# MICROBIAL AETIOLOGY IN RELATION TO INTRA-VAGINAL PRACTICES AMONG WOMEN PRESENTING WITH VAGINAL DISCHARGE IN DAR ES SALAAM, TANZANIA

BY

MTEBE VENANCE MAJIGO, M.D

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## CERTIFICATION

The undersigned supervisor certify that he has read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: *Microbial Aetiology in Relation to Intra-Vaginal Practices Among Women Presenting with Vaginal Discharge in Dar Es Salaam, Tanzania,* in partial fulfillment of the requirements for the degree of Master of Medicine (Microbiology/Immunology).

.....W.K. Urassa.....

Willy K. Urassa MD, M.MED, PhD

Department of Microbiology and Immunology,

Muhimbili University College of Health Sciences,

University of Dar es Salaam, Tanzania

Date...21st November 2005.....

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I, Mtebe Venance Majigo, declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

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## DEDICATION

This dissertation is dedicated to my wife Cornelia, my sons Norbert and Thaddeus.

## ABSTRACT

Genital discharge in women is caused by different aetiologic agents. A significant proportion of women globally practice douching and drying vagina before or during sexual intercourse which have been reported to be associated with some reproductive tract infections including HIV. The extent of these practices in our society is not well known. Between November 2002 and January 2004, a cross sectional study investigated the microbial aetiology of vaginal discharge and its relationship with intra-vaginal practices among women presenting with vaginal discharge attending reproductive health clinic.

Three hundred twenty six women presenting with vaginal discharge were enrolled. The median age was 26 years (SD 6.9). BV was the most prevalent RTI (41.2 %) while the HIV seroprevalence was 26.6%. Among 326 participants, 81.9% and 50.3% reported practicing douching and drying vagina before or during sexual intercourse respectively. Water (53.2%) and piece of cloth (78%) were the most often used materials for douching and drying vagina respectively. Douchers had higher prevalence of BV than non douchers (45.3% vs. 21.3%) $p = 0.002$. There was a significant association between petty business and BV as well as HIV infection, and between low educational status and Trichomoniasis as well as HIV infection. BV and trichomoniasis were more common among HIV seropositive than HIV seronegative women. In conclusion that the prevalence of BV and HIV among women studied is high. The intra-vaginal practices; douching and dry sex are widespread, and douching is significantly associated with BV.

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## LIST OF ABREVIATION

| | |
|---|---|
| AIDS | acquired immunodificiency syndrome |
| BV | bacterial vaginosis |
| CID | clinic of infectious diseases |
| DNA | deoxribose nucleic acid |
| EIA | enzyme immuno assay |
| GDS | genital discharge syndrome |
| HIV | human immunodeficiency virus |
| IDC | infectious diseases centre |
| IUCD | intra-uterine contraceptive device |
| KOH | potassium hydroxide |
| LPS | lipopolysacharide |
| MNH | Muhimbili National Hospital |
| MPC | mucopulurent discharge |
| MUCHS | Muhimbili University College of Health Sciences |
| PCR | polymerase chain reaction |
| PID | pelvic inflammatory disease |
| PMN | polymophonuclear cell |
| RTI | reproductive tract infection |
| STI | sexually transmitted infection |
| WHO | world health organization |

# 1.0 INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

Vaginal discharge is a common presenting symptom among patients seen in many health services like; primary health care, gynaecology, family planning, and Sexually Transmitted Infection (STI) clinics. Vaginal discharge may be physiological or pathological. Although abnormal vaginal discharge often prompts women to seek medical attention in STI clinics, vaginal discharge is a poor predictor of STIs.

All women have normal vaginal discharge that may be clear, cloudy or white, non-irritating, and odor-free. It may also contain white flecks and at times may be thin and stringy. However each woman has her own sense of normality and what is acceptable or excessive for her. The quality and quantity of vaginal discharge may vary over time. During the normal menstrual cycle the amount and consistency of vaginal discharge vary. Right after menstruation it may be of small amount, very thin or watery discharge, and around ovulation the discharge has thicker consistency. Changes in normal discharge in terms of amount and color can occur for many reasons, and may be a sign of a vaginal infection.

The pH of the vaginal secretion is acidic, which reduces chance of some infections. Any interference with the delicate balance of vaginal secretions sets up an environment conducive to infections that include genital discharge and genital ulcerations. The

human vaginal ecosystem is dominated by *Lactobacillus* species. Lactobacilli are gram-positive rods that maintain the normal vaginal pH between 3.8 and 4.4. They have been found to produce substances with antimicrobial properties, including lactacidin, acidolin, lactacin B, and hydrogen peroxide ($H_2O_2$) [1]. Therefore $H_2O_2$ producing *Lactobacillus* strains play a pivotal role in controlling the microenvironment of the vagina and in inhibiting the overgrowth of potentially pathogenic organisms.

Vaginal infections are very common, they may present with vaginal discharge, itching, rash or soreness, and burning micturation. A vaginal discharge that has an odor or that is irritating is usually considered abnormal. The irritation might be itching or burning or both. Both of these symptoms are usually made worse by sexual intercourse.

Reproductive tract infections (RTIs) including STIs are of major public health importance globally and have far-reaching health, social, and economic consequences. The highest prevalence of RTIs is found in developing countries mostly among underprivileged people and women in particular. RTIs are a major public health problem not only because of the morbidity of acute illness but also because they have serious chronic sequelae and may facilitate the transmission of human immunodeficiency virus (HIV) [2-4]. Two common conditions associated with pathological vaginal discharge are vaginitis and cervicitis.

## 1.2 Vaginal infection

Vaginal infections include vaginitis and vaginosis. Vaginitis is a word that is used to describe inflammation of the vagina. Vulvovaginitis refers to inflammation of both the vagina and vulva. This condition can result from an infection caused by organisms such as bacteria, yeast, or viruses, as well as irritations from chemicals in creams, or sprays. Vaginal infection is the most frequent reproductive tract infection in women encountered in STI clinics [5-7]. Symptoms of vaginal infection are nonspecific, and neither self-diagnosis nor diagnosis by a physician is adequate without laboratory confirmation. Its management remains largely empirical, and many clinicians assume that is never life-threatening and that empirical therapy is always harmless. Vulvovaginitis is frequently associated with mixed infections.

### 1.2.1 Aetiology

Currently, three major types of vaginal infections are recognized which include trichomonal vaginitis, candidal vaginitis, and bacterial vaginosis (BV) previously referred to as nonspecific vaginitis (NSV) or *Gardnerella vaginalis*-associated vaginitis. Trichomonal vaginitis is caused by *Trichomonas vaginalis,* while candidal vaginitis is caused by *Candida* species, commonly *C. albicans*. *Candida* species are among the normal microbial flora of the vagina and digestive tract. BV is seen when there is depletion of hydrogen peroxide-producing lactobacilli followed by overgrowth of *Gardnerella vaginalis, Mycoplasma hominis,* and anaerobic organism like *Bacteroides*

species. BV is believed to be caused by proliferation of these organisms [5, 8].

### 1.2.2 Epidemiology

The prevalence of vaginal infection is uncertain, in part because the condition is so often self-diagnosed, self-treated and is frequently asymptomatic in about one third to three quarters of affected women. Reported prevalence vary based on the population studied, it has been reported in 15 to 19% of ambulatory gynecologic patients, 10 to 30% of pregnant women and 24 to 40% of patients in STI clinics [8-10]. In the United States, BV is currently the most common cause of vaginal infection, accounting for 40 to 50% of cases in women of child-bearing age [8].

Even though higher rates of BV have been reported in STI clinics and in women with multiple sexual partners, the role of sexual transmission is unclear, as women who are not sexually active have been found to be infected, therefore treating the male sexual partner of a woman with BV was suggested not to be beneficial [11]. Additional risk factors for BV include the use of intrauterine devices (IUDs), douching and pregnancy [8].

Candidiasis is one of the most common causes of vaginitis [5], *Candida albicans* is the infecting agent in 80 to 90% of patients with candidal vaginitis [9]. However the frequency of non-albicans species such as *C. glabrata*, *C. parapsilosis, C. tropicalis*, has recently increased, possibly secondary to HIV infection and greater use of over-the-

counter antifungal products [12]. The epidemiological data on candida vulvovaginitis are incomplete, prevalence estimates rely mainly on self-reported history and diagnosis by attending clinician. Vulvovaginal candidiasis is routinely diagnosed clinically without the support of microscopy or culture, and as many as half of the women given this diagnosis may have other conditions [13].

Although candidiasis is rare before menarche, by the age of 25 years half of women attending clinic will have had at least one diagnosed episode and it has been reported to be less common in postmenopausal women [14, 15]. In other populations, at least one episode of vulvovaginal candidiasis is reported in up to 75 percent of premenopausal women [16].

The risk factors for candidiasis are difficult to determine but studies have shown increased risk in women who use oral contraceptive pills, vaginal diaphragm, spermicides, or IUD [17]. The risk has also been reported to be increased in some women who have diabetes, pregnancy or are taking antibiotics [18]. Establishing *Candida* species as the cause of vaginitis can be difficult because women have these organisms as part of their endogenous vaginal flora. *Candida* species are not normally transmitted sexually, and episodes of vulvovaginal candidiasis do not appear to be related to the number of sexual partners [19].

The protozoan *T. vaginalis* affects 180 million women worldwide and currently accounts

for 10 to 25% of vaginal infections [9]. However its incidence has been reported to be decreasing in most industrialized countries [5]. *T. vaginalis* are sexually transmitted and may be identified in 30 to 40% of the male sexual partners of infected women [9, 20]. Trichomoniasis, which is associated with a high prevalence of other sexually transmitted diseases, has been suggested to facilitate transmission of HIV [4, 21].

Differences in the prevalence of *T. vaginalis* between different populations are striking, particularly given the absence of any deliberate STIs control programmes in most of developing countries. The prevalence has been reported to range from less than 1% in urban women to more than 20% in under-served populations [22].

### 1.2.3 Pathogenesis and pathology

The normal physiologic vaginal discharge comprises vaginal secretions, exfoliated cells and cervical mucus. A dynamic interrelationship between *Lactobacillus acidophilus* and other endogenous flora, estrogen, glycogen, vaginal pH and metabolic by-products of flora characterize the normal vaginal environment. Vaginal infection is thought to occur because the vaginal flora has been altered by the introduction of pathogens or changes in the vaginal chemical balance that allow pathogens to proliferate. Antibiotics, contraceptives, sexual intercourse, douching, stress and hormones have been suggested to change the vaginal chemical balance and micro flora that allow pathogens to grow [6].

In BV, it is believed that some events decrease the number of hydrogen peroxide producing *L. acidophilus* organisms. The resultant increase in pH allows proliferation of organisms that are normally suppressed, such as *G. vaginalis, M. hominis* and *Mobiluncus* species [8]. These organisms produce metabolic by-products, such as amines, that further increase the vaginal pH and cause exfoliation of vaginal epithelial cells. The amines are also responsible for the characteristic malodorous discharge in BV.

Similarly, changes in the vaginal chemical balance such as, an increase in glycogen production in pregnancy or altered estrogen and progesterone levels from the use of oral contraceptives enhance the adherence of *Candida* species to vaginal epithelial cells and facilitate the germination of yeast. These changes may transform asymptomatic colonization into symptomatic infection.

Trichomoniasis is a sexually transmitted disease, *T. vaginalis* in females inhabits the vagina, and the life cycle consists only of a trophozoite stage which is transmitted by direct contact during sexual intercourse. The trophozoites attach to the epithelium of the urogenital tract, where they replicate by binary fission. The infection, once established, persists for long periods in females. The exact mechanism of *T. vaginalis* pathogenesis has not been clearly elucidated but it has been reported that *T. vaginalis* can cause the concentration of *L. acidophilus* to fall which lead to elevations of vaginal pH, the environment that favors its growth [23].

### 1.2.4 Clinical presentation

The common symptoms of vaginitis are itching, burning sensation, and vaginal discharge that are different from normal secretions. Itching and burning can be inside the vagina or on the vulva. Discomfort during urination or sexual intercourse may also occur. BV is a common clinical entity characterized by abnormal grayish white, homogenous, malodorous vaginal discharge that is more noticeable after unprotected sexual intercourse. Up to 50% of women having BV have been found to be asymptomatic [24]. A candidal infection produces a thick, white curd-like vaginal discharge. Although the discharge can be somehow watery, it is odorless. The infection is usually associated with vaginal and vulval itching. The pH of vaginal discharge in candidiasis may remain normal (pH of 4.0 to 4.5).

Trichomoniasis presents a wide variety of clinical patterns. The spectrum of clinical trichomoniasis in women ranges from the asymptomatic carrier state to flagrant vaginitis. Depending on the severity of the infection, trichomoniasis may be classified as acute, chronic, or asymptomatic. The clinical picture in the acute infection reveals diffuse vulvitis due to copious leukorrhea. The discharge is typically frothy, yellow or green, and mucopurulent. In chronic infections, the predominant symptoms are mild, with pruritus and dyspareunia, while the vaginal secretions may be very scanty and mixed with mucus. This form of the disease is particularly important from the epidemiological point of view because these individuals are the major source of transmission of the parasite.

### 1.2.5 Diagnosis

Evaluation of patients with suspected vaginal infection rests mainly upon both physical examination of the vagina and the discharge, and upon microscopic examination of the discharge and determination of its pH. The amount, consistency, and location of the discharge within the vagina are also important. For microscopic examination a sample of the discharge is taken from the lateral vaginal wall with a swab while avoiding contamination with cervical mucus.

The pH is determined directly by rolling the swab onto pH indicator paper or placing litmus paper in the pooled vaginal secretions. The color change is then compared to the color and corresponding pH values on a standard chart. A normal vaginal pH is between 3.8 and 4.2. A pH greater than 4.5 is found in 80 to 90 % of patients with BV and frequently in patients with trichomoniasis [6].

Traditionally, BV has been clinically diagnosed using Amsel's criteria, which requires the presence of three of the following four criteria: (1) a thin, homogeneous discharge, (2) vaginal pH greater than 4.5, (3) a positive "whiff" test or release of amine odor with the addition of base, and (4) clue cells on microscopic evaluation of saline wet preparation. Based on these criteria, about 90 % of women with BV can be diagnosed correctly [25].

#### 1.2.5.1 Microscopic examination

A wet mount preparation is made by diluting the vaginal discharge with one or two drops of 0.9% normal saline. Alternatively, the vaginal discharge can be put into a 2 ml test tube containing saline solution. The preparation is examined microscopically using high dry power (x 40). The scanning of several fields for motile trichomonads has been reported to have a sensitivity of 60% and a specificity of up to 99% [26].

Microscopic examination of a wet-mount preparation can also detect "clue cells," which are vaginal epithelial cells that are coated with coccobacilli. Detection of clue cells in wet-mount preparations has been found to have a sensitivity of 60% and a specificity of up to 98% for the diagnosis of BV [25]. This examination may also help to demonstrate the presence of fungal hyphae seen in vaginal candidiasis and increased numbers of polymorphnuclear cells seen in trichomoniasis.

Gram stain of vaginal discharge is another method used for diagnosis of BV. Typical infection is indicated by presence of mixed flora (gram-positive, gram-negative or gram-variable bacteria) and absent or decreased *L. acidophilus*. The Gram stain using Nugent's criteria is believed to be the gold standard for diagnosing BV. Its scoring system (zero to 7+) is a weighted combination of the following bacterial morphotypes: A: *L. acidophilus* (large gram-positive rods). B: *G. vaginalis* and *Bacteroides* species (small gram-variable or gram-negative rods). C: *Mobiluncus* species (curved gram-variable rods).

The total score is the sum of the weighted quantity of the three bacterial morphotypes. Scoring for each of the above bacterial morphotypes is as follows:

Zero = No morphotype per oil-immersion field,

1+ = Less than one morphotype per oil-immersion field.

2+ = One to four morphotypes per oil-immersion field.

3+ = Five to 30 morphotypes per oil-immersion field.

4+ = More than 30 morphotypes per oil-immersion field.

For the combined score (A + B + C), zero to 3 represents normal flora, 4 to 6 represents indeterminate, and 7 or higher is diagnostic for BV[ 27].

### 1.2.5.2 Culture

In patients with a negative microscopic examination but symptoms suggestive of candida vaginitis, a culture using Sabouraud's dextrose agar may be helpful [7]. Culture has also long been considered the gold standard for diagnosis of *T. vaginalis.* Several different media are available for culture, including self contained system. Diamond's modified media has been shown to be superior for isolation of *T.vaginalis*. Culture is more sensitive than microscopy, because of the lower minimum concentration of organisms required for a positive result. The drawbacks to culture include great cost and long duration of incubation of culture media which ranges from 3 to 7days [28].

#### 1.2.5.3 Nucleic acid amplification

Although not commercially available, Polymerase Chain Reaction (PCR) testing is much more sensitive than microscopy or culture for detecting *T. vaginalis* [29]. PCR has become increasingly attractive for diagnosis of infection with *T. vaginalis* because it requires only DNA, which are either viable or non-viable and in concentrations of as low as one organism [30].

### 1.2.6 Treatment

Accurate diagnosis is essential for the proper management of patient with vaginal infection. However the availability of over-the-counter medications increases the likelihood of inappropriate or partial treatment of this condition. The standard treatment for BV has been oral metronidazole 500 mg twice daily for 5 to 7 days. Intravaginal 0.75% metronidazole gel has also shown to be as effective as oral metronidazole [31]. Oral metronidazole at a dose of 2 g daily of varying duration has similar initial cure rates of 85%, 87%, 86%, and 87% for 1, 2, 5, and 7 days, respectively but it is more likely that single-dose therapy may improve adherence [32].

Vulvovaginal candidiasis has long been treated with intra-vaginal imidazoles. No difference has been seen in outcomes with the various imidazoles or with treatment durations of 1 to 14 days [33-35]. Oral treatment is popular, most commonly a single dose of fluconazole, however oral itraconazole and ketoconazole have also been used

successfully [36, 37]. The current treatment for trichomoniasis is oral metronidazole given as a 2g single dose, 250 mg 3 times daily for 7 days or 500 mg twice a day for 7 days. Intra-vaginal therapy has been reported not to be effective, probably due to the parasite's presence in inaccessible areas such as the vaginal glands and urethra [38]. Short-term treatment is comparable with long-term treatment [39].

Diagnosis of specific etiology of vaginal discharge may be difficult in resource poor settings. Therefore accurate treatment of a particular infection may not be possible since symptoms and signs are not specific. In such settings the World Health Organization (WHO) has promoted syndromic approaches for management of STI-related symptoms with a combination of antibiotics likely to cover the major causative agents. Syndromic management of vaginal discharge may not be very effective in women because cervical discharge is a confounding factor. Over-treatment using syndromic management compared to microbiological diagnosis of some infection has been observed [40, 41].

### 1.2.7 Complication

Evidence suggests that BV is a risk factor for premature rupture of membranes and preterm labor and treating the infection in pregnancy has been found to decrease that risk [9, 42]. Additional possible adverse outcomes include an increased frequency of abnormal Papanicolaou (Pap) smears, pelvic inflammatory disease (PID) and endometritis [43]. PID and endometritis can occur if invasive gynecologic procedures or

surgery is performed when a patient has BV [5, 8, 9].

Trichomoniasis in women ranges from an asymptomatic carrier state to severe, acute, inflammatory disease. If untreated in pregnancy, trichomoniasis has been reported to be associated with premature rupture of the membranes, prematurity, and post-hysterectomy cellulitis [44]. However, in a randomised controlled trial of screening for *T. vaginalis* and treating asymptomatic pregnant women to prevent preterm birth, results were disappointing [45]. *T. vaginalis* has also been reported to potentially promote the sexual transmission of HIV [4].

## 1.3 Cervicitis

### 1.3.1 Aetiology of cervicitis

Cervicitis is an inflammation of the uterine cervix. It may occur due to infectious or non infectious causes. However the infectious aetiologies are significantly more common than the noninfectious causes. Most possible infectious causes of cervicitis are sexually transmitted.

Noninfectious cervicitis might be caused by local trauma, radiation, or malignancy. However, in a few cases it may be attributed to chemical exposure or a foreign body, such as pessaries, cervical cap, or vaginal diaphragm. The condition may also be caused by an allergy to contraceptive spermicides or latex in condoms. In cases where cervicitis

is attributed to foreign objects, infection is still frequently the cause, but the presence of the foreign object may make the cervix more susceptible to infection.

Gonorrhea, chlamydia, and trichomoniasis are the commonest STIs that cause cervicitis. Infection with the herpes simplex virus (genital herpes) and Human papilloma virus (genital warts) are two viral STIs that can cause cervicitis. Organisms, such as S*taphylococcus*, *Streptococcus*, and *G. vaginalis* can also cause cervicitis. *Mycoplasma genitalium* is one of the newest agents of STI causing urethritis in men, and evidence is growing that it causes cervicitis in women [46, 47].

### 1.3.2 Epidemiology of cervicitis

Cervicitis is very common, affecting more than half of all women at some point during their adulthood. Increased risk is associated with intercourse at an early age, high-risk sexual behavior, multiple sexual partners, and history of sexually transmitted disease. Increased risk is also associated with having a partner who has engaged in high-risk sexual behavior or who has had a previous sexually transmitted disease.

Although the true incidence of mucopulurent cervicitis (MPC) is not known, recent reports suggest that, the presence of any signs of cervicitis (mucopus, friability, erythema, and ectropion) together with >10 pus cells/high power field is significant for the presence of chlamydial cervicitis [48]. Sexually active adolescents, in particular,

have a high risk of cervicitis, typically as a result *of C. trachomatis* infection [49].

*C. trachomatis* has been ranked as the commonest non-viral causes of sexually transmitted infections worldwide [50]. The actual prevalence of *C. trachomatis* infection is not well known as most infections in women are asymptomatic and laboratory investigation is rare in developing countries. Syndromic approach for management of STI may under or over estimate the true incidence of disease in the community since diagnosis is based on the clinical presentation. *Neisseria gonorrhoeae* infection was initially viewed as a desease confined to high-risk groups, but it might also be common among disadvantaged populations [51, 52]. With the emergence of AIDS, prevalence of gonorrheal cases has been reported to decline among female sex workers in Western Europe [53].

### 1.3.3 Clinical presentation of cervicitis

Because the female genital tract is continuous from the vulva to the fallopian tube, there is some overlap between vaginitis and cervicitis. MPC is a distinct clinical entity seen predominantly in sexually active women. It is characterized by a yellow, mucopurulent endocervical discharge with polymorphonuclear (PMN) leukocytes. Cervicitis presents with unusual vaginal discharge which can be persistent, grayish white or yellowish. The discharge may also be profuse and have an odor or associated with vaginal bleeding after sexual intercourse, between periods, and after menopause. The cervix may be tender to instrument application, palpation, or movement.

### 1.3.4 Diagnosis of cervicitis

A pelvic examination reveals redness of the cervix or evidence of a cervical discharge. It may also reveal inflammation of the vaginal walls caused by the infected discharge. There are various laboratory methods for detection of organisms causing cervicitis.

#### 1.3.4.1 Microscopic examination

A wet mount preparation of cervical discharge is examined microscopically using high dry power (x 40) to detect the presence of motile trichomonads for diagnosis of trichomoniasis. Gram staining of a smear made from a cervical swab for detection of Gram negative intracellular diplococci has been found to have a sensitivity of 45-65% and specificity of 90-95% [54]. The sensitivity of microscopy is greatly reduced in asymptomatic infections. In endocervicitis, the sensitivity of this test is less than 50%, when compared with properly performed culture. In general, detection rates are lower when Gram-stained smears are examined by inexperienced microscopists [54].

#### 1.3.4.2 Culture

Culture for isolation of *N. gonorrhoeae* from endocervical swab in women remains the gold standard diagnostic method, as information on antibiotic susceptibility is essential. Culture based systems for isolation of *N. gonorrhoeae* have a high degree of specificity, but require personnel trained in handling the fastidious gonococcus [54]. The two basic requirements for reliable gonococcal culture include use of enriched medium to support

the growth of the fastidious *N. gonorrhoeae* and selective media to suppress the often extensive normal vaginal flora. Both the nutrient and selective properties of gonococcal culture media have been steadily improved, so that the sensitivity of culture as a diagnostic tool has significantly increased. In addition to isolation media of demonstrable quality, elevated $CO_2$, humidity, and an incubation temperature of 35–37 °C are required to support gonococcal growth [55].

Identification of *N. gonorrhoeae* can be problematic, a number of techniques are available, and their applicability and use depend on resources, expertise and circumstance. Presumptive identification is usually based on growth on selective media, Gram stain morphology, and oxidase test; these are regarded as sufficient for diagnosis in many settings. Definitive identification relies on the above criteria plus one or more tests that explore carbohydrate utilization patterns and or immunological characteristics or enzyme profiles. Carbohydrate utilization tests are the most widely used [55]

#### 1.3.4.3 Enzyme immunoassay

Tests based on antigen detection and enzyme immunoassay have been developed and extensively investigated for diagnosis of Chlamydia infection. Chlamydia antigen extracted from genital specimen can be detected early and easily. The test does not depend on viable organisms. The sensitivities of this nonculture tests have been reported to range from 62 to 75%, with significant differences between tests [56].

### 1.3.4.4 Nucleic acid based methods

Because culture-based diagnosis of gonorrhea has specimen collection and transport requirements that may pose difficulties, there has been good development of tests based on nucleic acid detection tests, some of which reportedly have high sensitivity and specificity. For the diagnosis of urethritis and endocervicitis, a non-invasive first-catch urine sample usually suffices. A number of techniques are available for the amplification of gonococcal DNA using cycling probe technology. Theoretically, even a single DNA copy, from live or dead organisms, can be amplified to detectable levels. The sensitivity of this test, for sample collected from all anatomical sites, has been estimated to be about 95% [57].

Nucleic acid amplification techniques have largely replaced tissue culture, non-amplification DNA probe assays and other rapid antigen assays (e.g. direct immunofluorescence and enzyme immunoassay) as the "gold standard" for diagnosis of *C. trachomatis* because of their greater sensitivity. Nucleic acid amplification techniques such as PCR, ligase chain reaction or strand displacement amplification, may use samples collected from the cervix and vagina, but are also easily adapted to self-sampling with self-inserted swabs, tampons or first-void urine [58, 59]. These techniques can be performed in less than 24 hours, but they are expensive and technically demanding. A PCR assay for both *C. trachomatis* and *N. gonorrhoeae* is available in a single tube; specificity is reported to be lower for *N. gonorrhoeae* than for *C. trachomatis* [60].

### 1.3.5 Treatment of cervicitis

Treatment of gonorrhea infection has been changing with time due to recognized resistance to penicillins and tetracyclines, currently third-generation cephalosporins and quinolones are widely used as a single dose, but quinolones resistance is now well established, particularly in the Asia-Pacific region [61]. Treatment of Chlamydia infection depends on use of single-dose oral azithromycin or doxycycline for 1 week [62]. Results of clinical trials indicate that azithromycin and doxycycline are equally efficacious [63].

The syndromic approach of cervicitis is similar to that of vaginitis consisting of treating STI-related symptoms with a combination of antibiotics likely to cover the major causal agents. This approach is a pragmatic strategy that allows prompt treatment of symptomatic patients without relying on a medical and laboratory infrastructure [64]. Improved access to syndromic management has been reported to reduce the incidence of HIV by controlling STIs in Tanzania [65].

### 1.3.6 Complications of cervicitis

Chronic cervicitis may develop and persist for months to years. If genital chlamydial infection or gonorrhea remains untreated, the infecting organisms may ascend into the uterus and fallopian tubes and cause endometritis, salpingitis, or salpingo-oophoritis with the sequelae of tubal factor infertility and ectopic pregnancy. By compromising the

fallopian tubes, chlamydial PID leaves the woman vulnerable to recurrent PID with endogenous vaginal flora, and chronic pelvic pain [66].

## 1.4 Intra-vaginal practices

### 1.4.1 Vaginal douching

Douching is defined as rinsing or cleaning of the vagina by using water or other solutions. This practice is thought to have been around since ancient times. It has long been thought that women need to clean their vagina for hygienic purpose and reduce odor. Some women have reported to douche after their menstrual periods or after sex to avoid getting STIs, or prevent pregnancy [67].

Douching is quite prevalent in many populations around the world [68, 69], and often begins during adolescence. Motivation for the initiation and maintenance of this practice appears complex, and presents challenges to the intervention efforts. Douching has been implicated in numerous adverse reproductive health outcomes and RTIs [68, 70-72]

Women who douche on a routine basis have been found to have more RTI related problems than women who do not douche or who rarely douche [68, 70]. These problems include vaginal irritation, infections like BV and other STIs. Douching has



been suspected to predispose women to PID [73]. RTIs such as cervicitis and PID have, in turn, been thought as risk factors for HIV acquisition; hence, vaginal douching may indirectly have a facilitating role in the heterosexual transmission of HIV [74]. Douching after sex may not prevent pregnancy contrary to false concept of some women, instead it reduced fertility [75]. Frequent douching has been reported to greatly increase the chance of ectopic pregnancies which can be life-threatening [76, 77].

Douching is not recommended by health personnel due to the fact that it changes the delicate chemical balance in the vagina and the vaginal flora, which can make a woman more prone to bacterial infections and spread existing vaginal or cervical infections into the pelvic organs [68, 70- 72].

Although frequent vaginal douching may increase women's susceptibility to RTIs as discussed above through modification of the vaginal flora, what is not known is how frequent douching affects the microflora found in the vagina. Douching less than once per week, particularly with water has not been associated with BV [70]. However it has been reported that douching after menses though less than once per week is strongly correlated with BV [78].

### 1.4.2 Vaginal drying

This is the practice of reducing vaginal secretions before or during sexual intercourse (dry sex) aiming at drying, tightening, and sometimes warming of the vagina. Reports of this practice come mainly from African countries [79-81]. Despite these reports, little is known about the prevalence of this practice, and its risk to RTIs and HIV infection. The practice include inserting of a substance into the vagina, drying with cloths, use of tissue paper or drinking preparations containing local herbs believed to cause vagina drying [79-82]. Substances usually inserted into the vagina include powders and absorbent stones, leaves, and herbal preparations [79-83].

These agents are used when there is a cultural preference for a dry rather than a lubricated vagina. Reasons for this preference vary in different societies. The main reason being given for adopting the practice of dry sex is to increase sexual pleasure [79-82]. Some cultures regard vaginal wetness during sexual intercourse as an indication of woman's infidelity and drying agents may be used where there is a community belief that the vagina must bear the mark of artistic work [84]. Both men and women, educated and non-educated have expressed preference for vaginal dryness and tightness during intercourse which is reported to increase sexual satisfaction and pleasure for both. Drying agents in some areas are also used for self-treatment of STI symptoms such as itching and vaginal discharge. Pregnant women who commonly have genital itching have been found to commonly use drying agents to combat itching and discharge rather

than tightening the vagina [85].

Some of the methods used as mentioned above can cause a number of reactions including an inflammatory response and epithelial damage [79-83]. Genital lesions as a result of RTIs have been shown to increase the risk of HIV transmission [74]. This would suggest that dry sex practices could be a potential risk factor in the transmission of RTIs including HIV. Attempt to investigate whether there is an association between dry sex practice and HIV transmission has led to inconclusive results [81-83]. However physical effects of intra-vaginal substances are marked [83].

Although the literature does not uniformly support the assertion that vaginal drying practices are linked to increased risk of HIV infection, the relationship is plausible through numerous mechanisms. HIV transmission is known to be enhanced in the presence of genital lesions and ulcerations [86]. Since vaginal drying agents have been documented as producing lesions that disrupt vaginal membrane integrity, the practice may also increase the risk of HIV acquisition. Drying practices may also increase the likelihood of trauma and abrasions during intercourse because of the absence of natural lubrication [79]. In addition, intra-vaginal substances may alter the vaginal pH, which normally serves as a protective factor against HIV acquisition [72].

## 2.0 STATEMENT OF THE PROBLEM

Reproductive Tract Infections especially genital discharge syndrome and HIV infection, continue to be a major public health problem. In Dar es Salaam the prevalence of genital discharge syndrome (GDS) among women attending reproductive health clinic at IDC has been more than 50% [87]. Genital discharge although frequently the result of infections, may also have non-infectious causes and is due to different aetiological agents. Most patients in developing countries with genital discharge receive treatment without laboratory confirmation, in so doing some noninfectious cases are given antibiotic and patients with physiological discharge also may receive treatment.

Majority of women in most parts of the world practice douching and drying vagina before or during sexual intercourse for different purposes, and different materials have been reported to be used by different societies. These intra-vaginal practices have been reported to be associated with some RTIs including HIV infection. The prevalence of these practices, the reasons, material used and their possible role on RTIs in our society is not well known.

## 3.0 OBJECTIVES OF THE STUDY

### 3.1 Broad objective

To investigate the microbial aetiology of vaginal discharge and to determine its relationship with intra-vaginal practices among women presenting with vaginal discharge attending reproductive health clinic in Dar es Salaam, Tanzania.

### 3.2 Specific objectives

3.2.1 To determine the prevalence of etiology of genital discharge among women attending reproductive health clinic.

3.2.2 To determine the seroprevalence of HIV infection among women with genital discharge attending reproductive health clinic.

3.2.3 To determine the relationship between intra-vaginal practices and RTIs and HIV infection.

3.2.4 To relate the common RTIs and HIV infection with socio-demographic characteristics.

3.2.5 To determine the association between RTIs and HIV infection among women with genital discharge.

## 4.0 STUDY HYPOTHESES

**4.1** Intra-vaginal practices are associated with an increased risk of acquiring RTIs and HIV infection among women with genital discharge.

**4.2** There is no relationship between RTIs and HIV infection among individuals belonging to different socio-demographic groups.

**4.3** There is no relationship in prevalence of RTIs and HIV-serostatus.

# 5.0 MATERIALS AND METHODS

## 5.1 Study area

This study was conducted at an Infectious Disease Centre (IDC); reproductive health clinic in Dar es Salaam City, Muhimbili University College of Health Sciences (MUCHS) and Muhimbili National Hospital (MNH) in the Department of Microbiology/Immunology. The IDC clinic is located at the city centre and draws its patients from public and private institutions both within the city and outside the city. The clinic works closely with MUCHS and has a highly trained and motivated staff.

Dar es Salaam is the largest city in Tanzania and is the main business centre in the country. Its population is estimated to be around 2.5 million based on 2002 national population census. This makes the city to have a dynamic population, mixed cultures, many unemployed people, and many petty businesses. Dar es Salaam is one of the most hit regions by RTI and HIV/AIDS in the country [88].

## 5.2 Study design

This was analytical cross-sectional study conducted between November 2002 and January 2004.

### 5.3 Study population

All women with vaginal discharge attending reproductive health clinic at IDC during study period were eligible and were invited to participate in the study after signing a written informed consent.

### 5.4 Exclusion criteria

Women who were in their menses during the first visit, pregnant women and those on antimicrobial treatment within one week of the visit were not eligible for enrolment.

### 5.5 Sample size and sampling technique

The sample size was determined considering the proportion of gonorrhea infection of 4%, previously reported among pregnant women in Dar es Salaam [89]. The study aimed at detecting the pathogens with the confidence interval of 95% and precision of ± 2.5%. Therefore the minimum required sample size was expected to be 236. Consecutive women with genital discharge without exclusion characteristics who signed a written consent were enrolled until the sample size reached 326 participants.

### 5.6 Data collection

All women in the study were interviewed using standard structured questionnaire and assigned identity number. Information regarding social demographic characteristics,

intra-vaginal practice, symptoms related to reproductive tract infection and clinical findings after physical examination were recorded.

## 5.7 Physical examination

Physical examination was done by clinicians and included; general examinations, genital inspection, speculum examinations and bimanual palpation. During these examinations, vaginal pH and amine test were done, and genital specimens were collected.

## 5.8 Collection of Specimen

The specimens which were collected included; vaginal specimen, cervical specimen and blood. Vaginal specimens were collected after introduction of clean, non-lubricated speculum; high vaginal swabs were taken for preparing smear for Gram stain, wet saline preparation and other were immediately inserted into Stuart's transport media for culture to isolate *Candida* species.

Cervical specimens were collected after cleaning of the cervix and endocervical swabs were taken for preparing smear for Gram stain and other immediately inserted into Amie's transport media for culture to isolate *N. gonorrheae*; the second swab was collected and preserved using microTrak®II *Chlamydia* EIA specimen collection kit which include transport tube for detection of *C. trichomatis* antigen.

Two to three milliliters (mls) of venous blood were collected into sterile vacutainer tubes

without anticoagulant and left to clot to obtain serum for detection of antibodies against HIV. All specimens were labeled using numbers recorded in the questionnaire and transported to MUCHS and MNH, Department of Microbiology/Immunology for processing on the same day.

## 5.9 Laboratory methods

### 5.9.1 Microscopic examination

Three microscopic examinations included; Gram stain of high vaginal smear for detection of yeast cells and diagnosis of bacteria vaginosis using Nugent's criteria scoring system. Secondly Gram staining of cervical smear for detection of pus cells and Gram-negative intracellular diplococci. Lastly, a wet preparation of the vaginal specimen for detection of presence of pus cells, budding yeast cells, clue cells and motile trichomonads.

### 5.9.2 Culture of endocervical swabs

Endocervical swab preserved in Amies transport media were inoculated on Modified Thayer Martin agar (Oxoid, Unipath Limited, Basingstoke, UK) supplemented with vancomycin, nystatin, colistin, and trimethoprim. The inoculated plates were incubated at 35 - 37 $^{0}$C in 5-10% $CO_2$, and increased humidity for isolation of *N. gonorrheae.* After 48 hours of incubation, presumptive identification of *N. gonorrhoeae* was based

on characteristic of colony morphology, Gram stain reaction and oxidase test on suspected colonies. All isolates were confirmed to be *N. gonorrhoeae* by the Phadebact coagglutination test (Phadebact, Monoclonal GC test, Boule Diagnostics AB, Huddinge, Sweden).

### 5.9.3 Culture of high vaginal swabs

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

### 5.9.4 Chlamydia serology

Chlamydia infection was diagnosed by enzyme immunoassay of detection of *C. trichomatis* lipopolysaccharide antigen (LPS) in endocervical swab using *Chlamydia* EIA MicroTrak® II (Trinity Biotech pcl. IDA Business Park, Bray, Co. Wicklow Ireland) following the manufacturers package insert instructions.

### 5.9.5 HIV serology

Serum samples were screened for anti-HIV antibodies by Behring Enzygnost anti-HIV-1/2 plus ELISA (Behringwerke, Germany). Reactive samples were further tested by Wellcozyme anti-HIV-1 ELISA (Murex Diagnostic Ltd., Darford, UK) in an alternative confirmatory strategy [90]. Samples reactive on both assays were considered as HIV antibody seropositive. Those non-reactive on the first ELISA were considered as HIV antibody seronegative [90]. Discordant samples were subjected to Western blot for confirmation and interpreted according to WHO criteria [91].

## 5.10 Diagnostic criteria

Diagnosis of bacterial vaginosis, vaginal candidiasis and gonorrhoea were based on the following criteria:

*Bacterial vaginosis*: This was based on the Amsel's criteria; 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 a wet preparation. Where features required for Amsel's criteria were incomplete, the Nugent's criteria scoring system was used and seven or higher score was taken as positive for BV. It has been reported that the sensitivity and specificity of Amsel's criteria are comparable with that of Nugent criteria [92].

*Candidiasis*: Due to the presence of *Candida* species in the vagina as normal flora, the diaginosis of candidal infection was based on presence of curd-like vaginal discharge

during physical examination and demonstration of pseudohyphae on microscopic examination or isolation of *Candida* species on culture.

*Gonococcal infection*: Finding of intracellular Gram negative diplococci in cervical smear and /or positive culture on cervical swab on selective media were used for diagnosis of gonorrhea.

### 5.11 Definition of term

- Vagina douching: participant reporting rinsing or cleaning inside the vagina by using water or other liquids.
- Vagina drying: participant reporting insertion of material inside the vagina before or during sex to remove or reduce secretions.
- Lower level of education: education level reported by participant from none up to primary school level.
- Higher level of education: education level reported by participant who has attained secondary school level or above.

### 5.12 Analyses of data

Data obtained from the questionnaires and laboratory results were double- entered in SPSS for windows version 10.0 [93] then validated using Epi Info version 6.04, [94]. Simple descriptive frequency of variables was determined followed by comparison of proportions of selected variables using chi- square ($\chi 2$) test. Two tailed level of

significance was taken at $p \leq 0.05$. The 95% confidence interval (95% CI) around proportions, were set using the Fleiss quadratic approximation formula [94].

## 6.0 ETHICAL CONSIDERATIONS

This study was part of TANSWED HIV programme. Ethical clearance to undertake this study was obtained from MUCHS higher degrees ethical committee in Dar es Salaam, the Ministry of Health, National Institute of Research (NIMR) and Karolinsika Institute in Sweden. Written informed consent was obtained from each participant before enrolment into the study. Participants were provided with free treatment following national guideline on syndromic management of STIs, and counseling for HIV testing. Those found to be HIV seropositive were referred to Clinic for Infectious Disease (CID) at MNH which provides care for HIV/AIDS patients.

## 7.0 RESULTS

### 7.1 Socio-demographic characteristics

Three hundred twenty six women presenting with vaginal discharge were included in the study. The median age was 26 years ranged from 16 to 63 (SD 6.9 years). Majority of these women 280/326 (85.9%) were aged up to 34 years. About 50% of all participants were single while 43.8% were married. 151 (46.3%) were unemployed or housewives. Among participants 43.2% had education level of secondary school or above, while 3.1% had not attended any formal education (Table: 1)

**Table 1: Distribution of socio-demographic characteristics of the study participants**

| Variable | Frequency (n = 326) | Percentage (%) |
|---|---|---|
| *Age (years)* | | |
| <25 | 121 | 37.1 |
| 25 – 34 | 159 | 48.8 |
| >34 | 46 | 14.1 |
| *Marital status* | | |
| Single | 167 | 51.2 |
| Married/cohabiting | 142 | 43.6 |
| Previous married | 17 | 5.2 |
| *Occupation* | | |
| Unemployed/housewife | 151 | 46.3 |
| Petty business | 79 | 24.2 |
| Employed/skilled labour | 59 | 18.1 |
| Student | 37 | 11.3 |
| *Education level* | | |
| None | 10 | 3.1 |
| Primary | 175 | 53.1 |
| Secondary | 108 | 33.1 |
| Post secondary | 33 | 10.1 |

## 7.2 Clinical examination findings

Physical examination findings for features suggestive of RTIs are summarized in table 2. Fifty seven participants (17.5%) apart from having genital discharge they were also found to have genital ulcers. When the vaginal discharge was characterized, 59.4%, 14.6% and 13.9% of participants had whitish, yellowish and mixed discharge respectively. Regarding the amount of vaginal discharge, 47.5% had moderate amount, 16.6% had profuse while 33.8% had scanty amount of discharge. On speculum examination, 39.0% of participants had cervical erytherma, while 22.7% had obvious cervical pus discharge. Bimanual examination revealed that 125 out of 288 (43.4%) participants had tenderness.

## 7.3 Prevalence of RTIs and HIV infection

Although the numbers of participants were 326, some laboratory tests could not be performed on all patients, therefore the number of tests performed varies in different diagnosis. BV were the most prevalent RTI in this population of women presenting with vaginal discharge affecting 115 out of 279 (41.2 %) followed by vaginal candidiasis. Only 3 out 176 (1.7%) of study participants were found to have *C. trichomatis* infection and 79 out of 297 (26.6%) were HIV seropositive (Table: 3).

**Table 2: Findings on clinical examinations of the study participants**

| Findings* | Frequency | Percentage (%) |
|---|---|---|
| *Vulva findings (n = 326)* | | |
| Normal | 269 | 82.5 |
| Ulcer | 57 | 17.5 |
| *Type of discharge (n = 323)* | | |
| Whitish | 192 | 59.4 |
| Yellowish | 47 | 14.6 |
| Curd-like | 39 | 12.1 |
| Mixed | 45 | 13.9 |
| *Quantity of discharge (n = 325)* | | |
| Profuse | 54 | 16.6 |
| Moderate | 161 | 47.5 |
| Scanty | 110 | 33.8 |
| *Features of cervicitis (n = 326)* | | |
| Erythema | 127 | 39.0 |
| Fragility | 83 | 25.5 |
| Pus discharge | 74 | 22.7 |
| *Bimanual examination ( n = 288)* | | |
| Tenderness | 125 | 43.4 |
| No tenderness | 163 | 56.6 |

** Total number of participants in different findings varied because information on three was missing and bimanual examinations were done on 288 women only.*

**Table 3: Prevalence of RTIs and HIV infection among study participants**

| | Total number tested* | Total positive (%) |
|---|---|---|
| Bacterial vaginosis | 279 | 115 (41.2) |
| HIV infection | 297 | 79 (26.6) |
| Candidasis | 323 | 55 (17.0) |
| Trichomoniasis | 323 | 26 (8.0) |
| Gonorrhoea | 318 | 11 (3.5) |
| Chlamydia | 176 | 3 (1.7) |

** Total number tested in different infection differed because some laboratory tests could not be performed on all study participants.*

### 7.4 Intra-vaginal practices

Of the 326 women interviewed, 267(81.9%) reported practicing douching (Table 4), among them 53.2% reported using water only. However 46.1% were commonly using material such as commercial antiseptic and soap that could change the vaginal chemical balance and microflora (Figure: 1). Data on the frequency and reasons for douching was available in 191 participants only, of which over 90% were douching more than once a week and for hygienic purposes. One hundred sixty four (50.3%) participants reported drying vagina before or during sex (Table: 4). Most of the participants who practiced dry sex used a piece of cloth (78%) (Figure: 2). More than 90% of 113 women were always drying the vagina before or during sexual intercourse (Table: 4). The commonest reason for dying was the desire to be clean (Figure: 3).

**Table 4: The prevalence, frequency and reason for intra-vaginal practices among the study participants**

| | Frequency | Percentage (%) |
|---|---|---|
| *Douching ( n = 326)* | | |
| Yes | 267 | 81.9 |
| No | 59 | 18.1 |
| *Frequency of douching (n = 191)* | | |
| More than once a week | 180 | 94.2 |
| Once or less a week | 11 | 5.8 |
| *Reason for douching (n = 191)* | | |
| For hygiene | 177 | 92.7 |
| For treating STI | 11 | 5.8 |
| Others | 3 | 1.6 |
| *Drying vagina before or during sexual intercourse (n = 326)* | | |
| Yes | 164 | 50.3 |
| No | 162 | 49.7 |
| *Frequency of drying vagina (n = 113 )* | | |
| Always | 102 | 90.3 |
| Sometimes | 10 | 8.8 |
| Rarely | 1 | 0.9 |

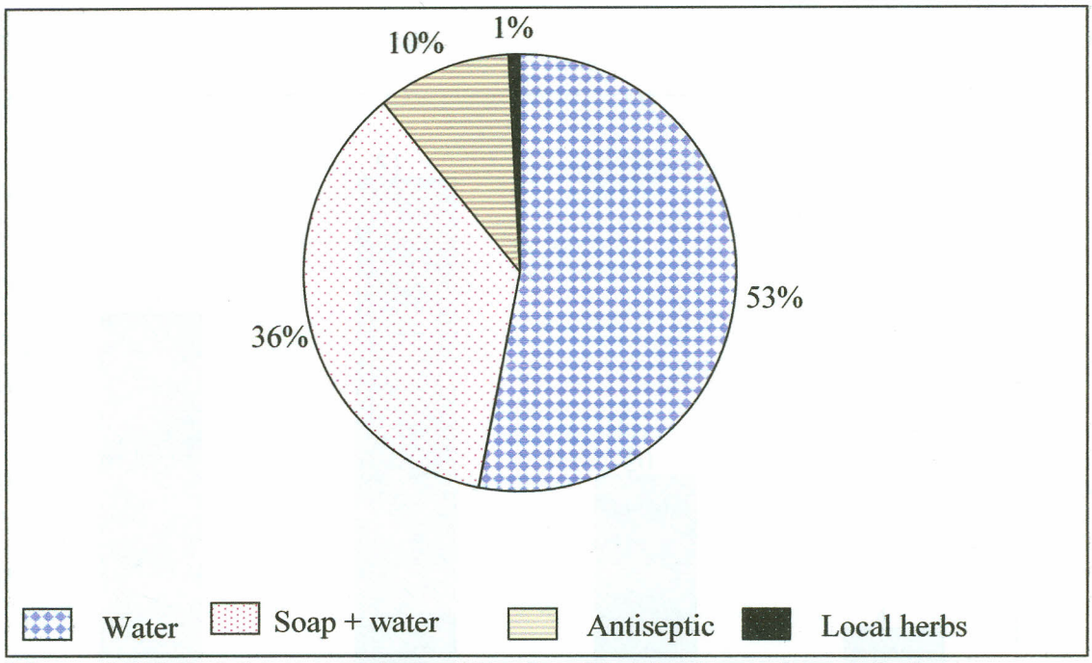



***Figure 1: Materials commonly used for douching by the study population***

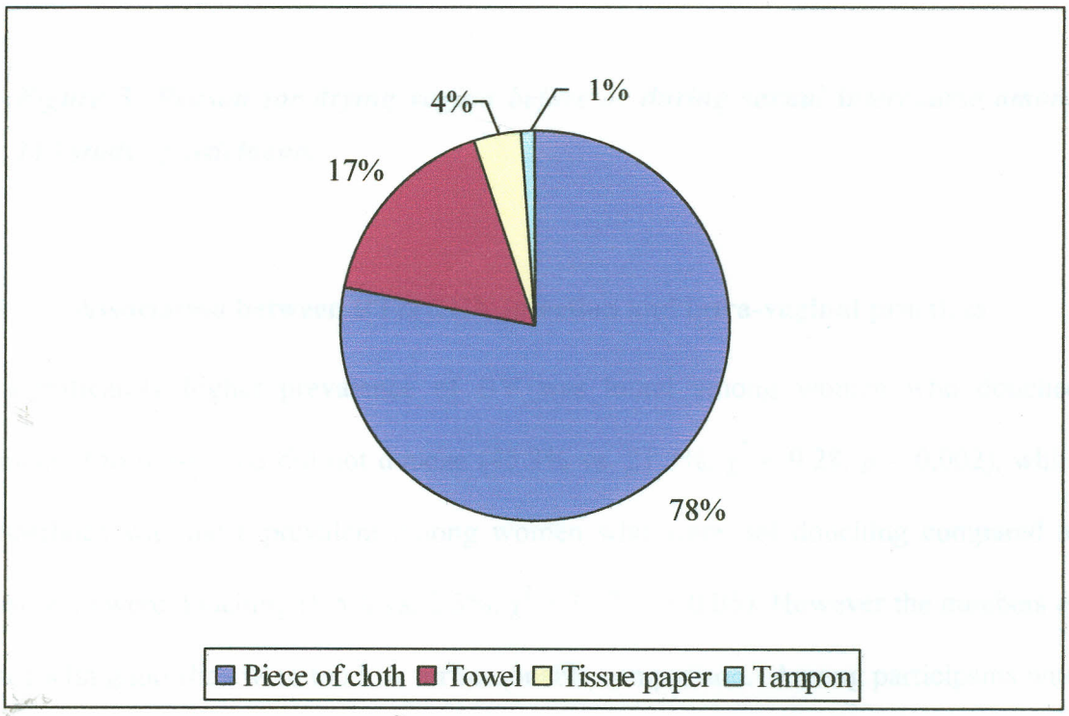



***Figure 2: Materials commonly used by the study participants for drying vagina before or during sexual intercourse***

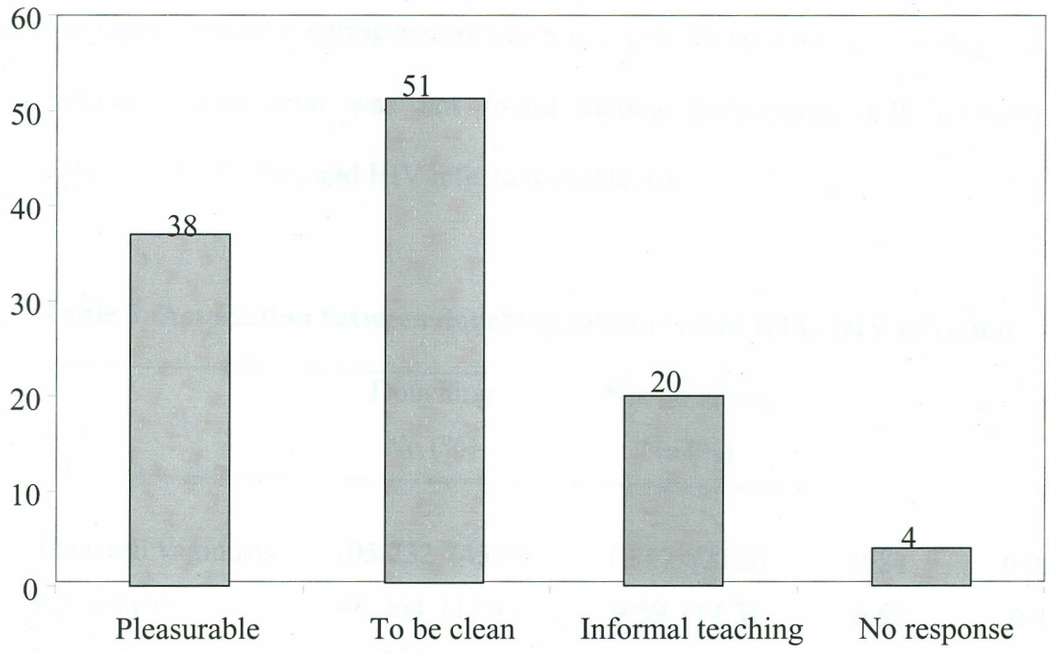



***Figure 3: Reason for drying vagina before or during sexual intercourse among 113 study participants***

## 7.5 Association between RTIs /HIV infection and intra-vaginal practices

A significantly higher prevalence of BV was found among women who douched compared to those who did not douche (45.3% vs. 21.3%, $\chi^2 = 9.28$, $p = 0.002$), while gonorrhoea was more prevalent among women who were not douching compared to those who were douching (8.5% vs. 2.3%, $\chi^2 = 3.77$, $p = 0.05$). However the numbers of cases with gonorrhea were too low for reasonable comparison. Among participants with candidasis, trichomoniasis, chlamydial infection, and HIV infection there were no significant difference between douchers and non douchers (Table: 5)

A significantly higher prevalence of trichomoniasis was found among women who dried the vagina before or during sexual intercourse (11.7% vs. 4.4%, $\chi^2 = 5.78$, $p = 0.02$). Similarly association was not found among participants with candidiasis, BV, chlamydial infection, and HIV infection (Table: 6).

**Table 5: Association between douching practices and RTIs/HIV infection**

| | Douching No (%) | Not douching No (%) | $\chi^2$ | $p$ - value |
|---|---|---|---|---|
| Bacterial vaginosis | 105/232 (45.3) | 10/47 (21.3) | 9.28 | 0.002 |
| Candidasis | 46/264 (17.4) | 9/59 (14.3) | 0.52 | 0.47 |
| Gonorrhoea | 6/259 (2.3) | 5/59 (8.5) | 3.77 | 0.05 |
| Chlamydia | 3/167 (1.8) | 0/9 | * | * |
| Trichomoniasis | 18/264 (6.8) | 8/59 (13.6) | 2.12 | 0.15 |
| HIV infection | 71/247 (28.7) | 8/50 (16.0) | 3.46 | 0.06 |

**Comparison was not done due to zero proportion on one of the groups*

**Table 6: Association between vagina drying practices before or during sexual intercourse and RTIs/HIV infection**

| | Drying vagina No (%) | Not drying No (%) | $\chi^2$ | *p* - value |
|---|---|---|---|---|
| Candidasis | 29/163 (17.8) | 26/160 (16.3) | 0.14 | 0.71 |
| Gonorrhoea | 6/161 (3.7) | 5/157 (3.2) | 0.07 | 0.79 |
| Chlamydia | 2/95 (2.1) | 1/81 (1.2) | 0.02 | 0.89 |
| Trichomoniasis | 19/163 (11.7) | 7/160 (4.4) | 5.78 | 0.02 |
| Bacterial vaginosis | 53/137 (38.7) | 62/142 (43.7) | 0.53 | 0.47 |
| HIV infection | 44/151 (29.1) | 35/146 (24.0) | 1.01 | 0.31 |

### 7.6 Association between RTIs/HIV infection and socio-demographic characteristics

The association between *T. vaginalis* infection and socio-demographic characteristic is shown on table 7. Marital status and education level were significantly associated with *T. vaginalis* infection. Women previously married were significantly more likely to be found with *T. vaginalis* infection compared to those who were single and currently married ($p < 0.001$). While those women with lower level of education were significantly more likely to be found with this infection compared to those with higher education ($p = 0.003$)

**Table 7: Association between *Trichomonas vaginalis* infection and participants' socio-demographic characteristics**

| Variable | Total tested | Positive (%) | 95% CI | *p*-value |
|---|---|---|---|---|
| *Age group ( years)* | | | | |
| <25 | 121 | 9 (7.4) | 3.7-14.0 | |
| 25-34 | 157 | 11(7.0) | 3.7-12.5 | 0.4 |
| >34 | 45 | 6 (13.3) | 5.5-27.5 | |
| *Marital status* | | | | |
| Single | 165 | 11(6.7) | 3.5-11.9 | |
| Married/cohabiting | 141 | 9 (6.4) | 3.1-12.1 | <0.001 |
| Previous married | 17 | 6 (64.7) | 15.3-61.4 | |
| *Occupation* | | | | |
| Student | 37 | 1 (2.7) | 0.1-15.8 | |
| Unemployed | 149 | 12 (8.1) | 4.4-13.9 | |
| Petty business | 78 | 10 (12.8) | 6.7-22.8 | 0.21 |
| Employed/skilled labor | 59 | 3 (5.1) | 1.3-15.1 | |
| *Education* | | | | |
| Lower level | 183 | 22 (12.0) | 7.8-17.8 | |
| Higher level | 140 | 4 (2.9) | 0.9-7.6 | 0.003 |

There was a significant association between occupation and BV. Participants engaged in petty business were more likely to have BV compared to employed/skilled labor ($p < 0.001$) Different age group, marital status, and education level were not associated with BV (Table:8).

**Table 8: Association between bacterial vaginosis and participants' socio-demographic characteristics**

| variable | Total tested | Positive (%) | 95% CI | *p*-value |
|---|---|---|---|---|
| *Age group ( years)* | | | | |
| <25 | 107 | 40 (37.4) | 28.4-47.3 | |
| 25-34 | 134 | 62 (46.3) | 37.7-55.1 | 0.243 |
| >34 | 38 | 13 (34.2) | 20.1-51.4 | |
| *Marital status* | | | | |
| Single | 144 | 55 (38.2) | 30.3-46.7 | |
| Married/cohabiting | 118 | 51 (43.2) | 34.2-52.7 | 0.4 |
| Previous married | 17 | 9 (52.9) | 28.5-76.1 | |
| *Occupation* | | | | |
| Student | 32 | 9 (28.1) | 14.4-47.0 | |
| Unemployed | 132 | 57 (43.2) | 34.7-52.1 | 0.001 |
| Petty business | 66 | 38 (57.6) | 44.8-69.4 | |
| Employed/skilled labor | 49 | 11(22.4) | 12.2-37.0 | |
| *Education* | | | | |
| Lower level | 161 | 68 (42.2) | 34.6-50.3 | |
| Higher level | 118 | 47 (39.8) | 31.1-49.3 | 0.7 |

There was no significance association between socio-demographic characteristics and Candida, gonorrhea and Chlamydia infections (Table 9, 10, 11 respectively)

**Table 9: Association between vaginal candidiasis and participants' socio-demographic characteristics**

| variable | Total tested | Positive (%) | 95% CI | *p*-value |
|---|---|---|---|---|
| *Age group ( years)* | | | | |
| <25 | 121 | 24 (19.8) | 13.3-28.3 | |
| 25-34 | 157 | 24 (15.3) | 10.2-22.1 | 0.6 |
| >34 | 45 | 7 (15.6) | 7.0-30.1 | |
| *Marital status* | | | | |
| Single | 165 | 32 (19.4) | 13.8-26.4 | |
| Married/cohabiting | 141 | 20 (14.2) | 9.1-21.3 | 0.5 |
| Previous married | 17 | 3 (17.6) | 4.7-44.2 | |
| *Occupation* | | | | |
| Student | 37 | 9 (24.3) | 12.3-41.6 | |
| Unemployed | 149 | 24 (16.1) | 10.8-23.2 | |
| Petty business | 78 | 8 (10.3) | 4.8-19.7 | 0.12 |
| Employed/skilled labor | 59 | 14 (23.7) | 14.0-36.9 | |
| *Education* | | | | |
| Lower level | 183 | 25 (13.7) | 9.2-19.7 | |
| Higher level | 140 | 30 (21.4) | 15.1-29.3 | 0.07 |

**Table 10: Association between gonorrhea and participants' socio-demographic characteristics**

| variable | Total tested | Positive (%) | 95% CI | *p*-value |
|---|---|---|---|---|
| *Age group ( years)* | | | | |
| <25 | 119 | 3 (2.5) | 0.7-7.7 | |
| 25-34 | 155 | 7 (4.5) | 2.0-9.4 | 0.6 |
| >34 | 44 | 1(2.3) | 0.1-13.5 | |
| *Marital status* | | | | |
| Single | 162 | 5 (3.1) | 1.1-7.4 | |
| Married/cohabiting | 139 | 6 (4.3) | 1.8-9.6 | 0.6 |
| Previous married | 17 | 0 | 0.0-22.9 | |
| *Occupation* | | | | |
| Student | 36 | 0 | 0.0-12.0 | |
| Unemployed | 146 | 4 (2.7) | 0.9-7.3 | |
| Petty business | 78 | 6 (7.7) | 3.2-16.6 | 0.1 |
| Employed/skilled labor | 58 | 1 (23.7) | 0.1-10.5 | |
| *Education* | | | | |
| Lower level | 180 | 6 (3.3) | 1.4-7.4 | |
| Higher level | 138 | 5 (3.6) | 1.3-8.7 | 0.9 |

**Table 11: Association between Chlamydia infection and participants' socio-demographic characteristics**

| variable | Total tested | Positive (%) | 95% CI | *p*-value |
|---|---|---|---|---|
| *Age group ( years)* | | | | |
| <25 | 76 | 1 (1.3) | 0.1-8.1 | |
| 25-34 | 79 | 2 (2.5) | 0.4-9.7 | 0.7 |
| >34 | 21 | 0 | 0.0-19.2 | |
| *Marital status* | | | | |
| Single | 93 | 0 | 0.0-4.9 | |
| Married/cohabiting | 70 | 3 (4.3) | 1.1-12.8 | 0.09 |
| Previous married | 13 | 0 | 0.0-28.3 | |
| *Occupation* | | | | |
| Student | 19 | 0 | 0.0-20.9 | |
| Unemployed | 77 | 1 (1.3) | 0.1-8.0 | 0.35 |
| Petty business | 43 | 2 (4.7) | 0.8-17.1 | |
| Employed/skilled labor | 37 | 0 | 0.0-11.7 | |
| *Education* | | | | |
| Lower level | 99 | 1 (1.0) | 0.1-6.3 | |
| Higher level | 77 | 2 (2.6) | 0.5-9.9 | 0.42 |

The association between HIV seropositivity and socio-demographic characteristics is summarized in table 12. Occupation and level of education were significantly associated with HIV seropositivity. Women doing petty business and unemployed/housewives had significantly higher prevalence of HIV infection compared to employed /skilled labor group ($p = 0.001$). The prevalence of HIV infection in participants with lower education was more than twice that of participants with higher education level ($p < 0.001$)

**Table 12: Association between HIV infection and participants' socio-demographic characteristics**

| variable | Total tested | Positive (%) | 95% CI | *p*-value |
|---|---|---|---|---|
| *Age group ( years)* | | | | |
| <25 | 116 | 30 (25.9) | 18.4-34.9 | |
| 25-34 | 140 | 35 (25.0) | 18.2-33.2 | 0.5 |
| >34 | 41 | 14 (34.1) | 20.6-50.7 | |
| *Marital status* | | | | |
| Single | 153 | 36 (23.5) | 17.2-31.2 | |
| Married/cohabiting | 127 | 35 (27.6) | 20.2-36.3 | 0.1 |
| Previous married | 17 | 8 (47.1) | 23.9-71.5 | |
| *Occupation* | | | | |
| Student | 34 | 5 (14.7) | 5.5-31.8 | |
| Unemployed | 135 | 43 (31.9) | 24.3-40.5 | |
| Petty business | 73 | 26 (35.6) | 25.0-47.8 | 0.001 |
| Employed/skilled labor | 55 | 5 (9.1) | 3.4-20.7 | |
| *Education* | | | | |
| Lower level | 169 | 59 (34.9) | 27.8-42.7 | <0.001 |
| Higher level | 128 | 20 (15.6) | 10.0-23.3 | |

## 7.7 Association between RTIs and HIV infection

Prevalence of RTIs stratified by HIV serostatus is shown in table 13. Prevalence of BV and trichomoniasis, were significantly higher among HIV seropositive compared to HIV seronegative participants. There was no significant difference between the presence of gonorrhea and Chlamydia infection among HIV seropositive and HIV seronegative individuals.

**Table 13: RTIs prevalence among participants with genital discharge by HIV serostatus***

| | HIV seropositive No (%) | HIV seronegative No (%) | $\chi^2$ | *p*- value |
|---|---|---|---|---|
| Bacterial vaginosis | 37/64 (57.8) | 67/188 (35.6) | 9.69 | <0.01 |
| Trichomoniasis | 10/79 (12.7) | 12/216 (5.6) | 4.23 | 0.04 |
| Candidiasis | 9/79 (11.4) | 46/216 (21.3) | 3.74 | 0.05 |
| Chlamydia | 2/39 (5.1) | 1/133 (0.8) | $\Phi$ | $\Phi$ |
| Gonorrhoea | 3/76 (3.9) | 8/214 (3.7) | 0.07 | 0.79 |

**Denominators differ because laboratory tests could not be performed on some study participants.* $^{\Phi}$ *Comparisons were not done due to very small proportion of one groups*

## 8.0 DISCUSSION

Different etiological agents are involved in causing vaginal discharge which is a common presenting symptom among patients with reproductive tract infections. Reports from different parts of the world have shown wide spread intra-vaginal practices and associate these practices with some RTIs including HIV infection. There is paucity of information of these practices in our society. This cross sectional study sought to determine the microbial causative agents, intra-vaginal practices and their association among women with genital discharge attending reproductive health clinic in Dar es Salaam.

Women who participated in the study had varied socio-demographic characteristics covering a wide range of these characteristics though majority were of middle age, low education, unmarried, and unemployed, which reflect population of low socioeconomic status. This study found high infection rates of some RTIs, HIV infection and high prevalence of intra-vaginal practices among participating women. BV was the RTI with the highest prevalence (41.2%) seen among participants. Similar results have been reported elsewhere ranging from 24 to 37% in women attending STI clinics [95].

The prevalence of typical STIs associated with vaginal discharge like gonorrhoea and Chlamydia was found to be low as compared to previous finding of 4% gonorrhoea infection and 5% Chlamydia infection among women in Tanzania [89, 96]. BV which was found to have the highest prevalence in the studied population is not a common

sexually transmitted infection, but rather indicate a higher rate of disturbance of normal genital microbial flora that lead to proliferation of pathogenic organisms than exposure to STIs.

The low prevalence of Chlamydia and gonorrhea infections in this population may be a reflection of decline of these infections or could be masked by other factors related to poor detection of organisms from specimens due to local genital practices. If the decline is real, it has to be interpreted in the context of an increasing use of public facilities for health care, and in the context of increasing diagnosis and treating STIs because of an extensive training programme of health workers in syndromic STI management in the country. The other possible explanation for decline of gonorrhea and Chlamydia is behavioral change in the community by increased condom use [97].

Majority of sexually transmitted infections are asymptomatic in women, ranging from 60 to 70% of gonococcal and chlamydial infections. Due to asymptomatic nature of these infections women tend not to seek treatment in STI clinics, which may lead to low prevalence of these infections among women attending STI clinics. In contrast other studies conducted in antenatal and family planning clinics, have reported high prevalence of similar infections [78, 89, 96]. However due to the setting of these studies possibilities of detecting asymptomatic cases is high.

The current seroprevalence of HIV found in this population has remained high (26.6%),

which is more than two fold the current seroprevalence among antenatal care attendees (12.8%) in the same region [98]. The finding of seroprevalence at that level does not surprise because the population studied is one among the high risk groups for HIV infection.

This study has confirmed that intra-vaginal practices, douching and dry sex are widespread practices among studied population. Findings of 81.9% of the women reporting vaginal douching and 50.3% reporting commonly drying their genitalia before or during sexual intercourse is an indication of a widespread of this practices in the studied population. These findings are comparable with what has been reported in other studies among pregnant women and other women attending STI clinics in Africa [68, 81, 85].

The main reason for douching found in this study was personal hygiene and most women used water for that purpose. Very few women (5.8%) reported douching because of presence of STIs, but published literature has shown that the use of vaginal products for the treatment of vaginal symptoms is widespread [85, 99]. These findings reflect the wide variation of specific preparations used for douching, most likely according to local cultural factors. In several African settings, herbs and dry leaves have been reported to be used for treatment of vaginal infections or for a "tightening effect" to achieve dry sex [83, 100].

The different purposes for douching reported in different studies can explain the

difference in materials used for douching in the current study compared to other communities studied [70, 85, 100]. Because majority of women in this study were douching for hygienic purposes, that could be the reason why water or water with soap were the commonest materials used for the practice and commercial products were rarely used. This study has found as several other studies in Africa and globally that vaginal douching is a routine practice among commercial sex workers, pregnant women, adult women and adolescents [68, 101, 69, 102]. Hence, understanding of factors associated with this practice and the effects of douching on genital infections may have implications for a large proportion of women in the world population.

Substantial proportion of women in this study was normally drying their vagina before or during sexual intercourse, the practices intended to decrease vaginal secretions to attain 'dry sex'. The prevalence of drying vagina for dry sex found in this study is comparable with finding from another study in Africa. A cross-sectional study conducted in a sexually transmitted diseases clinic in Zambia found that 50% of women had engaged in at least one dry sex practice [81].

Majority of women in this study were drying the vagina using a piece of cloth, which is different from what other studies have reported. One study in Zambia found that majority were drinking 'porridge' a liquid or suspension containing local herbs [81]. Use of local herbs also has been reported by studies done in other societies in Africa [79-82]. It is believed that local herbs help to dry secretions and more importantly make the

genital organ tighter [79]. The difference in materials used could be explained by the different reason perceived by women for drying vagina. Majority of women (45.1%) in this study population were commonly dying the vagina because they perceived that removing secretions led to a cleaner sexual intercourse. In populations where local herbs are commonly used, women report use of vaginal dying agents for tightening and thus enhancing their partner's sexual pleasure [79-82].

The current study has shown a significant association between douching with increased prevalence of BV and also drying vagina before or during sex with increased prevalence of *T. vaginalis* infection. Women who were douching were significantly found to have BV than those who did not douche. To establish a cause and effect relation is not possible with cross sectional nature of this study. Women with BV symptoms may douche more often to reduce the amount of foul smelling discharge. But it is also possible that douching may lead to BV by disturbance of vaginal chemical balance and microbial normal flora.

The finding of association between douching and BV is consistent with results from other studies [103, 104]. A case-control study has shown that douching accounts for a two-fold increase in the risk of BV [103]. A study among adolescent women found that douching after menses was strongly correlated with BV [104]. The finding in a prospective cohort study that acquisition of BV is associated with douching makes a

causal relation more likely [72]. In the studied population, women who did not douche were more likely to have gonorrhea as compared with those who were douching. It is not known whether douching reduces transmission of gonorrhea or is associated with poor detection of gonorrhea from the specimen. However the number of cases found with gonorrhea 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 this organism.

There was no association between douching or drying vagina and seroprevalence of HIV infection in the current study contrary to what other studies have reported [85, 100]. The potential mechanism whereby vaginal douching could possibly enhance HIV transmission is through irritation of the vaginal mucosa, thus promoting the proliferation of lymphocytes which are target cells for HIV [105]. For the case of drying vagina increased risk of HIV infection could be due to dehydration of the vaginal mucosa, rendering the vaginal epithelium more vulnerable to local trauma [106]. Due to the fact that majority of women in this study were using water only for douching, there is high likelihood of not finding a direct association because water alone is unlikely to cause mucosal irritation.

There is considerable evidence from other studies that the presence of BV has a role in the acquisition of HIV [107]. A study in Kenya found sex workers who douched were

more likely than those who do not douche to have BV, which in turn was associated with an increased risk of HIV infection [108]. Therefore, failure to find a direct association between douching practices and HIV seroprevalence, can not rule out the indirect relationship for facilitating HIV transmission.

The association of douching with RTIs mostly depends on the material used. The current study did not determine the association of specific material used for douching with a specific RTI. In this regard there is a possibility that different materials could have been used at different times. Some studies have found that women who always douched with local herbs or commercial agents had a substantially increased risk for having sexually transmitted infections [71]. Study in Cote d'Ivoire among pregnant women found that women using water douches had increased risk of *Ureaplasma urealyticum* infection and increased *C trachomatis* were associated with use of antiseptic solutions [68].

Other reproductive conditions that are associated with STIs have also been found to be increased in women who douche. These include the presence of PID [76, 109,-110] and ectopic pregnancies [76, 77]. This study did not determine the complications associated with STIs but studies referred above comprised predominantly women in industrialized countries, where most vaginal cleansing involved the instillation of a fluid into the vagina, often under some force with a bag applicator which easily may force pathogens to upper genital reproductive tract. These risks may or may not apply to genital cleansing

performed with the water or soap on the finger the technique performed by most women in our society.

The development of effective prevention efforts against HIV and RTIs 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 STIs [85]. The emphasis on dryness may influence women's willingness to use vaginal microbicides as well. In this studied population where 50% value a dry vagina during sexual intercourse, the lubricating qualities of microbicides may preclude overt use.

The current study found some of socio-demographic characteristics were associated with increased risk of acquiring RTIs and HIV infection. There was increased risk of *T. vaginalis* infection among women previously married and those with lower level of education, increased risk of BV among women engaged in petty business and increased risk of HIV infection among women engaged in petty business and unemployed/housewife and those with lower education level. Generally the association of RTIs and HIV found showed that the women with low socio-demographic characteristics are more likely to have some infections compared with those with higher

level socio-demographic characteristics. The relationship found is difficult to explain but it is understandable that the risks of acquiring specific infection depend on combination of various demographic factors and sexual behavioral practices.

There was a strong correlation between HIV infection and bacterial vaginosis and trichomoniasis. Current infection with *N. gonorrhoeae*, *C. trachomatis*, was not associated with increased seroprevalent HIV infection in this cross sectional study. Women with bacterial vaginosis, a recurrent imbalance of the vaginal microbial flora, were more likely to be HIV infected. The reasons for this association, which has also been found in other studies, [110-112] remain unclear. However the alteration of vaginal environments with a high pH and a lack of $H_2O_2$, found in bacterial vaginosis may predispose to HIV infection.

There are some limitations in the current study. To know that participants interviewed were douching or drying vagina before or during sexual intercourse depended on the response of the particular woman. Although interviewers were trying to explain what was meant by vaginal douching, it is possible that our definition of vaginal douching might not mean what actually some women understood. For example vulva washing by just separating labia apart, could probably be confused by some participants as douching which will exaggerate the prevalence.

To establish *Candida* species as the causative agent of vaginitis can be difficult because

as many as 50% percent of asymptomatic women have *Candida* species as part of their endogenous vaginal flora [113]. The current study restricted the diagnosis of candidiasis to include isolating *Candida* species on culture and presence of curd-like discharge, the later may be subjective. The criteria used might also underestimate the prevalence of infection by not including those with infection but the discharge is not clearly curd-like. However by basing on culture findings one could overestimate the prevalence of infection by including isolation of normal flora.

Lastly microscopic examination for detection *T. vaginalis* infection which was the only one method used has been reported to have a low sensitivity of about 60% [26], and thus underestimates the prevalence of this infection in the population studied.

## 9.0 CONCLUSION AND RECOMMENDATIONS

Prevalence of BV and HIV infection among women studied is high. The intra-vaginal practices (douching and drying vagina) among women attending reproductive health clinic is widespread, and douching is significantly associated with BV. Women with low socio-demographic characteristic are at more risk of RTIs and HIV infection and effort should be put to target such women in the effort to control such infections.

The findings of this study should prompt women to reconsider the practice of douching and drying the vagina due to their possible influence on RTIs and their implications for the new advocated method of HIV prevention interventions using microbicides. These observations reveal the need for providing proper counseling and health education to patients with RTIs on intra-vaginal practices besides treatment to control and prevent these infections. Further research is required to better understand the complex relation between douching, risk for BV, HIV infection, and other RTIs.

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