



Original Research Article

## **Microbial Aetiology of Vaginal Discharge in Relation to Intra-Vaginal Practices among Women Attending Reproductive Health Clinic in Dar es Salaam, Tanzania**

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### **ABSTRACT**

Genital discharge in women is due to different etiology. A significant high proportion of women globally practice douching, and drying vagina due to various reasons. These intra-vaginal practices have been reported to be associated with reproductive tract infections and HIV. The influence of these practices on vaginal discharge and reproductive tract infections in Tanzania was not well known. A cross sectional study was conducted between January 2003 and January 2004, investigating microbial etiology of vaginal discharge and its relationship with intra-vaginal practices among women presented with vaginal discharge at reproductive health clinic in Dar es Salaam. A structured questionnaire and laboratory forms were used to collect required information. Univariate and multivariate analysis were done.

Three hundred twenty six women were enrolled. The median age was 26 years (range 16 - 63). A proportion of 81.9% and 50.3% reported practicing douching and drying vagina before sex respectively. Water (53.2%) and piece of cloth (95.3%) were the most often used material for douching and drying vagina respectively. Candida infection (44.3%) was the most prevalent RTI followed by bacterial vaginosis (41.2 %), while HIV seroprevalence was 26.6%. Douching was associated with higher risk of bacterial vaginosis (OR, 3.10, 95% CI (1.36 – 7.06)). The intra-vaginal practices were found widespread among women in Dar es Salaam, douching being significantly associated with Bacterial vaginosis. Provision of health education and proper counseling at reproductive health clinic on intra-vaginal practices is warranted besides treatment to control and prevent infections. The findings should prompt women to reconsider on intra-vaginal practices

**Keywords:** Vaginal discharge, Intra-vaginal practices, Douching, RTI, Tanzania.

### **INTRODUCTION**

Vaginal discharge is a common presenting symptom among patients seen at reproductive health services globally, and has far-reaching health, social, and

economic consequences. The highest prevalence is found in developing countries mostly among underprivileged population. In Dar es Salaam Tanzania, unpublished data has shown that proportion of genital

discharge syndrome among women attending reproductive health clinic at Infection Disease Clinic has been more than 50%. Although frequently genital discharge is a result of infection, may also have non-infectious causes due to different etiological agents.

Intra-vaginal practices including douching (applying liquid into a vagina), drying vagina before sexual intercourse and inserting substances into the vagina have been around since ancient time and have been reported to be widespread in different regions and populations' throughout sub-Saharan Africa. Frequent use has been reported among female sex workers in Nairobi, Kenya; [1] pregnant women in Abidjan, Ivory Coast; [2] attendees at a sexually transmitted infections clinic in Bangui, Central African Republic; [3] and family planning, primary care, and postnatal clinic attendees in Harare, Zimbabwe. [4]

The intra-vaginal practices have been associated with a higher prevalence of reproductive tract infection (RTI) and Human Immunodeficiency Virus (HIV). [5,6] Increased susceptibility to RTI may be due to change of the delicate chemical balance in the vagina and vaginal flora that allow new infections and spread existing infections in the lower reproductive tract into the pelvic organs. [7,8] Some of these practices may lead to epithelial damage and inflammatory response thus increase risk for transmission of HIV. [9-11] Frequent douching has been suspected to predispose women to pelvic inflammatory disease which in turn, greatly increase the chance of ectopic pregnancies. [12,13]

Although intra-vaginal practices have been reported in many countries in sub-Saharan Africa their possible role on RTI and HIV in Tanzania is not well known. We therefore investigated the microbial etiology of vaginal discharge and determined its relationship with intra-vaginal practices among women presenting

with vaginal discharge, attending reproductive health clinic in Dar es Salaam, Tanzania.

## **MATERIALS AND METHODS**

### ***Study design, sample size and inclusion criteria***

Women presenting with signs and symptoms suggestive of genital discharge attending reproductive health clinic between January 2003 and January 2004 were invited to participate in the analytical cross-sectional study after signing a written informed consent. The study excluded those on menses, on antimicrobial treatment and pregnant women. The sample size was determined considering proportion of gonorrhea infection of 4%, previously reported among pregnant women in Dar es Salaam. [14] The minimum required sample size was 236.

### ***Data collection***

Participants underwent structured interview, general examination, pelvic examination and collection of genital and blood specimen. Information regarding social demographic characteristics, intra-vaginal practice (douching, drying vagina), and symptoms related to reproductive tract infection and clinical findings were recorded. Free treatment was provided syndromically for genital discharge as per guideline set by Ministry of Health and Social Welfare in Tanzania. Those who were found to be HIV seropositive were referred for HIV/AIDS care and treatment.

### ***Laboratory Methods***

RTI screening consisted of determination of vaginal secretion pH, amine test, microscopic examination of wet preparation for pus cells, *Trichomonas vaginalis*, *Candida* species and clue cells, and Gram staining of cervical smear for *N. gonorrhoeae*. Diagnosis of bacterial vaginosis was based on the Amsel's criteria [15] (*presences of three of the following: homogenous whitish vaginal discharge,*

elevated vaginal pH greater than 4.5, positive amine test, and presence of clue cells on wet mount preparation).

Endocervical secretion preserved in Amies transport media were cultured for *N. gonorrhoea* on Modified Thayer Martin media (Oxoid, Unipath Limited, Basingstoke, UK) supplemented with vancomycin, nystatin, colistin, and trimethoprim and incubated at 35 - 37 °C in 5-10% CO<sub>2</sub>, at increased humidity. Presumptive identification for *N. gonorrhoea* was based on characteristic of colonial morphology, Gram stain reaction and oxidase test that were confirmed by the Phadebact co-agglutination test (Phadebact, Monoclonal GC test, Boule Diagnostics AB, Huddinge, Sweden).

The high vaginal swab preserved in Stuart's transport media, were inoculated onto Sabourauds dextrose agar (Oxoid, Unipath Limited, Basingstoke, UK), cultured for *Candida* species and incubated at 37°C for 48 hours. Suspected colonies were identified as *C. albicans* by typical characteristic colonial morphology, Gram stain reaction and germ tube test. Other isolates with typical colonial morphology of *Candida* species but negative germ tube test were named as other *Candida* species.

Endocervical secretions collected specifically for detection of Chlamydia infection were tested for *C. trichomatis* lipopolysaccharide antigen by EIA (MicroTrak® II Trinity Biotech plc, IDA Business Park, Bray, Co. Wicklow Ireland) following the manufacturer's instructions. HIV serology was done by first ELISA (Behring Enzygnost anti-HIV-1/2 plus Behringwerke, Germany). Reactive sample was confirmed by second ELISA (Wellcozyme anti-HIV-1 ELISA, Murex Diagnostic Ltd., Darford, UK) in an alternative confirmatory strategy.<sup>[16]</sup> Samples reactive on both assays were considered HIV antibody seropositive.

Discordant samples on the two ELISA were tested using Western blot.<sup>[17]</sup>

### **Statistical Methods**

All data were double entered into data entry system of SPSS for windows then validated using Epi Info 6. Univariate analysis was used to initially identify association of intra-vaginal practices and demographic characteristics with RTI/HIV using chi-square ( $\chi^2$ ) test. Two-tailed level of significance was taken at  $p \leq 0.05$ . Intra-vaginal practices that showed statistical significant association with RTI and or HIV infection on univariate analysis were then evaluated in multivariate analysis using logistic regression models adjusted by other independent variables. The adjusted odds ratios (AOR) and 95% Confidence Interval (CI) were used to describe the strength of association.

### **Ethical clearance**

The ethical clearance to undertake this study was obtained from Muhimbili University college of Health and Allied Sciences higher degrees ethical committee in Dar es Salaam, Written informed consent was obtained from each participant before enrolment into the study.

## **RESULTS**

### **Socio demographic characteristics**

Three hundred twenty six women participated in the study; median age was 26 years (range 16-63). Majority 159 (48.8%) were aged between 25 to 34 years while 121 (37.1%) aged below 25 years. In all, 167 (51.2%) were single, while 142 (43.8%) were married or cohabiting. A total of 151 (46.3%) were unemployed or housewives and only 59 (18.1%) were formally employed. Majority 175 (53.1%) had primary education while 10 (3.1%) had not attended any formal education. Substantial number of participants 1162 (49.1%) had 2 or more parity. In average every woman had one partner past six months with 59 (18.1%) having multiple sexual partners (table 1)

**Table 1: Socio-demographic characteristics of the study participants attending reproductive health clinic in Dar es Salaam (n = 326)**

Variable	Number of Population	Percentage (%)
<b>Age (years)</b>		
<25	121	37.1
25 – 34	159	48.8
>34	45	14.1
<b>Marital status</b>		
Single	167	51.2
Married/cohabiting	142	43.6
Previous married	17	5.2
<b>Occupation</b>		
Unemployed/housewife	151	46.3
Petty business/work	79	24.2
Employed/skilled labour	59	18.1
Student	37	11.3
<b>Education level</b>		
None	10	3.1
Primary	175	53.1
Secondary	108	33.1
Post- secondary	33	10.1
<b>Parity</b>		
0	91	27.9
1	73	22.4
2-4	127	39.0
5+	35	10.7
<b>Sexual partner at 6 Months</b>		
0	27	8.3
1	240	73.6
2+	59	18.1

### ***Intra-vaginal practices***

Of the 326 women interviewed, 267(81.9%) reported practicing douching, among them, 142 (53.2%) reported using water only while 123 (46.1%) reported using other material like soap, antiseptic and local herbs. Data on the frequency and reasons for douching was available from 191 participants, among them 180 (94.2%) were douching more than once a week. Majority 177 (92.7%) were douching in order to be clean and reduce odor. One hundred sixty four (50.3%) participants reported drying vagina before or during sexual intercourse (table 2). In all, 151 participants who reported on materials used for vagina drying; 144 (95.3%) were drying using pieces of cloth including, 26 (17.2%) used towels, 6 (4.0%) were using toilet paper and only 1 (0.7%) reported using female tampons

### ***Prevalence of RTI and HIV infection***

Candida infection was the most prevalent RTI in studied participants affecting 143/323 (44.3%) followed by

Bacterial vaginosis 115/279 (41.2 %). Concurrent Candida infection and Bacterial vaginosis among 277 participants who had both tests performed was present in 47 (17.0%). *T. vaginalis* was detected in 26 (8.0%) while *N. gonorrhoeae* was detected in 11/318 (3.5%). All women with *C. trichomatis*, 3/179 (1.7%) had concurrent *N. gonorrhoeae* infection while no concurrent infection was detected in *T. vaginalis* and *C. trachomatis* or *N. gonorrhoeae*. There was substantial high number of women 79/297 (26.6%) who were HIV seropositive (table 3).

**Table 2: The prevalence and characteristics of intra-vaginal practices among study participants**

Characteristics	Frequency (%)
<b>Intra-vaginal practice (n = 326)</b>	
Vaginal Douching	267 (81.9)
vaginal Drying	164 (50.3)
<b>Material for douching (n = 267)</b>	
water only	142 (53.2)
water + soap	96 (36.0)
antiseptic	27 (10.1)
local herbs	2 (0.7)
<b>Frequency of douching (n = 191)</b>	
Douching > 1 a week	180 (94.2)
Douching ≤ 1 a week	11 (5.8)
<b>Reason for douching (n = 191)</b>	
For hygiene	177 (92.7)
For treating STI	11 (5.8)
Others	3 (1.6)

**Table 3: Prevalence of RTI and HIV seroprevalence among women presetting with genital discharge**

RTI/HIV	Proportion (%)
Candidiasis	143/323 (44.3)
Bacterial vaginosis	115/279 (41.2)
Trichomoniasis	26/323 (8.0)
Gonorrhoea	11/318 (3.5)
Chlamydia	3/179 (1.7)
HIV sero positive	79/297 (26.6)

### ***Association of RTI /HIV infection with intra-vaginal practices and demographic characteristics***

A significantly higher prevalence of Bacterial vaginosis was found among women reported douching (45.3% vs 21.3,  $p < 0.05$ ), unemployed/petty business and HIV positives (). *N. gonorrhoeae* was significantly more prevalent only among women who were not douching (8.5% vs. 2.3%, ( $p = 0.05$ ). HIV infection was associated with unemployment/petty

business and low education ( $p \leq 0.05$ ). There were no significance differences between douching and not douching among participants with candidiasis, trichomoniasis, and chlamydial infection while candidiasis was significantly associated with being student/employed and HIV infection. A significantly higher prevalence of *T. vaginalis* was found among women who reported drying vagina, low education and HIV positive ( $p \leq 0.05$ ) (Table 4)

Multivariate analysis was performed to determine the association of intra-vaginal practices with RTI after adjusting for socio demographic factors. Women who reported douching had three times risk of bacterial vaginosis (AOR, 3.10, 95% CI (1.36 – 7.06),  $p = 0.007$ ), and those reported not douching had around 16 times chance of detection of *N. gonorrhoea* compared to those reported douching. (AOR, 15.74, 95% CI, (2.96 - 83.82),  $p = 0.01$ ). The association between vagina drying practice and *T. Vaginalis* found in univariate analysis were not found during multivariate analysis (AOR, 2.4, 95% CI 0.92 - 6.03),  $p = 0.07$ ). (Table 5)

## DISCUSSION

Intra-vaginal practices can influence vaginal discharge in a positive or negative way due to one of different etiological agents. It is the paucity of information on association of intra-vaginal practices with RTI/HIV in our society that led on conduction of this study. Women who participated in the study had varied socio-demographic characteristics majority being of middle age, low education, unmarried, and unemployed, thus reflecting population of low socioeconomic status. Candidiasis and bacterial vaginalis which were found to be more prevalent RTI in this study are not commonly sexually transmitted, thus the findings could indicate a higher rate of disturbance of normal genital microbial flora

that led to proliferation of pathogenic organisms. This is supported by the low prevalence of typical Sexually Transmitted Infection (STI) associated with vaginal discharge like gonorrhea, Chlamydia and trichomoniasis.

The current study has confirmed high level of intra-vaginal practices, among studied population in Dar es Salaam comparable with what has been reported in other studies done in Africa. [7,13,18] The main reported reason for douching found in this study was personal hygiene as found in other studies and mostly using water for that purpose. [19] Very few women reported douching because of presence of STI, contrary to what found in other studies. [18,20] In several African settings, herbs and dry leaves have been reported to be used for treatment of vaginal infections or "tightening effect" to achieve dry sex. [1,21] Different purposes for douching have been reported, which explain the difference in common materials used, [18,19,21,22] the current study found majority of women were douching for hygienic purposes, that explain why water only was the commonest material used for the practice.

Substantial proportion of women in this study were drying vagina before or during sexual intercourse comparable with finding from Zambia. [10] But majority were using a piece of cloth, different from other studies that reported local herbs as the commonest preferred material for the purpose. [9,10,23,24] The difference in materials used could be explained by the different reason perceived by women for drying vagina. In the current study 45.1% clients perceived that removing normal vaginal secretions led to a cleaner sexual intercourse. In populations where local herbs are commonly used, women report use of vaginal drying agents for tightening and thus enhancing their partner's sexual pleasure. [9,10,23,24]

**Table 4: The univariate analysis result for factors association with RTI/HIV infection among women presetting with genital discharge**

Independent variable	Dependent variable											
	B. Vaginosis		Candidiasis		Gonorrhoea		Chlamydia		Trichomoniasis		HIV+	
	N (%)	P value	N (%)	P value	N (%)	P value	N (%)	P value	N (%)	P value	N (%)	P value
Douching												
Yes	105(45.3)	< 0.05	46(17.4)	0.470	6(2.3)	0.05	3(1.8)	*	18(6.8)	0.15	71(28.7)	0.06
No	10(21.3)		9(14.3)		5(8.5)		0(0)		8(13.6)		8(16.0)	
Drying vagina												
Yes	53(38.7)	0.47	29(17.8)	0.71	6(3.7)	0.79	2(2.1)	0.89	19(11.7)	< 0.05	44(29.1)	0.31
No	62(43.7)		26(16.3)		5(3.2)		1(1.2)		7(4.4)		35(24.0)	
Age (years)												
Below 25	40(37.4)	0.30	24(19.8)	0.30	3(2.5)	0.48	1(1.3)	0.73	9(7.4)	0.75	30(25.9)	0.82
25+	75(43.6)		31(15.3)		8(4.0)		2(2.0)		17(8.4)		49(27.1)	
Marital Status												
single	55(38.2)	0.29	32(19.4)	0.23	5(3.1)	0.71	0(0)	*	11(6.7)	0.35	36(23.5)	0.23
Married	60(44.4)		23(14.6)		6(3.8)		3(3.6)		15(9.5)		43(29.9)	
Occupation												
Unemployed/Pet business	95(48.0)	< 0.05	32(14.1)	< 0.05	10(4.5)	0.13	3(2.5)	*	22(9.7)	0.10	69(33.2)	< 0.05
Employed/student	20(24.7)		23(24.0)		1(1.1)		0(0)		4(4.3)		10(11.2)	
Education												
Low level	68(42.2)	0.7	25(13.7)	0.07	6(3.3)	0.9	1(1.0)	0.42	22(12.0)	< 0.05	59(34.9)	< 0.05
High level	47(39.8)		30(21.4)		5(3.6)		2(2.6)		4(2.9)		20(15.6)	
HIV status												
Positive	37(57.8)	< 0.05	9(11.4)	0.05	3(3.9)	0.79	2(5.1)	0.06	10(12.7)	< 0.05		
Negative	67(35.6)		46(21.3)		8(3.7)		1(0.8)		12(5.6)			

\*comparison not done due to zero proportional

**Table 5: Multivariate analysis results for association of intravaginal practices with RTI/HIV infection among women presetting with genital discharge**

Intra-vaginal Practice	Bacterial Vaginosis				Gonorrhoea				Trichomoniasis			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	N (%)	P Value	AOR (95%CI)	P value	N (%)	P Value	AOR (95%CI)	P value	N (%)	P Value	AOR (95%CI)	P value
Douching												
yes	105(45.3)	< 0.05	3.10(1.36-7.06)	0.007	6(2.3)	0.05	1	0.01	-	-	-	-
No	10(21.3)		1		5(8.5)		15.74(2.96-83.82)		-	-	-	-
Vagina Drying												
Yes	-	-	-	-	-	-	-	-	19(11.7)	< 0.05	2.4 (0.92-6.03)	0.07
No	-	-	-	-	-	-	-	-	7(4.4)		1	

This study has shown an association between douching with increased prevalence of bacterial vaginosis as well as drying vagina with increased prevalence of *T. vaginalis* infection. To establish a cause and effect relation is not possible with cross sectional nature of this study. But it is possible that frequent douching may lead to bacterial vaginosis due to disturbance of vaginal chemical balance and microbial normal flora. The finding is consistent with results from other studies. [8,25,26] Women who reported not douching were more likely to have gonorrhoea as compared with those who were douching. It is not known whether douching reduces transmission of gonorrhoea or is associated with poor detection of gonorrhoea from the specimen. However the number of cases found with gonorrhoea in the current study was too small for reasonable comparison. There is possibility that frequent douching may impair the isolation of *N. gonorrhoea* due to the fastidious nature of the organism.

The development of effective prevention efforts against HIV and RTI requires a more thorough understanding of different sexual preferences and intra-vaginal practices. The importance of such information or knowledge is underscored by previous finding that some materials used for vaginal drying prior to sexual intercourse may also compromise condom integrity and render the condom inadequate as a contraceptive or as prevention against STI. [18] The emphasis on dryness may influence women's willingness to use vaginal microbicides as well. In this studied population where about 50% of women value a dry vagina during sexual intercourse, the lubricating qualities of microbicide may preclude overt use. Some laboratory tests could not be performed on all patients due to failure to obtain representative sample and limited reagents for *C. Trichromatic* hence

variation of number of tests performed for different etiological diagnosis.

## CONCLUSION

There is high prevalence of RTI and intra-vaginal practices among women attending reproductive health clinic while douching is significantly associated with Bacterial vaginosis. The findings of this study should prompt women to reconsider intra vaginal practices due to possible influence on RTI and implications for advocated method of HIV prevention interventions using microbicides. These observations reveal the need for providing proper counseling and health education to patients with RTI on intra-vaginal practices besides treatment to control and prevention of infections

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