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Survey of possible pathogenic organisms found in urine and vaginal swab samples of selected female population in Lagos, Nigeria

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ABSTRACT

Urinary Tract Infection (UTI) is caused by the presence and growth of microorganisms and females are more at risk of the infection. This survey was carried out to determine the prevalence of pathogenic organisms other than bacteria in urine and vaginal swabs of some selected female individuals and to determine the suitable sample material appropriate for identification of such organisms. Samples were collected from 200 female students of Yabatech and 200 patients attending General Hospital Lagos Island. The female students' and patients' age ranged from 16-55 years. Questionnaires were also administered. A total of 400 samples were collected and examined microscopically and also cultured for organisms' growth. The organisms encountered were Yeast cells, Pus cells and *Trichomonas vaginalis*. 5(2.5%) positive samples with *Trichomonas vaginalis* were detected among the examined patients while none of the students were found infected with *Trichomonas vaginalis*. Yeast cells were the most frequently encountered organism while *Trichomonas* were the least encountered. The vaginal swab samples yielded more organisms than urine samples. Age group 31-35 years were observed to have the highest percentage growth rate of cultured organisms with students having 87.5% while the patients had 48.8%. Vaginal discharge was the commonest symptom identified among the examined individuals with 68(34.0%) responses. No statistical significance was observed among results obtained for both students and patients. Sex education should be introduced as a means of increasing students' awareness of infections and proper sanitation enforced by the institution.

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Keywords: UTI, *Trichomonas*, Yeast cells, Pyuria, Vaginitis, Hygiene.

INTRODUCTION

Urine, a liquid by-product of the body is usually inspected and examined by many physicians for medical purposes, the color and other attributes of urine serves as indicator of certain diseases. The presence of certain organisms in the urine is linked to urinary infection in humans. Yeastlike organisms when discovered in the urine signify infection of either the upper or lower urinary tracts,

colonization of the bladder, or contamination of the urine sample. Many yeast infections are superficial in nature, but some may represent life-threatening, invasive disease (Kauffman et al., 2011). The presence of Pus cells in urine (pyuria) is a definite indication of some infection in the lower or upper urinary tract. The organisms responsible for genital infections are generally fastidious and often difficult to culture (Ronald and Alfa, 1996).

Urinary Tract Infection (UTI) is described as the microbial invasion of any tissues of the urinary tract. The number of urine samples in comparison with the other specimens in medical microbiology laboratories is very large (Behzadi et al., 2010). Women are more prone to urinary tract infections. Organisms such as *T. vaginalis* infects the urethra as well as the vagina, as such, urine represents potential source for microscopic examination (Wallin et al., 1981). Sexually transmitted diseases (STI) and reproductive tract infections often present with vaginal discharge (Leon et al., 2009). The incidence of abnormal vaginal discharge in female of reproductive age is found to be the commonest problem in clinical medicine and gynecological.

Sexually transmitted infections (STI) are worldwide public health problem, with most infections affecting the reproductive system causing reproductive tract infections. The implicated organisms in the transmission of sexually transmitted infections belong to the group bacteria, viruses, yeast and protozoa (Oyewole et al., 2010). More than 95% of UTI cases are caused by Bacteria, which are the major causative organisms for UTI (Amaeze et al., 2013). The infection is common among sexually active males and females especially during the child bearing age (Egberé et al., 2009). These infections continue to present major health, social, and economic problems in the developing world. The prevalence rates apparently are far higher in developing countries where STI diagnostic tests are not easily available. Most of the sexually transmitted infection are prevalent in Nigeria and constitute one of the major public health problems (Okonko et al., 2012).

The most frequently seen STI include chlamydia, gonorrhoea, and syphilis which are bacterial infection, Viral STIs include genital herpes, AIDS, while Parasitic STIs include trichomoniasis. Trichomoniasis is a sexually transmitted infection caused by the protozoan parasite *Trichomonas vaginalis*, often referred to as the “neglected” sexually transmitted infection. It is the most common non-viral

sexually transmitted disease worldwide (Opara et al., 2009) and frequently encountered among 50% of women complaining of abnormal pain, vaginal discharge and itching. The infection often leads to vaginitis, cervicitis in women and urethritis in men and acute inflammatory disease of the genital mucosa, urinary tract, fallopian tubes and pelvic (Egberé et al., 2009). It may be asymptomatic, symptoms of infection includes frothy, yellowish green vaginal discharge with a strong odour, lower abdominal pain, low birth weight infants, preterm labor, predisposition to cervical cancer, atypical pelvic inflammatory disease, infertility, itching and burning sensation in women, urethral discharge in men, complication in the fetus and newborn (Valadkhani et al., 2010). Trichomoniasis is associated with important public health problems which including amplification of Human Immunodeficiency virus (HIV) transmission.

The estimated annual incidence of Trichomoniasis worldwide exceeds that of chlamydia and gonorrhoea combined, yet, there are no established screening, surveillance, or control programs for infected individuals (Van der Pol, 2007). The World Health Organization (2000) estimated that 180 million cases of Trichomonal infection are acquired annually worldwide (Johnston and Mabey, 2008). In tropical Africa, a prevalence rate among the female patients range between 5-37% (Bakare et al., 2002). In Nigeria, there are several documented reports on the prevalence of *Trichomonas vaginalis*. The prevalence of this infection depends on factors such as age, sexual activity, and number of sex partners (Leon et al., 2009).

Various studies have been establish to undertaken the etiologic agent of abnormal vaginal discharge in the environment. Nwankwo et al. (2010) collected High vaginal specimens from 587 patients and the major pathogen found were *Candida albicans* 177(70.0%), *Trichomonas vaginalis* 14(5.5%) and *Gardnerella vaginalis* 62(24.5%). The

unmarried ladies were found to have the higher positive result and it was concluded that multiple sexual partners increase the rate of infection (Nwankwo et al., 2010). In Nigeria, where human immunodeficiency virus (HIV) seroprevalence still appears to be on the rise, increase attention to sexually transmitted diseases (STDs) ,awareness and treatment would likely help slow the epidemic. Cases identification, which is one of the two important components of sexually transmitted diseases (STD) control program, is extremely difficult in most parts of the world as these diseases are considered as social problem and frequently surrounded by prejudices and poor information. The most obvious approach to STI diagnosis is direct detection of microorganisms through the use of microscopy and appropriate staining or wet preparation to visualize pathogens. Other more sensitive methods such as Culture, antigen detection, or nucleic acid detection may have more complex technical requirements for optimal test performance and may increase the interval between testing and the availability of test results. Microscopy, particularly when performed while patients are present, may provide immediate results to guide management decisions. This work was designed to survey the prevalence of other possible pathogenic organisms apart from bacteria in urine and vaginal swabs of sampled individuals, to determine the suitable sample material appropriate for identification of such organisms and to assess the knowledge and perception of the selected individuals.

MATERIALS AND METHODS

Study areas and population

Two centers were used to obtain samples, the Health Center of Yaba College of Technology ,which is an institution owned by the Federal Government of Nigeria and the Parasitology Laboratory of General Hospital Lagos, which is owned by the Lagos State Government of Nigeria.

Administration of Questionnaire

Questionnaires were administered to students of Yabatech at the Medical Center to collect their socio-demographic information including age, area of residence, behavioral variables, marital status, type of water used in showering and information about sexually transmitted diseases. The data and information of the patients attending the General Hospital were also collected.

Specimen collection, transport, and preparation

Urine and Vaginal swab samples were collected from students and patients who gave their consent. Each subject was given a sterile plastic bottle to collect mid-stream urine after they were taught how to collect it aseptically; the collected samples were transported to the laboratory. Bar-coded numbers were used to ensure obscurity of students and patients, to facilitate laboratory procedure and minimize the chances of error during the handling of samples. Cotton swabs for wet mount examination were placed into test tube containing saline while the ones for culture were placed into broth medium.

Sample analysis

Wet mount preparations of the vaginal swabs and urine samples were examined under the microscope for the presence of organisms. In most cases, urine and vaginal swabs was examined within 30 minutes to 50 minutes of collection. Urine specimens were centrifuged, the supernatant were decanted and the sediment were examined under the microscopically at high magnification for pus cells, yeast-like cells, red blood cells, epithelial cells, crystals and *Trichomonas vaginalis*. Pus cells > 5 per high power field were also considered significant for infection (Houston, 1969).

The culture for organisms was carried out using Broth culture technique. Urine and vaginal samples were inoculated into separate Bijou bottles containing broth and incubated for 5 days at 37 °C. Cultures were evaluated

daily, by microscopic examination for up to 5 days. Incubator temperature was checked and recorded daily. All observations and results were documented.

Statistical analysis

Calculation to determine prevalence was done by dividing the proportion of persons with samples positive for organisms by the total number of individuals sampled. Descriptive statistics (Mean, Chi-Square) with SPSS package 2007 was used to analyze the data obtained, for all statistical tests in this study, $P < 0.05$ was considered significant.

RESULTS

A total of 200 female students had their urine and vaginal swabs examined. None of them had *Trichomonas vaginalis* infection (0.0%), 86(43.0%) had yeast cells and 23(11.5%) had pus cells when their vaginal swabs were examined (Table 1). However, when the urine samples were examined, 41(20.5%) had Yeast cells, 30(15.0%) had pus cells and none had *Trichomonas vaginalis* as shown in Table 2.

About 200 female patients from the General Hospital had their urine and vaginal

swabs equally examined. 5(2.5%) had *Trichomonas vaginalis* infection, 33(16.5%) had yeast cells and 19(9.5%) had pus cells from the examination of their vaginal swabs (Table 1). The examination of the urine samples revealed 5 patients (2.5%) infected with *Trichomonas vaginalis*, 30(15.0%) with yeast cells and 19(9.5%) with pus cells as shown in Table 2.

The urine samples and vaginal swabs collected from both students and patients were cultured to observe the growth. Students in the age group 26 - 30 had the highest observed growth from the culture sample while patients in the age group 36 - 40 had the highest observed growth (Table 3).

The questionnaire administered to students was used to assess symptoms observed by the sampled individuals. Vaginal discharge was the commonest symptom observed which was followed by itching and abdominal pain as shown in Table 4. Students belonging to age group 21-25 had the highest number of complaint for both vaginal discharge 25(28.4%) and itching 20(22.7%).

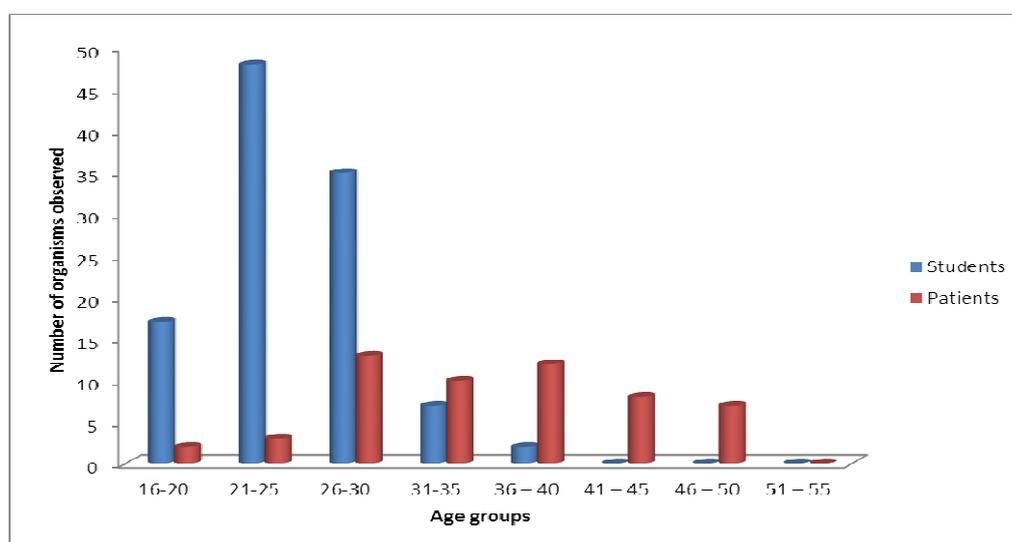


Figure 1: Total number of Organisms observed from vaginal swabs of both Students and Patients.

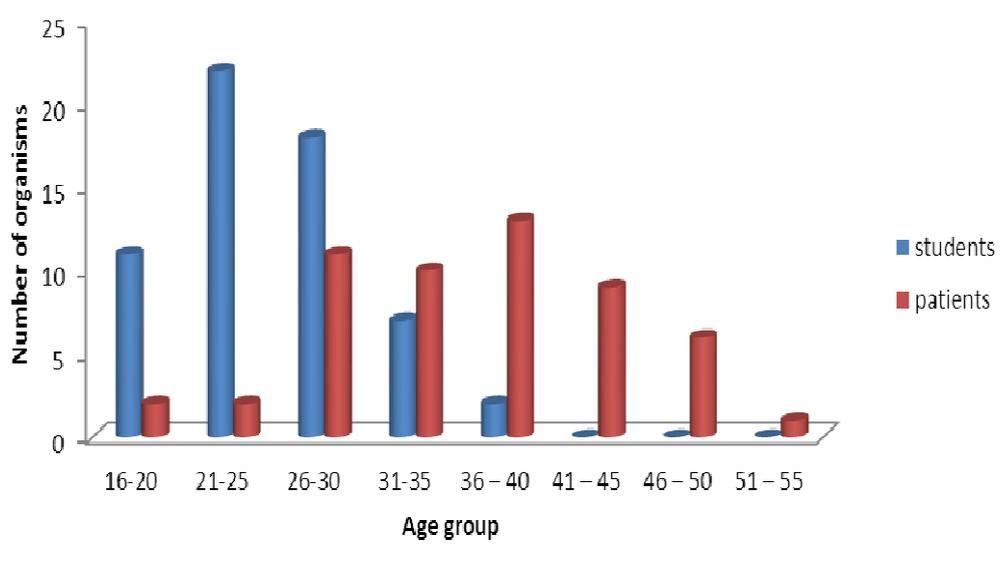


Figure 2: Organisms Observed from Urine of sampled students and patients.

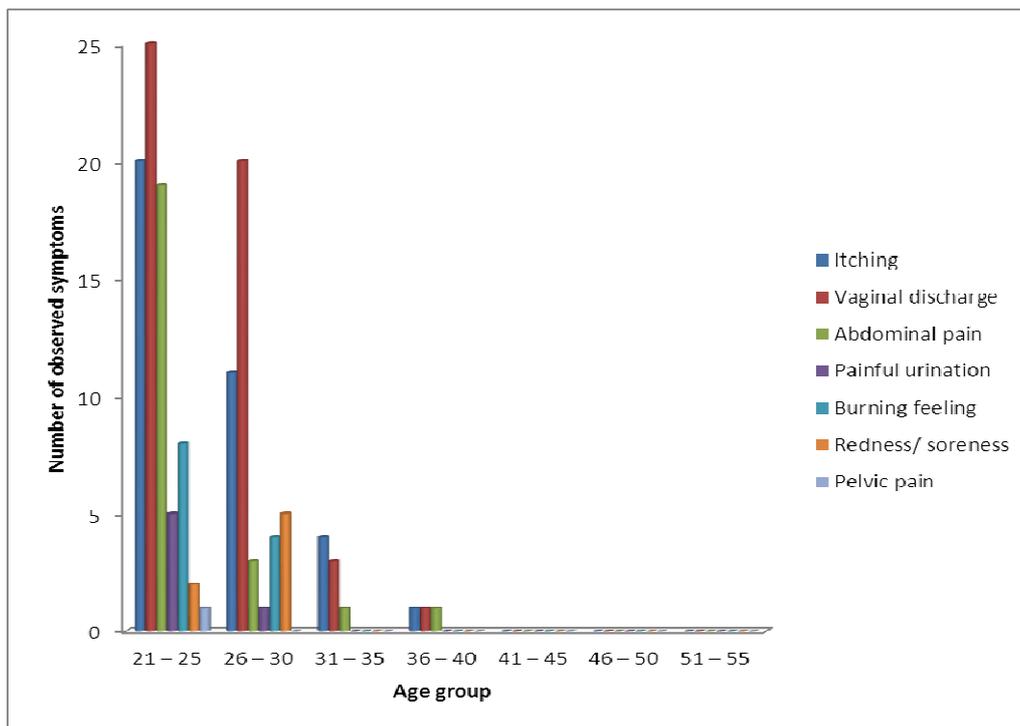


Figure 3: Multiple Bar chart of observed symptoms in samples individual with identified organisms.

Table 1: Organisms Observed from vaginal swabs of sampled students and patients.

Age Group	No Examined		<i>Trichomonas. vaginalis</i>		Yeast		Pus Cells		Total No with Organisms observed		% of Organisms observed	
	Students	Patients	Students%	Patients%	Students /%	Patients/ %	Students/ %	Patients/ %	Students	Patient	Students	Patients
16-20	44	10	0 (0.0)	0 (0.0)	13 (29.5)	2 (20.0)	4 (9.1)	0 (0.0)	17	2	38.6	20.0
21-25	88	10	0 (0.0)	0 (0.0)	40 (45.4)	1 (10.0)	8 (9.1)	2 (20.0)	48	3	54.5	30.0
26-30	57	36	0 (0.0)	1 (2.8)	29 (50.8)	8 (22.2)	6 (10.5)	4 (11.1)	35	13	61.4	36.1
31-35	8	34	0 (0.0)	2 (5.9)	3 (37.5)	5 (14.7)	4 (50.0)	3 (8.8)	7	10	87.5	29.4
36 - 40	3	41	0 (0.0)	1 (2.4)	1 (33.3)	6 (14.6)	1 (33.3)	5 (12.2)	2	12	66.6	29.3
41 - 45	0	33	0 (0.0)	0 (0.0)	0 (0.0)	5 (15.1)	0 (0.0)	3 (9.1)	0	8	0.0.	24.2
46 - 50	0	32	0 (0.0)	1 (3.1)	0 (0.0)	5 (15.6)	0 (0.0)	2 (6.3)	0	7	0.0.	21.9
51 - 55	0	4	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0	1	0.0	25.0
Total	200	200	0 (0.0)	5 (2.5)	86(43)	33(16.5)	23 (11.5)	19 (9.5)	109(54.5)	56(28)		

Table 2: Organisms Observed from Urine of sampled students and patients.

Age Group	No Examined		<i>Trichomonas vaginalis</i>		Yeast		Pus Cells		Total of Organisms Observed		% of Organisms Observed	
	Students	Patients	Students/ %	Patients %	Students/ %	Patients/ %	Students/ %	Patients/ %	students	Patient	Students	Patients
16-20	44	10	0 (0.0)	0 (0.0)	8 (18.2)	2 (20.0)	3 (6.8)	0 (0.0)	11	2	25.0	20.0
21-25	88	10	0 (0.0)	0 (0.0)	15 (17.0)	1 (10.0)	7 (8.0)	1 (10.0)	22	2	25.0	20.0
26-30	57	36	0 (0.0)	1 (2.8)	13 (22.8)	7 (19.4)	5 (8.8)	3 (8.3)	18	11	31.6	30.6
31-35	8	34	0 (0.0)	2 (5.9)	4 (50.0)	4 (11.8)	3 (50.0)	4 (11.8)	7	10	87.5	29.4
36-40	3	41	0 (0.0)	1 (2.4)	1 (33.3)	5 (12.2)	1 (33.3)	7 (17.1)	2	13	66.7	31.7
41-45	0	33	0 (0.0)	0 (0.0)	0 (0.0)	5 (15.2)	0 (0.0)	4 (12.1)	0	9	0.0	27.3
46-50	0	32	0 (0.0)	1 (3.1)	0 (0.0)	5 (15.6)	0 (0.0)	0 (0.0)	0	6	0.0	18.3
51-55	0	4	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0	1	0.0	25.0
Total	200	200	0(0.0)	5(2.5)	41(20.5)	30(15)	19(9.5)	19(9.5)	60(30.0)	54(27.0)		

Table 3: The results of growth of Cultured Samples observed from students and patients.

Age Group	Total No of cultured samples		No with observed Growth		% with Observed Growth	
	Students	Patients	Students	Patients	Students	Patients
16 – 20	44	10	20	2	45.5	20.0
21 – 25	88	10	60	3	68.	30.0
26 – 30	57	36	45	14	79	38.9
31 – 35	8	34	7	20	87.5	58.8
36 – 40	3	41	2	21	67	51.2
41 – 45	0	33	0	10	0.0	30.3
46 – 50	0	32	0	9	0.0	28.1
51 – 55	0	4	0	1	0.0	25.0

Table 4: Assessment of Observed Symptoms in sampled individual with identified Organisms.

Age Group	No examined	Itching	Vaginal discharge	Abdominal Pain	Painful urination	Burning feeling	Redness/ soreness	Pelvic pain
16 – 20	44	6 (13.6)	7 (15.9)	10 (22.7)	4 (9.1)	6 (13.6)	2 (4.5)	0 (0.0)
21 – 25	88	20 (22.7)	25 (28.4)	19 (21.6)	5 (5.7)	8 (9.1)	2 (2.3)	1 (1.1)
26 – 30	57	11 (19.3)	20 (35.1)	3 (5.3)	1 (1.8)	4 (7.0)	5 (8.8)	0 (0.0)
31 – 35	8	4 (50.0)	3 (37.5)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
36 – 40	3	1 (33.3)	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
41 – 45	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
46 – 50	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
51 – 55	0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TOTAL	200	42(21.0)	56(28.0)	34(17.0)	10(5.0)	18(9.0)	9(4.5)	1(0.5)

DISCUSSION

Urinary tract infections are common, and are most often caused by uropathogenic bacteria. UTI was usually diagnosed if the bacteria or pus cell count, or both were significant in an individual (Oladeinde et al., 2011). Less emphasis has been placed on other uropathogenic organisms. In view of this, the present survey focused on other causative agents of UTI other than bacteria. The potential pathogenic organisms encountered in this survey when the urine and vaginal swabs of sampled individuals were examined are *Trichomonas vaginalis*, yeast cells (*Candida spp.*) and pus cells. A total of 200 female students and 200 female patients had their urine and vaginal swabs examined.

When the wet mounts were examined microscopically, from 200 urine samples collected from the female students, 60(30%)

had various pathogenic organisms while 54(27%) out of the 200 urine samples collected from the female patients indicated the presence of pathogenic organisms. Examination of the wet mounts of the Vaginal swab samples from 200 female student revealed that 109(54.5%) had the above mentioned organisms while 56(28%) out of the 200 female patients sampled had these organisms.

The vaginal swabs examination showed 86(43.0%) of the students, 38(16.5%) patients had yeast cells and 23(11.5%) of students and 19(9.5%) of patients had pus cells in their vaginal swabs. However, when the urine was observed 41(20.5%) of the students, 30(15.0) patients had yeast infection and 19(9.5%) of the students and 19(9.5%) of patients had pus cells in their urine. Similar pattern of infection was reported by Battikhi and Battikhi (2015)

where they discovered that 53 of their positive samples revealed 32(26.0%) samples with pus cells, 12(9.7%) samples with red blood cells, 2 samples (1.6%) with yeast cells and 1 (0.8) with *Trichomonas vaginalis* infection. Onuoha and Fatokun (2014) in their study found a high percentage of pus cells (35.5%) and yeast (20%) cells out of the 200 urine samples they analyzed and they concluded that the high percentage of pus cells in the urine samples is an indication of the presence of urinary tract infection.

It was observed that the vaginal swab samples used in this study yielded more positive results than the urine samples for both students and patients, this findings agree with that of Amali et al. (2008) where the *Candida sp.* they found in HVS samples was higher (19.31%) than was found in the urine sample (8.45%) of same sampled individuals, but oppose the findings of Blake et al. (1999) where they found that 9 patients among their study population with negative vaginal wet mounts had positive urine specimens and therefore, they concluded that addition of a spun urine microscopic examination would significantly enhance detection of *T. vaginalis* without substantially increasing the cost of detection.

There were very few cases of trichomoniasis in this study, 2.5% (5 positive patients), and they were found distributed among the different age groups of the sampled female patients attending the hospital used. None of the students examined was found infected with *Trichomonas vaginalis*. There was no significant difference in the prevalence of trichomoniasis among the students and patients examined. This result is lower than the result obtained by Amali et al. (2008), they found out that *Trichomonas vaginalis* occurred in only 12(5.63%) HVS samples, and was the least cause of infection. They however found the infection in female students which is contrary to what was obtained in this study.

In a study carried out by Adeoye and Akande (2007) to determine the prevalence of

Trichomoniasis in outpatient women from two different hospitals in Lagos, they found a prevalence rate of 1.9% and 5.4% from each of the hospitals. They further reported a prevalence of 1.8% in the age group 21-30 years and they observed that none of the patients below 21 years had Trichomoniasis.

Egbere et al. (2009) in their findings reported a prevalence of 1.81% *Trichomonas vaginalis* infection in patients aged between 20-40 years who also had vaginal discharge. In this recent survey, prevalence of 2.5% infected with *Trichomonas vaginalis* was obtained from patients in General Hospital, which falls in line with what these researchers obtained. The absence of *Trichomonas vaginalis* infection from the students examined in this study may possibly be as a result of the time spent between urinating, submission of sample and examination in the laboratory that was experienced during the survey.

Van der pol et al. (2007) also reported 3.1% of *Trichomonas vaginalis* infection among women of reproductive age in the United States. These they explained, may be due to keeping of multiple sex partner, consequently resulting to high rate of infection.

In line with what Adeoye and Akande, (2007) observed, this recent survey also recorded that the age group 21-25 had the highest prevalence (45.4%) obtained for yeast cells and pus cells (9.1%) in vaginal swabs of students. The high number recorded is a clear indication of UTI. This may be attributed to the fact that females within this age group are sexually active young adult. It is however clear from this finding that the prevalence of infection was much higher in females of younger age compared to older ones.

In recent times, the incidences of yeast infections have been reported to have greatly increased especially due to the widespread use of broad-spectrum antibiotics and immunosuppressive treatment (Olowe et al., 2014). The results obtained from the present study showed a high prevalence of yeast cells

(*Candida spp.*) among students, and this may be due to the poor personal and sanitary behavior of the students, which are factors enhancing the transmission of the infection since the organism could be transmitted through common use of toilet seat.

Okonko et al. (2012) in their survey reported 27% prevalence of *Candida albican* and 1.5% *Trichomonas vaginalis* and they concluded that these organisms are the predominant STIs pathogen. Fusi Ngwa et al. (2012) also found a high rate of yeast vaginitis in women that they examined. Nwankwo et al. (2010) in their research, collected vaginal specimens from 587 patients attending Aminu Kano Teaching Hospital where the major pathogen found in the patients were *Candida albicans* (70.5%) and *Trichomonas vaginalis* (5.5%). The result they obtained is higher than the present study conducted among the students and patients. These differences in prevalence could be explained on the basis of differences on cultural, social and environmental factors. A study conducted by Moodley et al. (2002) had a lower prevalence of 6.0% infection with vulvovaginal candidiasis compared to the result observed in this study. Nimorsi et al. (2001) recorded the occurrence of *Trichomonas* in the genital urinary tract of 14(6.3%) female inhabitant of Ikao village in Owan, Edo State, this also differs from the result obtained in this present study.

The pattern of infection according to age group obtained in this study revealed that both students and patients within the age group 31-35 had the highest observed growth of organisms in the samples cultured, followed closely by age group 26-30, and the lowest observed growth rate was found from age group 41-45 and above. The higher rate of isolation found in the age groups is probably related to the higher level of sexual activity in the females of the age group. Closely related to what was obtained in this study, Battikhi and Battikhi (2015) discovered in their study that age group 38-42 exhibited the highest prevalence of UTI while age group 28-32

showed higher percentage of isolates, they concluded that UTI increase with age. When Abdulazeez et al. (2006) assessed infection rates according to age in their study, they found the highest prevalence rate of 1.1% within 16-24 years of age bracket while the least (0.2%) was recorded within the 45-54 years age-group. Their result is slightly in agreement with what was obtained in this present study.

A research conducted among women attending a health center in Iran by Mazloumi et al. (2008) to determine the prevalence of trichomoniasis showed a prevalence of 9.2% by culture and 3.1% by wet method. The result obtained in this present survey, differs from result of the study in Iran due to difference in location and to the unreliability of detection method.

According to Dahab et al. (2012), alteration in the balance of normal vaginal organisms can cause excessive growth of the bacteria that create vaginal discharge. In this present study the age group 21-25 had the highest recorded complain of vaginal discharge as symptom for UTI, followed by age group 26-30(35.1%). Itching symptom was the next highest observed complain followed by abdominal pain among sampled individuals. In the research carried out by Dahab et al. (2012), vaginal discharge was the most common symptom in women with UTI (15.1%), while other frequent symptoms were pruritus (12.3%) and abdominal pain (12.5%). Occasionally, many of the students may have been involve in unprotected sexual activities or unhygienic practices that could lead to these symptoms. There was no statistically significant difference between the organisms observed from the students and patients sampled.

Conclusion

The prevalence of Urinary infection in the community is a public health risk and other STIs constitute major burden that can enhance the susceptibility of an individual to acquire or transmit HIV through sexual

contact. It will continue to be a non-reportable disease with no government funded control programs, quietly perpetuating the spread of HIV. As we wait for more experimental evidence, the clinicians should routinely screen all pregnant women for the infection and appropriate treatment be given early to prevent the spread of STIs and possible implication on the newborn baby. Emphasis must be placed on the youths; education programs should be implemented to combat the endemicity of urinary infection in the world. The policy makers need to enlighten the community on girl child education, safe sex and good hygiene, and to institute policies that will make health care service accessible, affordable and standard. Although, a relatively low rate of the infection was recorded in this study, this research finding could still be very useful in the management and control of the infection in the society and could also serve as baseline information for future studies. Proper sanitation and personal hygiene should be strictly observed among the females.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

TSO-J designed the study and revised the final draft of the manuscript while both authors (TSO-J and VMK) carried out the laboratory work and drafting of the manuscript.

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