPs, single nucleotide polymorphism; rsID, reference SNP identification; Chr, chromosome; OR, odds ratio; eQTL, expression quantitative traits.

Given the role of multiple pathways in fungal infection, we hypothesized that the 14 genetic loci that show suggestive association with RVVC ($P < 9.99 \times 10^{-5}$) are enriched for susceptibility pathways for RVVC. To test this, we ran a pathway enrichment analysis using FUMA48. We found a significant enrichment of the identified RVVC susceptibility genes in metabolic pathways and cell adhesion pathways (Fig. 1, panels A–C).

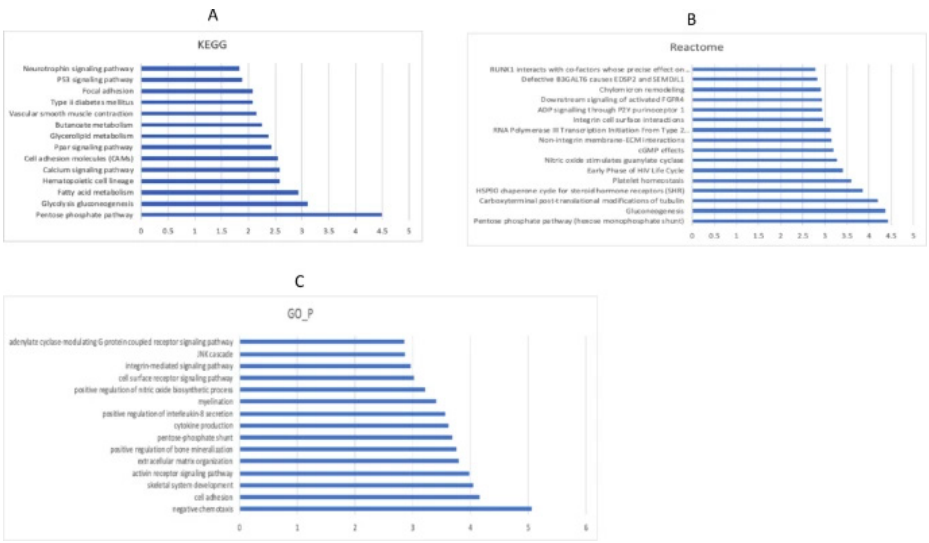

Fig. 1. RVVC-associated genes are enriched in metabolic and adhesion pathways based on (**A**) KEEG; (**B**), Reactome; and (**C**) GO database. – log (10) *P* values are shown on the x-axis and the significant pathways are named on the y-axis. RVVC, recurrent vulvovaginal candidiasis; SNPs, single nucleotide polymorphism; rsID, reference SNP identification; Chr, chromosome; eQTL, expression quantitative traits.

GWAS of VVC and annotation of susceptibility SNPs

Prior to comparing RVVC and VVC groups, we performed a GWAS analysis to identify genetic variants associated to VVC. After quality control, the cohort comprised 444 women (137 VVC and 307 Controls) and 7.18 million variants. A region on chromosome 14 was significantly associated to VVC (rs76123164, $P = 2.94 \times 10^{-8}$, OR = 4.61). While this SNP is closest to the *ADAM20* gene, we also found it to be eQTL for *TTC9* and *SLC8A3*. Additionally, 18 other regions showed suggestive associations ($P < 10^{-5}$). (Table 3).

Table 3. VVC versus controls: SNPs associated with susceptibility to VVC ($P < 10^{-5}$).

rsID	Chr	Position	*P* value	OR	Closest gene	eQTL	Association with other traits
rs76123164	14	70,963,806	2.94E–08	4.608	ADAM20	TTC9, SLC8A3	FINNGEN_R6_N14_GENITOUROTH, Other disorders of the genitourinary system
rs12645101	4	189,495,641	7.26E–07	2.895	TRIML1		
rs12148912	15	38,441,998	9.26E–07	2.326	SPRED1		vascular endothelial growth factor D levels
rs4259993	15	49,224,372	1.33E–06	2.877	SHC4	EID1, CEP152	
rs11147913	13	44,092,097	1.39E–06	0.318	ENOX1		OTU99_17 (Parabacteroides) abundance
rs220016	6	12,269,373	1.39E–06	2.706	EDN1		FINNGEN_R6_N14_ENDOMETRIOSIS_UTERUS
rs6082788	20	286,047	2.68E–06	2.087	ZCCHC3		FINNGEN_R6_R18_SYMPTOMS_SIGNS_INVOLVI_URINARY_SYSTEM
rs12112921	7	91,069,107	2.80E–06	3.112	FZD1		
rs7141605	14	63,880,446	2.98E–06	2.021	GPHB5		
rs2598007	7	37,932,490	3.33E–06	2.09	EPDR1	EPDR1, NME8	Adhesion G protein-coupled receptor E2 levels, testosterone
rs12015	13	43,597,865	4.13E–06	0.436	DNAJC15	DNAJC15, EPSTI1	

Table 3. Continued

rsID	Chr	Position	*P* value	OR	Closest gene	eQTL	Association with other traits
rs7333036	13	24,783,796	6.58E–06	0.414	C1QTNF9	PABPC3	TestASV_13 (Bacteroides) prevalence, OTU97_12 and OTU99_12 (Bacteroides) prevalence
rs7221982	17	9,386,531	6.81E–06	2.720	STX8	STX8	FINNGEN_R6_ AB1_VIRAL_NOS, Other viral diseases, not elsewhere classified"
rs140843488	7	38,006,925	7.66E–06	4.060	EPDR1		
rs71460779	12	127,779,575	7.74E–06	2.645	TMEM132C		
rs148657506	7	134,395,396	8.53E–06	3.666	CALD1		
rs78024235	12	89,198,265	9.11E–06	2.318	KITLG		Scavenger receptor cysteine-rich domain-containing group B protein levels, Leucocyte count
rs644014	11	109,183,666	9.40E–06	3.326	C11orf87		Peripheral vascular disease
rs11843172	13	112,123,643	9.67E–06	2.342	TEX29		

VVC, vulvovaginal candidiasis; SNPs, single nucleotide polymorphism; rsID, reference SNP identification; Chr, chromosome; OR, odds ratio; eQTL, expression quantitative traits.

GWAS of RVVC versus VVC and annotation of susceptibility SNPs

To test whether genetic variants were playing a role in conferring recurrent infections, we performed a GWAS analysis comparing 158 RVVC patients with 142 VVC patients. Although we found no genome-wide significant loci, we identified 12 independent loci with $P < 10^{-5}$, suggestive for an association with RVVC with the strongest SNP being on chromosome 21 ($P = 1.22 \times 10^{-6}$, OR = 0.33) located next to the *H2BC12L* gene. We also found several of these SNPs to be eQTLs. For example, we found the expression of transmembrane protein 39A gene (*TMEM39A*) to be associated with rs58936172 locus ($P = 8.96 \times 10^{-6}$, OR = 2.42), rendering *TMEM39A* an important gene for RVVC susceptibility. (Table 4).

Table 4. RVVC versus VVC: SNPs associated with susceptibility to RVVC ($P < 10^{-5}$).

rsID	Chr	Position	*P* value	OR	Closest gene	eQTL	Association with other traits
rs59298188	21	45,010,840	1.22E–06	0.333	H2BC12L		
rs4256198	4	164,690,560	2.72E–06	0.401	TMA16		
rs9544178	13	76,706,109	3.60E–06	0.385	LMO7		Bladder cancer
rs7608036	2	146,602,191	4.41E–06	0.344	ZEB2		FINNGEN_ R6_AB1_ CANDIDIASIS
rs7355834	3	78,227,862	4.52E–06	2.327	ROBO1		
rs34944783	19	11,684,646	5.48E–06	0.209	ACP5	ACP5, ZNF823, ZNF627	Neutrophil, white blood cells and reticulocyte cell count
rs62128804	19	48,342,011	6.97E–06	0.375	CRX	SULT2A1, CABP5, SELENOW	Vitamin D, Cholelithiasis, Disorders of gallbladder, biliary tract and pancreas
rs9608	9	34,998,003	7.28E–06	0.387	DNAJB5	VCP, DNAJB5, FANCG, PIGO, PHF24, STOML2	Platelet, monocyte and reticulocyte cell counts
rs10420116	19	55,030,897	8.08E–06	0.428	LAIR2	LAIR2, LILRB4	OTU99_558 (Bacteroidales) abundance
rs12491867	3	181,448,558	8.36E–06	0.248	SOX2		
rs58936172	3	119,127,883	8.96E–06	2.424	TMEM39A	POGLUT1, TMEM39A, TIMMDC1	Lymphocyte and eosinophil counts
rs73787097	5	110,951,123	9.90E–06	3.359	STARD4		

rsID, reference single nucleotide polymorphism identification; Chr, chromosome; OR, odds ratio; eQTL, expression quantitative traits.

From pathway enrichment analysis on genes from these loci, there was a significant enrichment of genes in metabolic pathways and disease signalling pathways, with the main ones being linoleic acid metabolism and fibroblast growth factor receptor (FGFR) signalling. (Fig. 2, panels A–C).

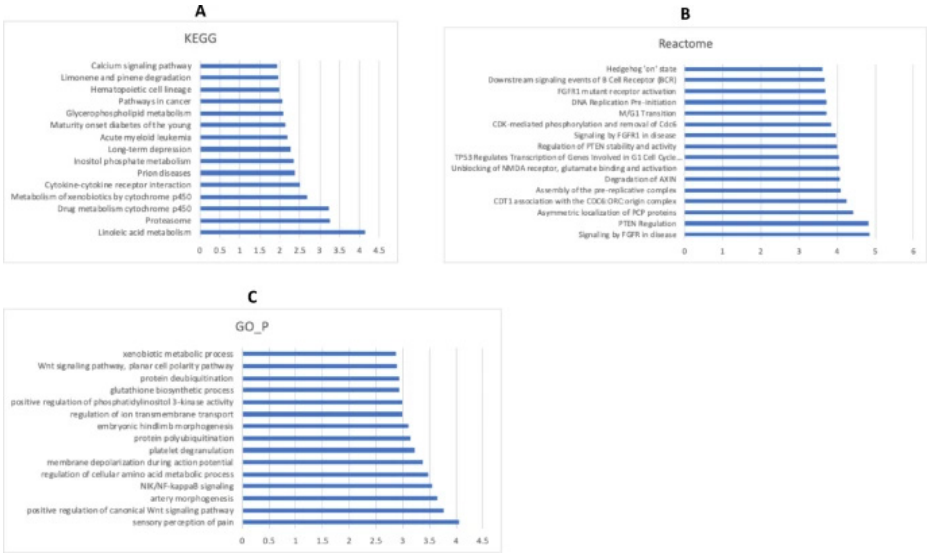

Fig. 2. RVVC versus VVC—Pathways enrichment analysis based on (**A**) KEEG; (**B**), Reactome; and (**C**) GO database. – log (10) *P* values are shown on the x-axis and the significant pathways are named on the y-axis.

Discussion

In this study we performed the first genome-wide association analysis in an African population of women reporting recurrent episodes of VVC. We identified several genomic variants associated with RVVC, with the strongest being the SNP rs8181503, located close to the *MS4A12* gene on chromosome 11; as well as the SNP rs58936172 next to the *TMEM39A* gene on chromosome 3. We found no overlap in the susceptibility SNPs for RVVC and VVC. The polymorphisms associated with RVVC were linked to dysregulated metabolic, cell adhesion and disease signalling pathways relevant for RVVC susceptibility, with the top pathways being gluconeogenesis, fatty acid metabolism, linoleic acid metabolism, pentose phosphate, chemotaxis, and FGFR signalling pathways.

During encounters with pathogens, the immune cells' metabolic processes are reprogrammed towards providing the energy and substrates necessary for phagocytosis and the mounting of a successful immune response[49,50]. In line with this, monocytes exposed to *Candida* exhibit an upregulation of glycolysis, and the biosynthesis and catabolism of glycogen is important for *Candida albicans* metabolism and virulence[51,52,53,54]; in gluconeogenesis-associated polymorphisms therefore, the immune response is bound to be inadequate. Linoleic acid inhibits hypha formation by *Candida* thus interfering with the

morphogenic processes which are key to the fungus's virulence55. Therefore, women with polymorphisms that affect the metabolism of linoleic acid may exhibit an insufficient immune response against *Candida*. It is thus interesting to observe that genes related to these metabolic processes are among the most enriched in genetic variants associated with susceptibility to RVVC.

The *TMEM39A* gene influences immune pathways for cytokine production and hence may affect the IL-17 and IL-22 cytokines, which are important components in the host's immune response and mucosal barrier against *Candida* infection, respectively[56,57]. Indeed, use of interleukin IL-17 blockers (in comparison with use of anti-TNFα) is associated with increased vulnerability to VVC in patients with psoriasis58. In women bearing the rs58936172 SNP, we speculate that the *Candida*-host commensal association[59] is easily tilted in favour of the fungus as compared to others who despite exposure to the *Candida* are able to maintain the commensal association always or most times.

Studies from non-African populations revealed genes associated with RVVC susceptibility via effects on the host immune processes[4,60]. From a European population, Jaeger et al.[39] identified the SIGLEC15 to be associated with RVVC. We speculate that the upregulation of the *MS4A12* and *PROC* genes in African women may be associated with the hyperinflammation to the *Candida* fungus in RVVC. An upregulated *MS4A12* gene likely promotes hyperinflammation via an exaggerated IgE-mediated inflammatory response[61], while that by the *PROC* gene is via protein C[62]. Since *TMEM39A* polymorphism is associated with autoimmune diseases[63,64], we have reason to suggest that for women with this SNP, RVVC is likely akin to an autoimmune condition with the immunogen being the *Candida* fungus during its commensal existence in the host. Our finding of no upregulation of hyperinflammation genes in VVC despite the VVC-associated inflammation aligns with this view. To elucidate the exact mechanisms involved, functional studies are warranted in the future, including assessment of the influence of the unique environmental factors and endemic infections in Africa, on the identified polymorphisms for RVVC.

In previous RVVC genetic studies, patients with RVVC were mainly compared with healthy women without vaginal symptoms. In our study, we expanded the comparator group to include women with symptoms but without an etiology as well as those with sporadic VVC, and one may argue that these control groups are the ideal—since susceptibility to recurrent *Candida* infections is also explored in contrast to occasional *Candida* infection or symptoms that are not related to the

fungus. To explore further on this conjecture, we recommend that further RVVC genetic studies involve the expanded comparator groups, and also integrate these comparator groups in RVVC-related genital mucosal immunological studies.

A key limitation of our study is the sample size, which impacts the statistical power to detect genome-wide significant associations. Based on standard GWAS power calculations, our cohort of 174 cases and 347 controls provides limited power (< 30%) to detect an odds ratio of 1.5 at a MAF of 5%, using a genome-wide significance threshold of 5×10^{-8}. Given these constraints, our study is primarily exploratory and aimed at identifying suggestive genetic associations that warrant further validation. Larger sample sizes, replication in independent cohorts, and meta-analyses will be necessary to confirm the observed associations and achieve robust genome-wide significance. A second limitation is that in this study's setting, diagnosis of VVC is conventionally symptom based without microbiological testing. Our definition of RVVC thus included reliance on participants' recall of previous VVC episodes, which may have introduced recall bias. Future studies should aim to obtain longitudinal microbiological confirmation of *Candida* infection for all VVC episodes. Third, our cohorts were not without co-infections (NG, TV, CT, MG, and BV)8; however, these being acquired conditions, we believe that the co-infections did not impact the SNPs identified, and are in any case a true reflection of these characteristics in the RVVC group. Finally, we lacked a comparative African RVVC cohort because studies and data on RVVC are scarce as the diagnosis of RVVC is not recognized in the syndromic approach frequently/widely used in African settings. This nevertheless means that the data that we present here is a treasure for future studies.

Despite these limitations, our study is notable, being the first to associate relevant SNPs with RVVC in an African population, hence affords valuable insights and can serve as a foundation for future research and clinical application. The RVVC-associated polymorphisms and pathways identified in the present work could be advanced for the development of novel personalized therapies, biomarkers, and tailored preventive strategies, to improve the management of patients with RVVC.

Conclusion

RVVC susceptibility is due to multiple factors, genetic predisposition being among the newer revelations. Our findings are a valuable addition for better understanding of the pathogenesis of RVVC in an African population, and an important resource for the future search for novel therapies and preventive strategies.

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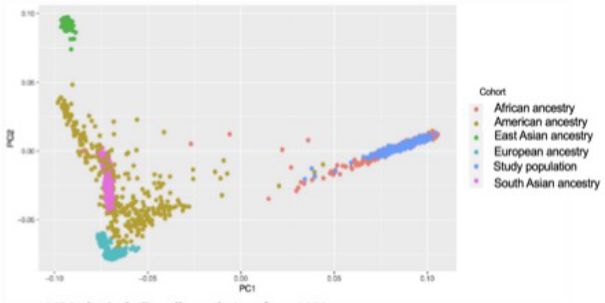
Contributions

GSO-M, IRP, DR, MGN, AJAMdV, and JtO conceptualised the study, and contributed to the design of the study; GSO-M, IRP, DR, MB, NWK, and MJ contributed to the acquisition and sorting of the data; GSO-M, IRP, DR, MJ, MGN, VK, JtO, and AJAMdV contributed to the data analysis; GSO-M, IRP, DR, MB, MGN, VK, MMO, JtO and AJAMdV contributed to interpretation of data; GSO-M, IRP, and DR wrote the initial draft of this manuscript; and all authors provided input and feedback on succeeding drafts. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of this work.

Supplementary figures

Supplementary Fig.1A

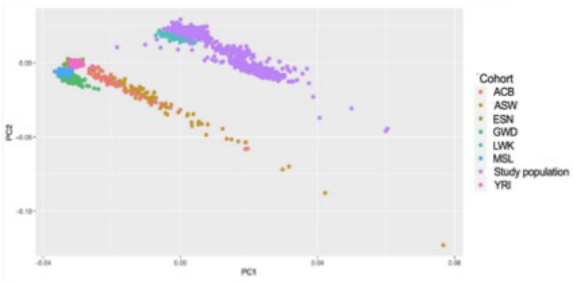


MDS plot including all populations from 1000g

Multidimensional scaling analysis (MDS) comparing the study population with populations from the 1000G project [1]. Abbreviations: MDS - multidimensional scaling analysis; 1000g - 1000G Project.

1. Abecasis, G.R., et al., *A map of human genome variation from population-scale sequencing.* Nature, 2010. **467**(7319): p. 1061-73.

Supplementary Fig. 1B

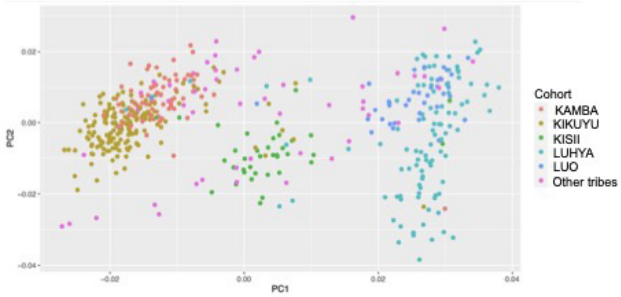


MDS plot including African populations from 1000g

Multidimensional scaling analysis (MDS) comparing the study population with African populations from the 1000G project [1]. Abbreviations: MDS - multidimensional scaling analysis; 1000g - 1000G Project; ACB – African Caribbean in Barbados; ASW – African ancestry in South West USA; ESN – Esan in Nigeria; GWD – Gambian in Western Division; LWK – Luhya in Webuye Kenya; MSL – Mende in Sierra Leone; YRI- Yoruba in Ibadan Nigeria.

1. Abecasis, G.R., et al., *A map of human genome variation from population-scale sequencing.* Nature, 2010. **467**(7319): p. 1061-73.

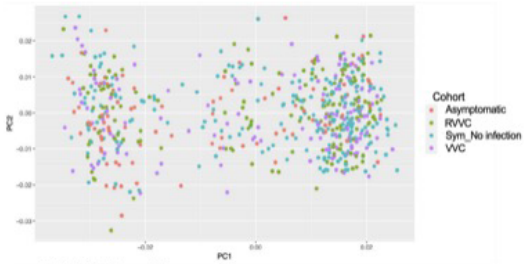
Supplementary Fig. 1C



MDS plot by tribe/ethnic group

Multidimensional scaling analysis (MDS) by study population ethnic groups/tribes to identify ethnic outliers. Abbreviations: MDS - multidimensional scaling analysis.

Supplementary Fig. 1D



MDS plot by disease status

Multidimensional scaling analysis (MDS) distribution by study population disease status. Abbreviations: MDS - multidimensional scaling analysis; Asymptomatic – without genital symptoms/healthy; RVVC – recurrent vulvovaginal candidiasis; Sym_No infection – with genital symptoms but no infection; VVC - vulvovaginal candidiasis.

CHAPTER 7: PLASMA INFLAMMATORY PROTEOME PROFILE IN A COHORT OF PATIENTS WITH RECURRENT VULVOVAGINAL CANDIDIASIS IN KENYA

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J. Fungi 2024, 10, 638

Abstract

Vulvovaginal candidiasis (VVC) affects up to 75% of women at least once during their lifetime, and up to 8% of women suffer from frequent recurrent episodes of VVC (RVVC). A lack of a protective host response underlies vaginal *Candida* infections, while a dysregulated hyperinflammatory response may drive RVVC. This study aimed to investigate the systemic inflammatory protein profile in women with RVVC in an African population, considering the potential influence of hormonal contraceptive use on systemic inflammation. Using multiplex Proximity Extension Assay technology, we measured 92 circulatory inflammatory proteins in plasma samples from 158 RVVC patients and 92 asymptomatic women (controls). Hormonal contraceptive use was not found to have a statistically significant correlation with a systemic inflammatory protein profile in either RVVC patients or the asymptomatic women. RVVC women had lower circulating Fibroblast Growth Factor 21 (FGF-21) concentrations compared with healthy controls (adjusted p value = 0.028). Reduced concentrations of FGF-21 may be linked to the immune pathology observed in RVVC cases through IL-1β. This study may help to identify new biomarkers for the diagnosis and future development of novel immunomodulatory treatments for RVVC.

Introduction

Vulvovaginal candidiasis (VVC) is a clinical condition affecting up to 75% of women of childbearing age worldwide, with approximately 138 million women suffering annually from more than three episodes of acute VVC, known as recurrent VVC (RVVC) [**1**]. Symptoms of VVC include genital itching, redness, swelling, and pain, ultimately contributing to substantial physical and psychological stress [**2**]. While the pathogenesis of RVVC has not been fully elucidated, several predisposing factors have been reported to increase susceptibility to VVC/RVVC. One of the factors involved in the pathogenesis is oestrogen; this is supported by epidemiological data, which show that the incidence of VVC/RVVC rapidly increases after menarche, that pregnancy is a particularly susceptible period, and that a decrease in incidence of VVC episodes occurs after menopause [**3**]. Mechanistically, oestrogens promote adhesion, microbial virulence, and the immune evasion of *Candida* [**3**,**4**]. Studies on healthy women also show the systemic effects of oestrogens, as hormonal contraceptives affect the plasma concentrations of proteins involved in inflammatory and immune-related pathways [**5**,**6**].

The central feature of symptomatic VVC/RVVC is aggressive neutrophil-induced mucosal inflammation [**7**], which is associated with increased NLRP3 inflammasome activation and more production of pro-inflammatory cytokines, such as Interleukin (IL)-8 and IL-1β [**8**]. The evidence of a dysregulated systemic host response also comes from a study showing inappropriately strong cytokine production by the circulating immune cells of RVVC patients upon stimulation with *Candida* hyphae [**9**]. Despite these studies, a deeper understanding of the mechanisms driving non-protective *Candida* infections is urgently needed to provide women with access to new treatment options.

Studying the local dysregulated inflammatory immune response requires invasive and unpleasant investigations such as vaginal lavages or vaginal biopsies, and also poses logistical challenges. Proximity Extension Assay (PEA) technology has enabled the simultaneous targeted large-scale analysis of many inflammatory proteins in plasma, which offers possibilities to identify biomarkers of pathophysiological systemic processes that may lead to new treatment options and/or identify biomarkers of disease susceptibility [**10**]. To date, no published studies have investigated the circulating inflammatory protein profiles of women with RVVC. Therefore, we conducted a cohort study on Kenyan women with RVVC to determine if they display a distinct plasma proteomic signature, considering the potential influence of hormonal contraceptive use on systemic inflammatory proteins.

Methods

Study Population and Inclusion Criteria

This study is part of a larger prospective observational study on women with lower genital tract symptoms (LGTS) [**11**] and an asymptomatic comparator group, conducted between October 2018 and March 2020 at seven outpatient health facilities in Nairobi City County (NCC), Kenya. The Kenyatta National Hospital-University of Nairobi's Ethics and Research Committee granted ethical approval for this study (P980/12/2016). A research license (permit) was obtained from the National Commission for Science Technology and Innovation, and authorization to conduct this study was obtained from NCC. Briefly, 813 symptomatic women aged between 18 and 50 years old were included after giving informed consent. The exclusion criteria were pregnancy, menopause, genital malignancy, HIV infection, and glycosuria. Social, demographic, and clinical data, including contraceptive use (hormonal and non-hormonal), were obtained from the eligible women who provided their consent. A vaginal swab was taken for microbiological examination and blood was drawn for use in proteome analysis. *Candida* infections were confirmed by direct microscopic examination and culturing on Sabouraud dextrose agar. RVVC was defined as experiencing three or more episodes of VVC in the preceding 12 months, at least one of which needed to be laboratory-confirmed (positive microscopy and/or culture). In some women with RVVC, microbiologically confirmed co-infections were present (including bacterial vaginosis [BV] and sexually transmitted infections caused by *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Mycoplasma genitalium*—for more details, see [**11**]). In addition, we included 104 asymptomatic women with similar exclusion criteria as the symptomatic women. From them, we also obtained EDTA plasma and performed a vaginal swab for microbiological analysis.

Proteomic Profiling of Circulating Inflammatory Proteins

Plasma samples were obtained from ethylenediaminetetraacetic acid (EDTA) blood by centrifugation and stored at −80 °C for proteomic analysis. Circulating plasma proteins were measured using the commercially available Proximity Extension Assay (PEA) technology from Olink® Proteomics, Bioscience AB (Uppsala, Sweden) [**12**], using the inflammation panel (92 circulating proteins). Briefly, proteins were recognized by PEA probes, which are oligonucleotides linked to antibodies containing unique DNA sequences. Upon binding, the probes were hybridized, and the sequence was extended by a polymerase reaction. The amplified sequence was quantified by real-time PCR. The readout was expressed as log2 transformation of the Normalized Protein Expression (NPX) value and this was proportional to the

protein concentration in the plasma sample. Samples were randomized and inter-plate controls were used to minimize variation. As quality control, proteins detected in less than 75% of the samples and samples that deviated by more than 0.3 NPX from the median were removed. Protein concentrations below the limit of detection were replaced by the lower limit of detection. As the samples were measured in two batches, bridge sample normalization was performed using 19 bridging samples and confirmed by principal component analyses (**Supplementary Figure S1**). The data analysed in this manuscript are included as **Supplementary File S1**.

Statistical Analysis

Protein abundances were assessed using a linear model included in the R limma package [**13**]. The linear model for contraceptive use included age as a covariate, while the model comparing RVVC women with controls included contraceptive use. Finally, the linear model used to account for the effect of co-infections included contraceptive use and co-infections as covariates. Results from these analyses were corrected for multiple testing using the Benjamini–Hochberg method, and an adjusted *p* value (adj.*p*.Val) less than 0.05 was considered statistically significant. All statistical analyses were performed in R version 4.3.2.

Results

Cohort Description

Drawing from the cohort of 813 women with LGTS [11] plus the 104 asymptomatic women (control), 269 women were included in this study. From these, we excluded 19 samples—18 samples failed to meet the quality control criteria and 1 sample did not have information on contraceptive use—thus leaving 250 participants for further analysis. Finally, the study population consisted of 158 (63%) symptomatic women with RVVC (cases) and 92 (37%) asymptomatic women (controls) (Figure 1). The mean age of the participants included in this study was 28 years, with a range from 19 to 50 years; for the RVCC women, the median age was 28 years (range 20–49), and for the controls it was 29 years (range 19–50). Of the total study population, 136 women (54%) used hormonal contraceptives; contraceptive use was adopted by 100 (63%) and 36 (39%) women in the cases and controls, respectively. Overall, 89 participants (36%) tested positive for at least one co-infection (BV, TV, NG, CT or MG); this was 32% and 41% of the RVVC and control groups, respectively. We did not have this information for 19 women, 16 of which were RVVC patients. *Candida* colonization was present in 8 (9%) members of the asymptomatic control group (Figure 1).

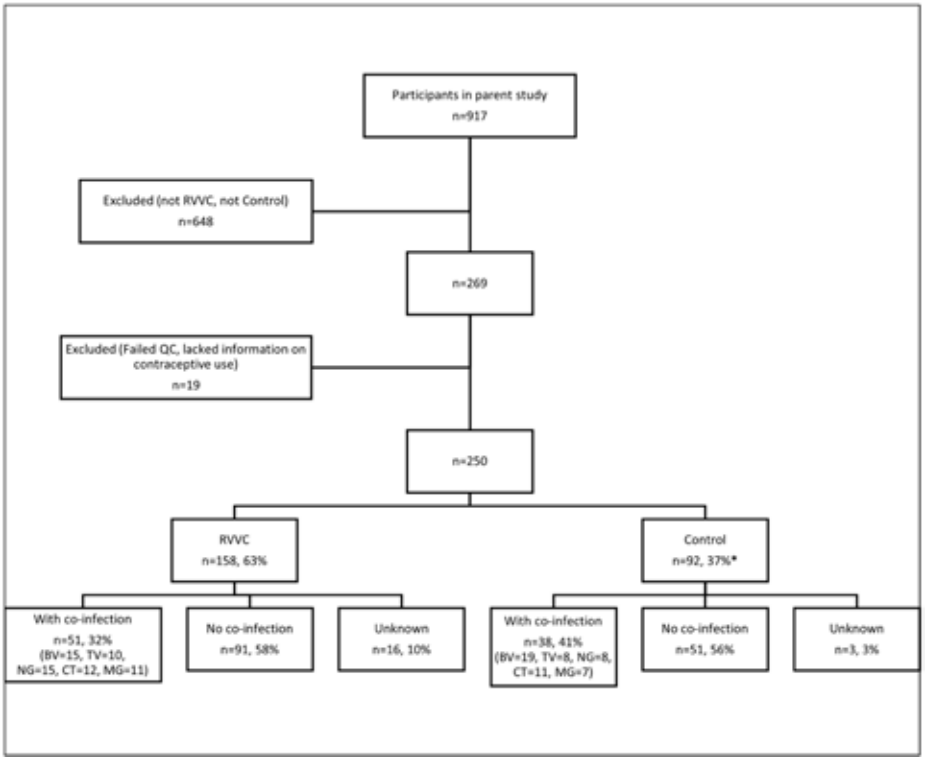

Figure 1. Participant inclusion flow chart. RVVC—recurrent vulvovaginal candidiasis; cases—women with RVVC; controls—asymptomatic women. BV—bacterial vaginosis; TV—Trichomonas vaginalis; NG—Neisseria gonorrhoeae, CT—Chlamydia trachomatis; MG—Mycoplasma genitalium. Key: * *Candida* colonization = 8 (8.7%).

Women with RVVC Display the Downregulation of Circulating Fibroblast Growth Factor 21 (FGF-21) Concentration

Of the 92 plasma proteins tested, 74 (80%) were detected in at least 75% of the plasma samples and were included in the analysis. We did not observe any separation between cases and controls when using PCA that includes all the proteins (Supplementary Figure S2).

We first investigated the effects of contraceptive use on the concentrations of inflammatory proteins in the entire cohort. For this, a differential abundance analysis was performed using a linear model that corrects for age. The differential abundance analysis showed that 13 proteins were significantly downregulated in women using contraceptives (Figure 2A left panel, Supplementary Table S1). Although these differences were not significant after adjustment for multiple testing (Figure 2A right panel), a clear tendency towards less inflammation was observed in women suffering from RVVC.

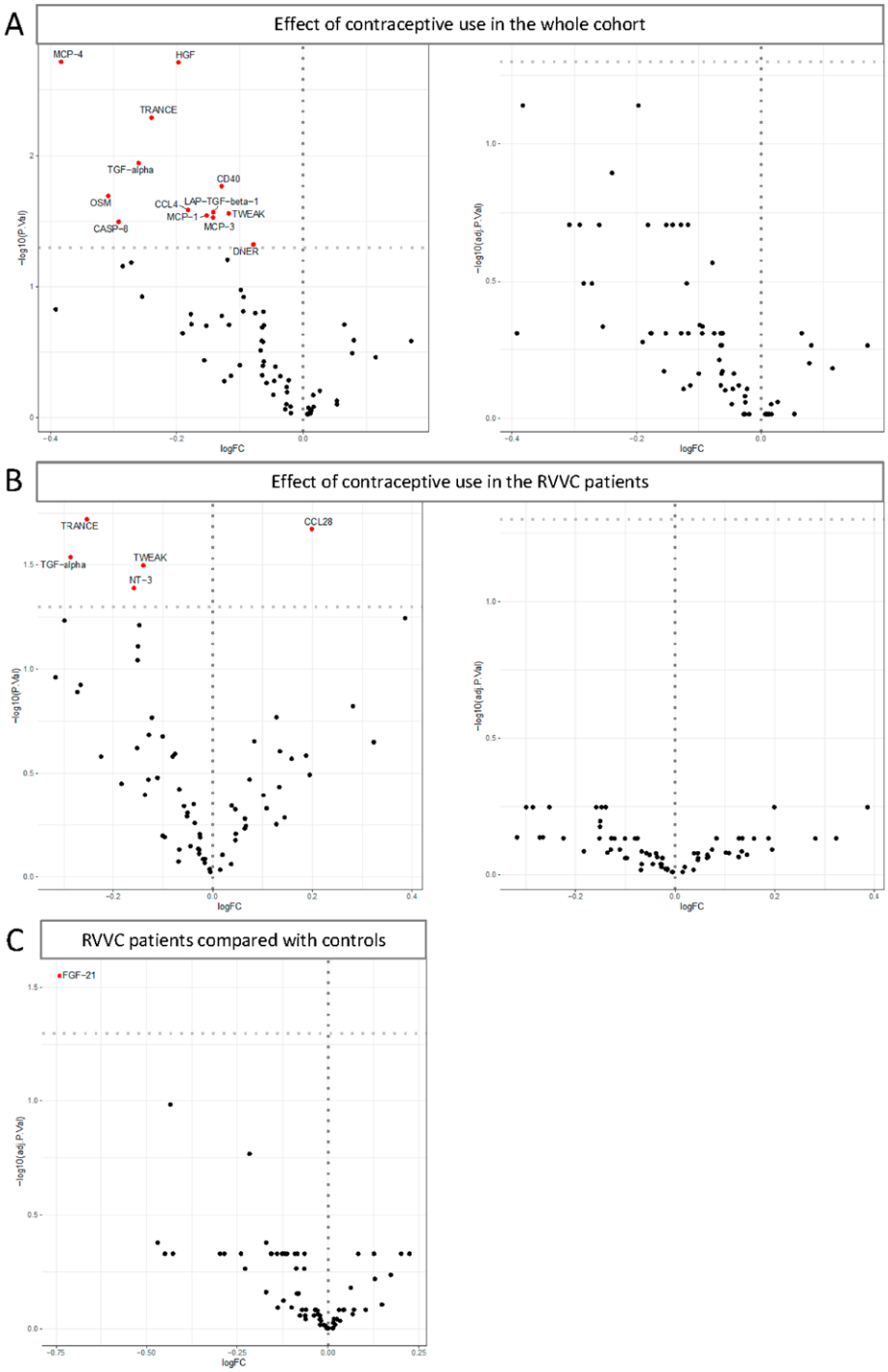

Figure 2. Volcano plots of 74 circulating proteins in plasma samples.

Figure 2. Continued

(**A**) The effects of contraceptive use on inflammatory proteins concentrations in our cohort, before (**left**) and after (**right**) adjustment for multiple testing. The X axis represents the logarithm of the fold change (Log FC) of women using contraceptives compared with women that did not use contraceptives and the Y axis represents the negative logarithm base 10 of the adjusted *p* value (adj.*p*.Val). Proteins on the left are proteins that are downregulated in women using contraceptives. (**B**) The effects of contraceptive use on inflammatory proteins concentrations within the RVVC patients' group, before (**left**) and after (**right**) adjustment for multiple testing. Proteins on the right side of the vertical dash line are higher in RVVC patients using contraceptives. The X axis represents the logarithm of the fold change (Log FC) of RVVC patients using contraceptives compared with RVVC patients that did not use contraceptives and the Y axis represents the negative logarithm base 10 of the adjusted *p* value (adj.*p*.Val). (**C**) A comparison of circulatory inflammatory proteins between RVVC patients and asymptomatic controls, adjusted for multiple testing. The X axis represents the logarithm of the fold change (Log FC) of RVVC patients compared with controls and the Y axis represents the negative logarithm base 10 of the adjusted *p* value (adj.*p*.Val) for multiple testing correction. Each protein is represented by a black dot and proteins showing a p value < 0.05 are depicted in red.

We then assessed the effects of contraceptive use within the RVVC group. We observed lower concentrations of four inflammatory proteins in RVVC patients using contraceptives compared to RVVC patients that did not use contraceptives, namely, TNF-related activation-induced cytokines (TRANCE), Transforming Growth Factor Alpha (TGF-alpha), TNF-related weak inducer of apoptosis (TWEAK), and Neurotrophin 3 (NT-3), and a higher concentration of Chemokine (C-C motif) ligand 28 (CCL28). While it was not statistically significant after adjustment for multiple testing (Figure 2B, right panel, Supplementary Table S2), we still included contraceptive use as a covariate in our subsequent analysis due to the observed association in unadjusted analysis.

Additionally, we compared the inflammatory profile of RVVC women with that of the controls. The differential abundance analysis showed that Fibroblast Growth Factor 21 (FGF-21) concentration (adjusted p value = 0.028) was significantly downregulated in RVVC patients when compared to controls (Figure 2C, Supplementary Table S3). As the women included in our cohort had various co-infections that could alter their inflammatory profile, we compared RVVC women (N = 142) with controls (N = 89), including contraceptive use and co-infection status as covariates. None of the proteins remained statistically significant after correction for multiple testing; however, FGF-21 was the most strongly downregulated protein (p value = 0.0007 and adjusted p value = 0.054, Supplementary Figure S3A,B).

Discussion

The present study is part of a larger investigation [11] aimed at characterizing the local and systemic factors contributing to susceptibility to VVC/RVVC. Here, we explored the systemic inflammatory profile in RVVC women attending seven outpatient clinics in Nairobi, Kenya. Using a targeted proteomic approach, we identified a significantly lower circulating concentration of FGF-21 in RVVC patients compared to healthy controls. To our knowledge, this is the first study to assess a broad range of circulating immunological markers in African women with RVVC.

FGF-21 is a pleiotropic hormone mainly produced by the liver and it is a regulator of glucose and lipid metabolism [14]. Higher FGF-21 plasma concentrations were found in healthy individuals from a sub-Saharan Africa population (Tanzania) compared to healthy Europeans [15]. Elevated concentrations of FGF-21 have been associated with systemic inflammatory conditions such as Crohn's disease [16], diabetes [17], colitis [18], sepsis [19], hyperglycaemia [20], acute COVID-19 [21], and normo-uricemic gout [22]. The latter study revealed that FGF-21 attenuates IL-1β and IL-1RA production using peripheral blood mononuclear cells [22]. Extending this finding to RVVC, reduced concentrations of FGF-21 may be linked to the immune pathology observed in RVVC. This could be explained by the reduced inhibition of IL-1β production. Supportive evidence that low FGF-21 concentrations are deleterious for patients suffering from *Candida* infections comes from a study showing an upregulation of FGF-21 concentrations in patients with chronic mucocutaneous candidiasis after successful treatment with a JAK inhibitor [23].

We hypothesized that hormonal contraceptives have an immune-modifying effect, as was suggested by a recent study [6]. The absence of a statistically significant association in our study may be due to the smaller sample size of our study. Moreover, Dordevic et al. [6] examined other inflammatory proteins and also focused on the plasma proteins involved in coagulation, metabolism, and cardiovascular pathophysiology, finding the strongest associations for angiotensinogen.

Our study also has several limitations. First, plasma sampling was conducted on the day of symptomatic diagnosis and not before or in between episodes. As such, our analysis may be limited to interpretations during inflammation. To support the hypothesized causative role of FGF21 in RVVC, future studies should also sample between VVC episodes. Furthermore, the presence of co-infections may have influenced the observed inflammatory response in some women. When we adjusted for this in one of our analyses, FGF21 was no longer found to be significant after

multiple testing, indicating that this may be the case. An alternative explanation for this finding is that it may be attributable to a reduction in power, given that data on co-infections were unavailable for some women. However, unlike RVVC, these infectious diseases are not typically associated with immune dysregulation. Second, we investigated the systemic protein profile without examining vaginal fluids from RVVC women, while experimental evidence suggests the importance of local dysregulation of inflammation [24]. Third, our study lacks an independent replication cohort to validate our finding. Since genetic variants may differentially affect circulatory inflammatory proteins, it would be interesting to (1) extend our study to other African populations, (2) compare our observations with the plasma proteome of RVVC women from non-African countries, and (3) investigate whether genetic variants affect any of the reported inflammatory proteins (protein quantitative trait loci, or pQTLs). This may provide a more specific signature and identify at-risk patients. Finally, the Olink platform provides relative quantification rather than absolute protein concentrations. Nevertheless, relative quantification is the predominant method used in multiplex assays in biomarker discovery due to its high specificity. The further validation of FGF-21 using commercial sandwich-based enzyme-linked immunosorbent assays (ELISAs) could be considered.

Despite these limitations, this study points out that the plasma protein FGF-21 is differentially expressed between RVVC and controls. Future functional immune measurements are warranted to validate these findings and to identify markers related to RVVC immunopathology, thereby improving diagnosis and management.

Supplementary Materials

The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jof10090638/s1, Supplementary Figure S1. Principal component analysis (PCA) depicting the sample distribution between the two batches. Supplementary Figure S2. Principal component analysis (PCA) of samples distribution between women with RVVC and controls. Supplementary Figure S3. RVVC patients compared with controls correcting for contraceptive use and co-infections status. (A). After correcting for multiple testing. (B). Without correcting for multiple testing. The X axis represents the logarithm of the fold change (Log FC) of RVVC patients compared with controls and the Y axis represents the negative logarithm base 10 of the adjusted *p* value (adj.*p*.Val) and the *p* value. File S1. Metadata of the RVVC cohort. Table S1. Results from the differential abundance analysis comparing women using contraceptives with women that do not use.

Table S2. Results from the differential abundance analysis comparing women with RVVC using contraceptives with women with RVVC that do not use. Table S3. Results from the differential abundance analysis comparing women with RVVC with women without RVVC.

Author Contributions

Conceptualization, M.G.N., A.J.A.M.v.d.V. and J.t.O.; methodology, D.R., I.R.P., V.K., M.G.N., A.J.A.M.v.d.V. and J.t.O.; software, I.R.P. and V.K.; formal analysis, D.R., I.R.P., V.K., M.G.N. and J.t.O.; investigation, D.R., G.S.O.-M., M.B., N.W.K. and M.J.; resources, G.S.O.-M., V.K., M.G.N., A.J.A.M.v.d.V. and J.t.O.; data curation, D.R., I.R.P., M.B., V.K., M.G.N. and J.t.O.; writing—original draft, D.R., I.R.P., G.S.O.-M. and J.t.O.; writing—review & editing, D.R., I.R.P., G.S.O.-M., M.B., N.W.K., M.J., V.K., M.G.N., A.J.A.M.v.d.V. and J.t.O.; visualization, D.R., I.R.P., M.G.N. and J.t.O.; supervision, V.K., M.G.N., A.J.A.M.v.d.V. and J.t.O.; project administration, G.S.O.-M. and J.t.O.; funding acquisition, G.S.O.-M., M.G.N., A.J.A.M.v.d.V. and J.t.O. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The Kenyatta National Hospital-University of Nairobi Ethics and Research Committee granted ethical approval for this study, (P980/12/2016). Research license (permit) was obtained from the National Commission for Science Technology and Innovation, and authorization to conduct the study from NCC. The study was conducted in accordance with the Declaration of Helsinki.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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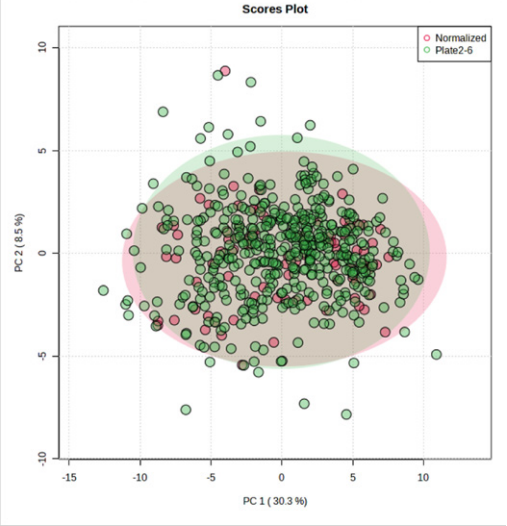
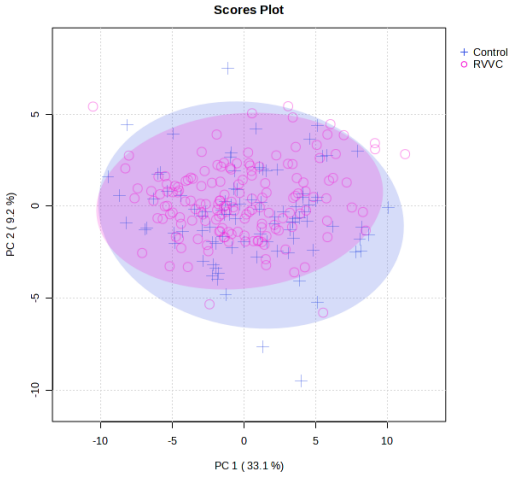
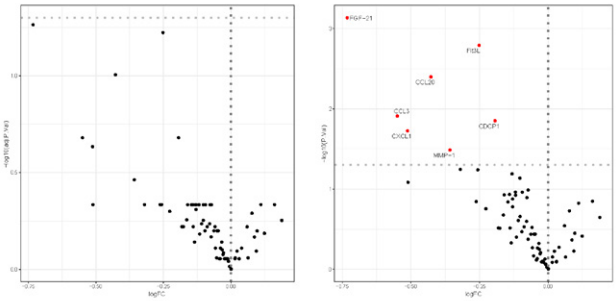
Supplementary figures



Supplementary Figure S1. Principal component analysis (PCA) depicting the sample distribution between the two batches.



Supplementary Figure S2. Principal component analysis (PCA) of samples distribution between women with RVVC and controls.



Supplementary Figure S3. RVVC patients compared with controls correcting for contraceptive use and co-infections status. A. After correcting for multiple testing B. Without correcting for multiple testing. The X axis represents the logarithm of the fold change (Log FC) of RVVC patients compared with controls and the Y axis represents the negative logarithm base 10 of the adjusted P value (adj.P.Val) and the P value.

CHAPTER 8: GENERAL DISCUSSION

DISCUSSION

Lower genital tract infections (LGTI) are a common problem affecting the health, social and economic wellbeing of women, which may even extend to their sexual partners and offspring (1-3). Women in Sub-Saharan Africa (SSA) seem to be more affected (4-8) than women in other parts of the world (9-12).

Control of the LGTI is associated with numerous challenges, i.e. understanding the pathogenesis, patients' perception and reporting of symptoms, correct diagnosis, and ultimately accurate, comprehensive and timely care with minimum sequalae. Also, costs and the availability of trained healthcare workers may be a problem, especially in low income countries (13, 14), including Kenya (15, 16). Despite many years' efforts to contain genital infections, the LGTI burden remains a worry in a country like Kenya (17-19). Therefore, healthcare workers are dealing with large numbers of patients that are mostly treated using an algorithm based on symptoms and some physical examination but without microbiological testing (20). A vaginal candida infection is part of this algorithm, however recurrent vulvovaginal candidiasis (RVVC) is not considered. This is remarkable as RVVC is not uncommon and causes great distress (21-23). Therefore, being a condition neglected from the syndromic guidelines, patients with RVVC suffer mismanagement; they receive repeated misplaced antibiotic treatment which may increase susceptibility vulvovaginal candidiasis (VVC) and development of antimicrobial resistance (AMR); also, RVVC patients are falsely labelled as having cervicitis/sexually transmitted infection (STI) with the associated stigma effects. If left untreated, VVC poses other dire consequences including poor pregnancy outcomes and congenital candidiasis (24, 25), and increased risk for HIV (26-28) and perhaps STI as well. Furthermore, RVVC pathogenesis is poorly understood (29). Consequently, a better understanding and unravelling of the underlying risks and pathogenesis for improved and satisfactory care is paramount. This thesis therefore not only aimed to improve the management of LGTI, but also tries to improve our understanding of the underlying factors driving RVVC in particular.

As an initial step, in **Chapter 2**, I conducted a large-scale review of records at 12 Nairobi City County (NCC) health facilities to assess the routine clinical management of non-pregnant Kenyan women with lower genital tract symptoms (LGTS) and guideline adherence, while using this opportunity to train the healthcare personnel expected to participate in my research. Out of 6,516 records of patients with LGTS, two-thirds were clinically categorized as cases of vaginitis i.e. VVC plus bacterial vaginosis (BV). Compliance with the treatment guidelines' recommendations for

vaginal discharge syndrome was wanting (overall rate 56%) and variant (range 0-83%) across the healthcare facilities; a vaginal examination was missed almost a half of the time (interfacility range 2%-100%) while the aetiologic/non- syndromic diagnosis of VVC was applied frequently. The glaring discrepancies in practice at the health facilities that should be similarly staffed, equipped, and applying the same guidelines was concerning. We did not conduct an in-depth analysis to explore the reasons behind the significant variation between healthcare facilities and the potential for improving care. However, previous studies suggest that factors such as insufficient supplies and medications, deficiencies in staff training and supervision, staffing shortages, and staff absenteeism (30-32) may contribute to the observed.

With my findings of a high frequency of women with LGTS, inconsistencies in practice and low adherence to treatment guidelines by healthcare workers, and published reports of a wanting performance of the syndromic approach in management of STI (33-36), in **Chapter 3**, I revealed the LGTS's aetiologies and their predictors in over 800 women recruited at the NCC health facilities. Additionally, I assessed the performance of the currently used vaginal discharge syndrome algorithm, and developed and evaluated an alternative improved algorithm. Eighty percent of the women reported multiple episodes of LGTS within 12 months, with the average being 3 episodes per woman per year (range 1-15), and 70% of the women reported negative psycho-socio-sexual effects due to the LGTS. We detected 790 infections in two-thirds (n=540) of the women with one third of them having multiple infections; 41% of the infections detected were an STI. Although my study's overall LGTI rate is comparable to reports from other populations in SSA, my findings of a predominance of candidiasis (40%) and lower prevalence of BV (17%) deviate from these reports by others in the region (4, 5, 34, 37). I also noted lower VVC and trichomoniasis rates compared to past studies from Kenya (38), but for STI the frequency was higher. I speculate that in an environment of significant vaginitis-STI coinfection (37, 39), reliance on the vaginal discharge syndrome algorithm, although catering for vaginitis, likely results in delayed or even missed treatment for bacterial STI. Indeed, my study findings support this conjecture: multiple/mixed infections were frequent, affecting one-third of those with an aetiology, and the vaginal discharge syndromic algorithm failed to give treatment to two-thirds of women with bacterial STI.

My other concerning revelation is that one-third of my study participants did not have an infection, yet the conventional algorithm, being symptom-driven, classifies such women as vaginitis and offers them treatment for BV, trichomoniasis and VVC. It is thus no wonder that the guideline gave an assortment of inappropriate

antimicrobials to two-thirds of the patients – mainly antifungal and metronidazole prescriptions. These findings corroborated my venture to improve the treatment algorithm; hence, in **Chapter 3** I designed an improved version. My substitute algorithm had advantages over the conventional one. First, I was able to reduce inappropriate prescriptions in 306 sampled patients by 36%, i.e. down from 359 prescriptions to 230. Secondly, my algorithm avoided 'blanket treatment' by differentiating between patients needing antifungal from those for metronidazole treatment resulting in an improved BV-trichomoniasis treatment accuracy score of 74% compared to 40%, and a notably lower rate of inappropriate metronidazole prescriptions (82% versus 32%). Thirdly, unlike the present algorithm, mine identified some women not needing any treatment (accuracy of 64% versus 0%), hence reduced unnecessary prescriptions. However, similar to the conventional algorithm, my alternative one also did poorly in delineating mixed infections, a phenomenon common in my study population, particularly involving STI. Hence, in agreement with other researchers (35, 36, 40), a syndromic-only approach can be misleading as a diagnosis and treatment tool for female LGTI; the performance is limited by indistinguishable behavioural factors, and symptoms and signs which are also shared across aetiologies. Moreover, the symptom-based flowcharts do not cater for women with asymptomatic infections; in my case-control analysis for genetic factors for RVVC (**Chapter 6**) asymptomatic STI were detected - 13% *Chlamydia trachomatis* (CT), 8% *Neisseria gonorrhoea* (NG), 8% *Trichomonas vaginalis* (TV), and 7% *Mycoplasma genitalium* (MG). These findings, similar to others' elsewhere (37), reaffirm that sole reliance on clinical presentation with no aetiological confirmation contributes to treatment incongruities and likely results in further transmission of STI. Therefore, only with integration of point-of-care (POC) testing into the algorithms is good discriminative power achievable. Inclusion of POC diagnostics in the treatment flowcharts has indeed been shown to improve the performance of a syndromic treatment approach (41, 42). To demonstrate the possibility to incorporate laboratory diagnostics in the LGTI flowcharts in Kenya and other similar settings, my **Chapter 4** established that repeated and varied genital mucosal sampling is feasible and accepted by women in Nairobi. This is corroborated in findings from our other work on the feasibility and approaches to implementation of cervical self-sampling in Kenya (43). Further, through my South-South regional mucosal training initiative for multi-centre collaborations (**Chapter 5**), we have built local capacity in mucosal studies that can be leveraged for local African scientists to engage in studies towards development of POC tests for R/VVC and other LGTI.

Further, in **Chapter 3**, I additionally revealed that besides the frequent VVC, RVVC was prevalent -constituting one-half of VVC, equivalent to one-fifth of all patients with

LGTS. This finding corroborates with rates from elsewhere in SSA (44), but is higher than findings from non-African populations (45). These variations are likely due to intrinsic and external inter-population differences in risk factors for RVVC: - The syndromic approach (46, 47) commonly used in SSA and other low- middle-income settings for management of LGTS does not recognize RVVC hence promotes disease persistence - the longest duration of antifungal treatment offered in the syndromic guidelines is 7 days for sporadic VVC, yet RVVC treatment duration should last about 6 months (48). Behavioural differences may also be contributory - in low- middle-income settings, indiscriminate antibiotic use is common more so due to scarcity of laboratory diagnostic support and AMR programs (49); in SSA also, there are higher rates of vaginal practices such as douching (50, 51) which can interfere with the natural vaginal antifungal ecosystem (52). Differences in RVVC rates may also have biological explanations, including variations in immune-associated genes as already demonstrated in non-African populations (53-55). Related to this and a first ever, in **Chapter 6**, I explored the genetic factors associated with susceptibility to RVVC in the African population. Although they did not reach the predefined significance level, several genomic variants were detected in RVVC with the main ones being, the SNP rs8181503 located close to the *MS4A12* gene on chromosome 1, and the SNP rs58936172 next to the *TMEM39A* gene on chromosome 3. These polymorphisms were linked to dysregulated metabolic and cell adhesion pathways relevant for RVVC susceptibility, likely linked to distorted immune responses on exposure to *Candida*.

Cervicovaginal proteins and metabolites are markers of various biological processes including cell organization and differentiation, enzyme activity, metabolism, and immune responses (56). Accordingly, profiling inflammatory markers would be important in the development of biomarkers and new therapeutic agents for RVVC. Hence, in **Chapter 7**, I sought to delineate the plasma proteomic profiling associated with RVVC, to obtain insights into the relevant immune mechanisms that would be valuable for future therapeutic endeavours for RVVC. This is the first study, to my knowledge, assessing a large number of circulating immunological markers in African women with RVVC. We were able to establish differential expression of circulating Fibroblast Growth Factor 21 (FGF-21) concentrations between RVVC patients and controls. Although the difference was not statistically significant, I speculate that the observed downregulation of FGF-21 may be linked to the immune pathology observed in RVVC through IL-1β. Additionally, profiling inflammatory markers may unravel the reasons behind the symptoms in those without an infection, as seen in one-third of our patients - **Chapter 3**. Future works can establish the mechanisms and suitable treatments for symptomatic but uninfected patients, potentially averting the unnecessary use of antimicrobials.

My studies also revealed that a majority of women reported significant disturbance to their social, sexual and psychological wellbeing due to the LGTS; other researchers elsewhere made similar observations (57, 58). The unfavourable psycho-socio-sexual effects may negatively impact a patient's health seeking behaviour and disease prevention efforts – my assumption is that women with recurrent or persistent LGTS, especially post-treatment, may lose trust in the medications and the health care providers/facilities. My findings and speculation should be explored further in follow-up qualitative studies, to understand the patients' perspectives and for designing mitigation measures.

CONCLUSION

The consequences of missed or mismanagement of LGTI and RVVC are wide and dire for individual patients, their communities and governments; they include clinical and psychosocial complications, economic loss, and development of AMR. This thesis work aimed to improve certain aspects of the problem and highlights important challenges, gaps and developments in the diagnosis, care, and research infrastructure surrounding female LGTI and RVVC, provides insight into the pathophysiology of RVVC, as well as emphasizes the need for improved practices, training, and standardization of research and patient care procedures, in low-resource settings. More specifically, my work addressed:

Inadequacies in service delivery for LGTI

There was variation in care with treatment disparities and overall suboptimal care for women with LGTI including RVVC, which might contribute to poor outcomes such as recurrence of LGTI, overall burden of LGTI and related sequalae.

Evaluation of syndromic practice

My evaluation of the syndromic management for female LGTI highlights the drawbacks of the symptom-based approach commonly used in many low-resource settings including Kenya. Despite the reasons promoting the use of syndromic management in these settings, the approach often lacks diagnostic precision especially for patients with multiple infections, leading to inaccurate and missed treatments for various LGTI and RVVC. To compound this are the shortcomings in the low-resource settings' healthcare delivery systems that hamper accurate application of the syndrome-driven protocols.

Identification of pathogens causing LGTI

Locally, microbiological testing is not routinely done in women with LGTI and treatment is empirical. The consequence is that healthcare workers are not informed about which pathogens are causing LGTI in Kenya and how common they are. The present thesis provides such insight and shows the prevalence of the pathogens and whether antimicrobial treatment is correctly given, and that incorrect treatment and persistence of complaints may falsely suggest that a sexually transmitted pathogen is causing the complaints. My thesis work includes a strong plea for inclusion of POC tests in the management of LGTI.

Genetics

The RVVC-associated genetic variations detected in this work may be an important contribution to explain the prominence of RVVC in the Kenyan population. Advancing such works may identify individuals with RVVC-susceptibility genetic markers for personalized therapies and tailored preventive strategies.

Proteomics

Proteomic profiling adds to the understanding of the pathophysiology of recurrent infections like RVVC, and in identifying biomarkers as well as customized treatments. Hence, my findings of altered biomarkers in RVVC need to be explored in larger studies with power to confirm the observed association as well as establish the pathophysiologic mechanisms involved.

Mucosal studies

The incorporation of innovative techniques such as genital mucosal specimen sampling for diagnosis, proteome profiling, genetic analysis, and other relevant research could transform how LGTI are addressed across the African continent, especially in resource-scarce settings. Mucosal studies and diagnostics utilize a variety of sample types and corresponding sampling techniques. Importantly, the specimen sampling procedures should be standardized for reliable and credible outcomes, and also should be feasible and acceptable locally. My regional mucosal training is a boost to research competencies and collaborations for conducting advanced research, such as RVVC-associated mucosal inflammatory proteome profiling, and development of locally-relevant POC diagnostic tools.

RECOMMENDATIONS

My thesis work on female LGTI and RVVC centres on understanding the disease determinants, accuracy of diagnosis, adequacy of treatment, consistency in diagnostics and research methods, and the search for targeted therapeutics, while advocating for a healthcare model that is more adaptive, precise, and sustainable - towards meeting the needs of the affected women in Kenya. In Figure 1, I summarize my proposed future direction for better management and pathobiological understanding of LGTI and RVVC.

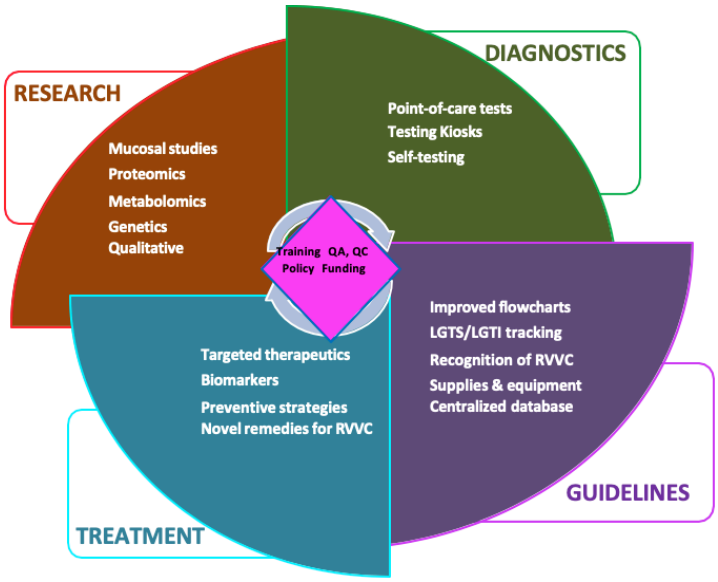


Figure 1. A summary future direction for improving management and pathobiological understanding of LGTI and RVVC

QA – quality assurance; QC – quality control; LGTS - lower genital tract symptoms; LGTI – lower genital tract infections; RVVC – recurrent vulvovaginal candidiasis

Recommendations to improve quality of care in LGTI and RVVC

I have revealed clear areas for improvement that should be subjected to further investigation to understand the determinants that hinder or facilitate adherence to the prescribed guidelines for management of LGTS in women in Kenya. As a first step to decrease variation and to improve guideline adherence overall, it would be imperative to delve further and gain an understanding of the reasons for the observed low adherence to guidelines and suboptimal patient care, as well as the wide variations in practice between health facilities. Understanding

the determinants of the behaviours behind the observed suboptimal practice by healthcare providers is essential for improvement interventions.

Therefore, I recommend follow-up qualitative studies to unravel the barriers and facilitators of optimal performance by the healthcare workers, and to guide and design interventions for improvement. This would include ethnographic studies especially in the one health facility which had optimal practices in patient care, to observe and learn valuable lessons and get insights into existing enablers and workable practices for replication in other health facilities. Additionally, for comprehensive management of LGTI, robust qualitative socio- behavioural research on the health facility clients should be conducted to obtain patient experiences and perspectives for improvement of care, as well as to explore further the reported negative psycho-socio-sexual effects of LGTS/LGTI, and to design appropriate interventions.

In the year 2016, almost all (85%) of the African Union countries conducted 5,562 clinical trials altogether; Kenya conducted 345 trials (59). From my several years' experience conducting clinical trials in Kenya (60-63), I attest that adherence to stringent standards of practice is possible in these resource-limited environments. With systematic training of the staff, use of standard operating procedures, monitoring of practice, institution of processes for quality control and quality assurance, and timely corrective actions, adherence to standards and production of quality work is possible. Therefore, to promote adherence to treatment guidelines and improve quality of patient care, I propose that the approach and standards used in clinical research be adopted in clinical practice; implementation of this would require well-trained and dedicated personnel to ensure execution. Towards this, studies designed to test the feasibility and acceptability of this approach in clinical care should be conducted.

Additionally, I recommend that all patients' health records be stored electronically in a centralized database to enable consolidation of individual patient's data irrespective of the facility visited; this would facilitate tracking of LGTI frequency and treatment outcomes, as well as enable monitoring of performance of health care providers and the health facilities. The Kenya Ministry of Health recently rolled out the national Afyangu system (64) which integrates patient-specific health records – my recommendations for performance monitoring can be incorporated into Afyangu and tested.

Beyond implementation challenges, the dependability of the current syndromic guideline is also challenged by the multiple factors that influence the occurrence and management of female LGTI and RVVC. I therefore dare recommend that for better

performance, the present syndromic algorithms should be re-designed advised by local data on infection patterns, unique patient characteristics, the likelihood of dual/multiple, recurrent and antimicrobial- resistant infections, while incorporating feasible laboratory-based diagnostics, research, and oversight. My specific recommendation is to design and test an amended guideline for the management of vaginal discharge syndrome. The guideline revisions that I propose are: - 1) Addition of a question specifically addressing symptom recurrences to identify and triage patients with 3 or more LGTI episodes in 12 months, for aetiological diagnosis/POC test and/or referral to a physician for RVVC care; assessing for presence of vulvovaginal pruritus/itch to identify patients for VVC-only treatment; assessing for presence of foul smelling vaginal discharge to identify patients for BV-trichomoniasis treatment; and referral to aetiological diagnosis sooner (7 days) for patients with persistent symptoms (Figure 2). 2) Introduction of an individualized LGTS tracking tool/card for documentation and tracking of LGTS episodes (Figure 3) - the tool would be provided to each patient to take away and present at each visit irrespective of the health facility visited. 3) Embedment within the guidelines of processes for training, monitoring, evaluation, and corrective action.

My recommendations will be communicated to the Kenya Ministry of Health and other stakeholders through a policy brief, to be published.

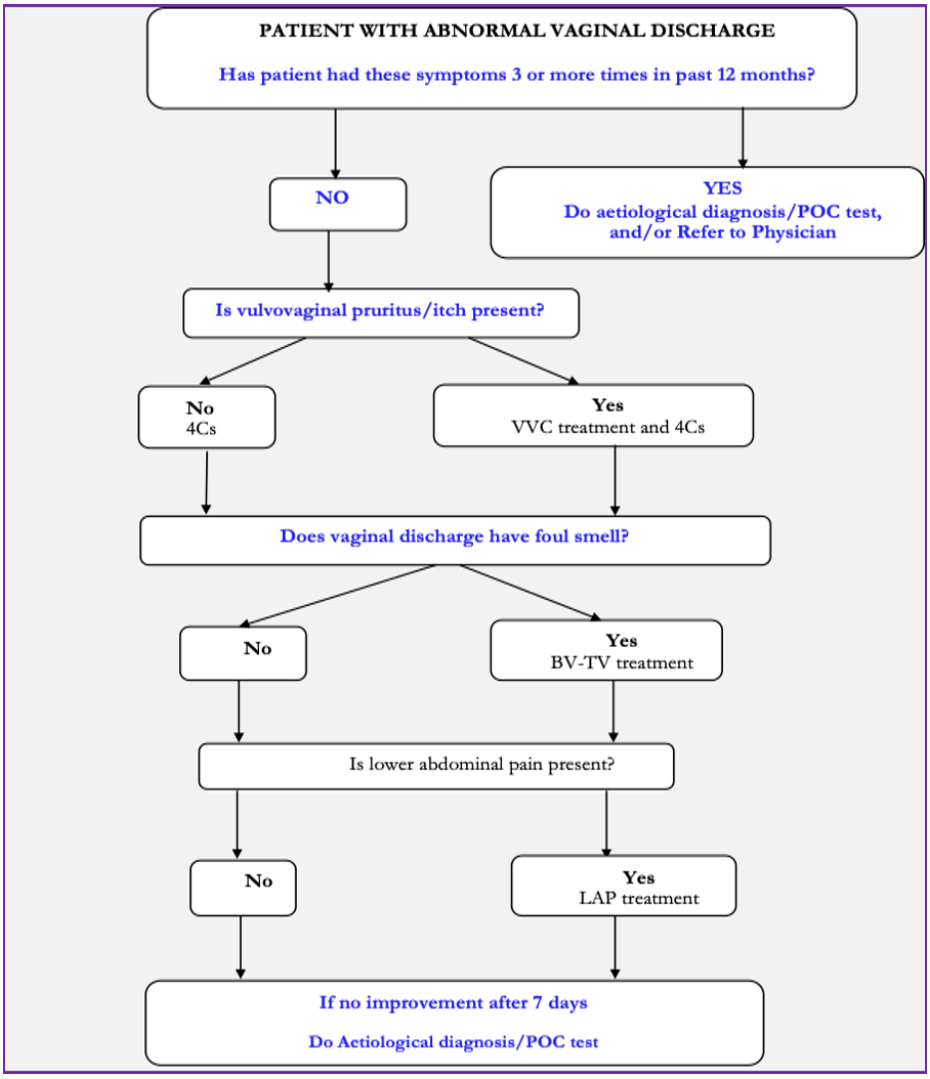


Figure 2. Amended Vaginal Discharge Syndromic algorithm - improvements/revisions are shown in blue text

POC - point-of-care; 4Cs – counselling, compliance, condoms, contact tracing; VVC – vulvovaginal candidiasis; BV – bacterial vaginosis; TV – Trichomonas vaginalis; LAP – lower abdominal pain

NAME:			
Date	**Symptoms**	**Treatment**	**Notes**
	Discharge Itch	Antifungal Antibiotic	
	Pain Other	Other None	
	Discharge	Antifungal	
	Itch Pain	Antibiotic Other	
	Other	None	
	Discharge Itch	Antifungal Antibiotic	
	Pain Other	Other None	

Figure 3. Lower female genital tract symptoms tracking tool

Understanding the pathophysiology in RVVC for improved care

Future studies should use an integrated approach incorporating vaginal microbiome and mycobiome, metabolome, and proteome, while factoring in the role of genetic influence, to allow for a more comprehensive understanding of the vaginal mucosal pathophysiology in RVVC. An integrated mucosal studies approach would allow for development of effective preventive strategies, biomarkers and novel treatments and approaches with durable effects. This is indeed possible – for example, Bilal et al (2024) showed an increased vulnerability to VVC following administration of interleukin 17 (IL-17) blockers (65); the reverse -use of IL-17 promoters- may be protective against VVC hence should be tested. As a follow-up to my work therefore, I recommend replication studies in independent and larger cohorts, as well as functional validation studies of the findings. For valid and reliable data, such works will likely need to be multisite involving large and diverse cohorts, including cohorts in SSA where we already built the capacity for mucosal studies. Existing RVVC cohorts, including my cohort in Nairobi of close to 200 women with RVVC, would be of great value for such future studies.

Mucosal studies to support improved care of LGTI/RVVC

The diagnosis of LGTI and particularly vaginal candidiasis need not be complex or complicated. Vaginal self-sampling has been shown to be feasible and acceptable in African populations (43, 66, 67). Linked to this, I recommend that for timely and accurate diagnosis of LGTI, self-testing and/or use of strategically located sampling and testing kiosks with linkage to treatment centres for care/prescriptions be explored. To support this, I also propose further genital mucosal research to develop POC tests for in/out-of-health facility testing. Such POC tests should preferably be multi-test, detecting multiple aetiologies using one vaginal swab sample.

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APPENDIX

Short summary

Korte Nederlandse samenvatting

Research Data Management

List of publications

Acknowledgments

Curriculum vitae

PhD Portfolio

SHORT SUMMARY

The consequences of missed or mismanaged LGTI and RVVC are wide and dire for individual patients, their families, communities and governments; they include psychosocial and clinical complications, economic loss and wastages, and development of resistance to anti- microbials. My thesis work exposes the weightiness of the LGTI and RVVC problems and the challenges in management among women in Kenya, reveals preliminary RVVC-linked genomic and proteomics data unique to the local population, and showcases aspects of mucosal work relevant for LGTI and RVVC diagnostics and research. I detected concerning gaps in patient care. While syndromic treatment has been considered to be the befitting approach in LGTI management for Kenya and other resource-constrained environments, the approach has weaknesses and shortfalls including poor implementation, misuse of antimicrobials, and failures in treatment especially for patients with mixed or recurrent infections including RVVC. As crucial foundational pioneer ventures in an African population, I revealed distinct RVVC related genetic variations and the systemic proteome signature connected to RVVC.

To improve patient outcomes in Kenya and similar environments, LGTI and RVVC management should be targeted and wholistic, with an interlinked approach advised by accurate diagnostics, including mitigating measures for any associated unfavourable psychosocial effects. This calls for further research to understand the enablers and barriers that impact accurate implementation of the syndromic treatment guidelines, design better performing treatment guidelines, develop feasible POC tests with easy and favourable implementation modes, and research towards a better understanding of the pathophysiology for targeted biomarkers and therapeutics for RVVC. There is thus a need for novel complementary and synergistic multidimensional approaches to the management of LGTI and RVVC, which calls for continued investment in healthcare infrastructure, training, oversight, timely corrective actions, and research.

KORTE NEDERLANDSE SAMENVATTING

De gevolgen van niet gediagnosticeerde of niet goed behandelde vaginale infecties (VI) kunnen groot zijn en zeer onaangenaam voor individuele patiënten, hun families, gemeenschappen en overheden. Mogelijke consequenties zijn psychosociale problemen, klinische complicaties, financieel verlies en toegenomen resistentie tegen antimicrobiële medicatie. Candida is een belangrijke ziekteverwekker die ook herhaalde infecties kan veroorzaken: dit laatste noemen we recurrente vulvo-vaginale candidiasis (RVVC). Mijn proefschriftwerk beschrijft de problemen en uitdagingen van VI en RVVC in Kenia. Ook rapporteer ik associaties met genetisch en circulerende eiwit data die uniek zijn voor de Keniaanse bevolking. Tevens worden belangrijke aspecten voor het doen van mucosaal onderzoek bij Keniaanse vrouwen beschreven.

Mijn onderzoek toont aan dat er belangrijke lacunes zijn in de patiëntenzorg voor vrouwen met vaginale infecties. Ondanks het feit dat de syndromale behandeling als zeer geschikt wordt beschouwd voor vaginale infecties in landen met beperkte middelen, kent deze aanpak forse tekortkomingen, waardoor de patiënten niet altijd de juiste behandeling ontvangen. Daardoor kunnen klachten persisteren en worden er weer een nieuwe antimicrobiële middelen voorgeschreven. Het gevolg is misbruik van antimicrobiële medicatie met resistentie tot gevolg. Ook de kosten nemen daardoor toe. Opmerkelijk is dat patiënten met RVVC, niet afzonderlijk herkent worden in het algoritme, waardoor de management van deze patiënten te wensen overlaat

Er valt dus het nodige te verbeteren aan de diagnose en behandeling van vaginale infecties, in Kenia. Noodzakelijk is ook dat RVVC in een algoritme worden meegenomen en dat bij een falende behandeling niet automatisch en alleen aan een seksueel overdraagbare infectie wordt gedacht. Er is behoefte aan een beter presterende behandelrichtlijn, waarbij in de toekomst eventueel gebruik gemaakt wordt van gemakkelijk toepasbare diagnostische testen en met een goed begrip van de implementatie. Specifieke biomarkers en nieuwe therapieën kunnen geïdentificeerd worden door onderzoek te doen naar de pathofysiologie van de verschillende vaginale infecties. Vanwege de omvang van het probleem van vaginale infecties in Kenia, de complexiteit en variabiliteit blijven voortdurende investeringen in infrastructuur, training, onderzoek, toezicht en tijdige corrigerende maatregelen noodzakelijk.

RESEARCH DATA MANAGEMENT

This thesis is based on the results of research conducted on humans in Nairobi Kenya. The studies were conducted in observance of the principles of the Declaration of Helsinki. The studies received ethics approval and research license from the Kenyatta National Hospital- University of Nairobi Ethics and Research Committee (KNH-UONERC) and the National Commission for Science Technology and Innovation, respectively. The studies were not subject to the Dutch Medical Research Involving Human Subjects Act (WMO).

For Chapters 3-7, written informed consent was obtained from participants to collect and process their data for this research project, prior to participation. Data for Chapter 2 were obtained from health facility charts by trained personnel from the respective facilities, after stripping the data of identifiers. Confidentiality was ensured by pseudonymization and use unique study numbers which were linked only to the informed consents; the signed informed consents were stored separately from the research data. The raw study data, including informed consent documents, are stored at the University of Nairobi's KAVI Institute of Clinical Research, in Kenya.

All data were collected onto case report forms then entered into Castor EDC where they were stored and only accessible by project members. Data were later obtained from Castor EDC and analyzed on the device of the principal investigator. This thesis's data are archived according to the Findable, Accessible, Interoperable and Reusable (FAIR) principles. The data used in data analysis are published in Data Sharing Collections in the Radboud Data Repository, ru.rumc.rvvcvmb_t0000194a_dsc_970.

The datasets will be stored for 15 years after termination of the research.

All the remaining study samples for this thesis are stored at KAVI-ICR and Radboudumc laboratories for at least 10 years and available for possible future tests upon receipt of additional ethical approval from the KNH-UONERC.

LIST OF PUBLICATIONS

Omosa-Manyonyi GS, Ponce IR, Rosati D, Bruno M, Kamau NW, Obimbo MM, Jaeger M, van der Ven AJAM, Netea MG, Kumar V, Oever JT. Genetic susceptibility to recurrent vulvovaginal candidiasis in an African population from Nairobi, Kenya. Sci Rep. 2025 Apr 9;15(1):12149. doi: 10.1038/s41598-025-95772-7. PMID: 40204783; PMCID: PMC11982394.

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I give all glory to God.

CURRICULUM VITAE

Gloria Susan Omosa-Manyonyi is a Lecturer and Clinical Researcher at the Faculty of Health Sciences, University of Nairobi (UON), Kenya. She has long and broad experience as a Physician, a lecturer in Medical Microbiology and Infectious Diseases, and a Clinical Researcher i.e. Principal Investigator/co-investigator in infectious diseases research - epidemiological studies, and clinical trials including HIV vaccine clinical trials. Gloria is also a trainer and mentor in Clinical Research, Scientific Writing, and Bioethics. She has several publications in peer- reviewed journals (https://scholar.google.com/citations?user=aGlr7IQAAAAJ&hl=en).

Gloria holds the Bachelor of Medicine and Bachelor of Surgery degree from UON Kenya, postgraduate degree in Infectious Diseases from the University of London UK, the Diploma of the London School of Hygiene & Tropical Medicine UK, and postgraduate diplomas in - Public Health from the Swiss Tropical Institute, and in Control and Management of HIV/AIDS and Sexually Transmitted Infections from the UON. She is passionate about advancement of clinical research through mentorship and experiential training.

In her PhD research titled "Lower Genital Tract Infections (LGTI) in women in Kenya: Studies to improve management and insight into vulvovaginal candida infections", Gloria aimed to improve management and pathobiological understanding of LGTI and recurrent vulvovaginal candidiasis (RVVC) in Kenyan women. She enrolled and characterized close to 1000 female patients with lower genital tract symptoms at outpatient clinics in Nairobi, established a cohort of women with RVVC and profiled their genetic and proteomic characteristics - the first ever in the African population. Additionally, Gloria spearheaded a technology transfer initiative in mucosal studies including training of staff at regional establishments in eastern Africa, an expertise that can be leveraged for LGTI research and care. In the course of her PhD she also supervised and mentored to completion, 7 Masters students involved in infectious disease research. Furthermore, Gloria pioneered a regional mentorship and training program in manuscript and grant writing targeting postgraduate students and researchers. She also has established valuable research collaborations.

Gloria's other notable involvements include: - Global Assessor for The Royal Society of Tropical Medicine and Hygiene, Editorial Board member for the East African Journal of Applied Health Monitoring and Evaluation, member of the Strathmore University Institutional Scientific and Ethics Review Committee, also previously Board member/vice- Chair of Aga Khan University Hospital Nairobi Institutional Ethics Review Committee.

PhD portfolio of Gloria Susan Omosa-Manyonyi

Department: Internal Medicine
PhD period: 18/04/2018 – 31/01/2025 (with breaks)
PhD Supervisor(s): Prof. dr. M.G. Netea, Prof. dr. A.J.A.M. van der Ven
PhD Co-supervisor(s): Dr. J. ten Oever

Training activities	Hours
Courses	
• Successful grant applications: getting it right (2016) Elsevier Publishing Campus	12.00
• Epidemiology: The Basic Science of Public Health (2016)	56.00
• Plagiarism (2017)	8.00
• How to become a Reviewer and what do Editors expect? (2017)	16.00
• Creating a good research data management plan (2017)	10.00
• RIHS - Introduction course for PhD candidates (2018)	15.00
• Understanding Clinical Research: Behind the Statistics (2018)	60.00
• Pedagogy, Andragogy and Mentorship Training (2019) University of Nairobi	34.00
• Good Clinical Practice Course (2020) CITI (Collaborative Institutional Training Initiative)	40.00
• Human Subjects Protection (2020) CITI (Collaborative Institutional Training Initiative)	12.00
• Radboudumc - Scientific integrity (2020) CITI (Collaborative Institutional Training Initiative)	20.00
• IRB Members - Basic ethics course (2020) CITI	60.00
• GRASP IT - Management of Anaphylaxis (2021)	8.00
• General Data Protection Regulation (2021)	24.00
• Turnitin Similarity Checker (2022)	4.00
• Basic Biostatistics using R (2022)	40.00
• 2022 Kevin Mitnick Security Awareness Training (2023) KnowBe4	0.25
• Governance of Research Compliance (2023) FNWI	14.00
• VIRT2UE Train the Trainer program (2023) University of Nairobi	40.00
Seminars	
• Study initiation training (2017)	16.00
• Study protocol training (2018)	36.00
• Kenyatta National Hospital-University of Nairobi weekly Webinars (2020, 2021, 2022, 2023, 2024)[§]	100.00
Conferences	
• Keystone symposium - Role of the Genital Tract Microbiome in Sexual and Reproductive Health (2018)[Ψ]	40.00
• NACC Maisha HIV and AIDS Conference, Nairobi (2019) [#]	16.00
• Biennial Infectious Disease Conference 2019, Nairobi, Kenya (2019) [*]	16.00
• The 2021 HIV Prevention, Care and Treatment Scientific Conference (2021)	32.00
• Biennial Infectious Disease Conference 2021 (2021)	16.00
• Regional Conference on Strengthening Research Ethics (2022) [#]	24.00
• IDSA IDWeek 2023 (2023)	15.00

Other	
• Global Assessor – The Royal Society of Tropical Medicine and Hygiene (2018 - 2024)	120.00
• Editorial Board Member – East African Journal of Applied Health Monitoring and Evaluation (2018 - 2024)	50.00
• Member - Aga Khan University, Kenya, Institutional Ethics Review Committee (2020 – 2022)	80.00
• Vice-Chair of Aga Khan University, Nairobi, Institutional Ethics Review Committee (2021-2022)	24.00
• Postgraduate Journal Club, Faculty of Health Sciences, University of Nairobi (2019, 2020, 2021, 2022)	48.00
• RCI PhD meet-up (2022)	7.00
• Radboudumc Research newsletter – Published article "Problems of the private parts" https://www.radboudumc.nl/en/news/2023/problems-of-the-private-parts (2023)	10.00
• Peer reviewer (2023) Dove Medical Press	3.00
• Reviewer for evaluating research projects (2024) Marató de TV3 2023	18.00
• Member of Strathmore University Institutional Scientific and Ethics Review Committee (2024)	32.00
Teaching activities	
Lecturing	
• Medical Microbiology, Infectious Diseases - undergraduate students (2018 -2024)	600
• Medical Microbiology, Infectious Diseases - postgraduate students (2018 -2024)	200
• Research Ethics (2021)	9.00
Supervision of internships / other	
• Supervision and mentorship of 7 Masters students to completion (2018 - 2023)	200.00
• Internship supervision – 6 research interns (2018, 2019, 2020)	100.00
• Supervision of postgraduate research intern (2019, 2020)	52.00
• Scientific Manuscript Writing Mentorship (2021, 2022, 2023, 2024)[¶]	200.00
• Clinical Trials Monitoring Training, SCALE-IT Project (2024)	16.00
• Grant writing training and mentorship (2024)	60.00
Total	**2613.25**

[*]Poster presentation, [§]Oral presentation, [#]Organizing committee member, session Chair [¶]Awards – Travel award/Training grant

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