Original Research Article

TRICHOMONAS VAGINALIS AND *CANDIDA ALBICANS* INFECTIONS AMONG WOMEN OF REPRODUCTIVE AGE IN IHIALA, ANAMBRA STATE, NIGERIA

ABSTRACT

Co-infection of *T. vaginalis* and *C. albicans* continues to present major health, social and economic problems in Nigeria. A study on the prevalence and co-infection of *T. vaginalis and C. albicans* among women of reproductive age in Ihiala community, Anambra state, was conducted between January and March 2015. A total of 734 women from the four villages in Ihiala participated in the study. Wet mounts were used to examine the high vaginal swabs of the women. Jerky movement and flagella were used to confirm the presence of *T. vaginalis.* Germ- tube test was used to confirm *C. albicans* after culturing on sabouraud dextrose agar. Of the 734 women studied, 150(20.4%) were positive to *C. albicans*, 7(1.0%) were positive to *T. vaginalis* and 4(0.5%) had co-infection of *T. vaginalis and C. albicans.* Women in the age group 40-49 years had the highest infection 22(37.9) of *C. albicans* and the age group 20-29 years had the lowest 96(17.0). Women in the age group, 30-39 years had the highest co-infection of *C. albicans* and *T. vaginalis* 2(1.8%), and the age group 20-29 years had the lowest 2(0.4%). Co-infections of the pathogens were highest 2(6.7%) among the traders and none among civil servants and students. Co-infection of the pathogens was observed in married women only and highest 1(3.6%) among wives of polygamous men. Co-infections of *T. vaginalis and C. albicans* was highest 1(14.3%) among the women with non-formal education and none among the women with tertiary education. Co-infections of the pathogens were observed only among non-pregnant women. Low co-infection rate of the parasites observed in this study could be attributed to improved life style, especially in personal hygiene and literacy in reproductive health, as well as the use of metronidazole in the treatment of other protozoal infections. Since most of the co-infections were observed among women with no formal education and among women in polygamous families, it is suggested that mass education on reproductive health to both men and women will help to reduce the scourge of these infections in the study area.

Keywords: *Trichomonas vaginalis*, *Candida albicans, women, Ihiala, Anambra State.*

1.0 INTRODUCTION

Trichomonas vaginalis is likely the most common non-viral sexually transmitted infection in the world. It is an important source of reproductive morbidity, a facilitator of Human Immuno-deficiency Virus (HIV) transmission and acquisition, and thus it is an important public health problem among women of reproductive age (Kissinger, 2015). The incidence of trichomoniasis depends on the population examined. Certain factors such as poor personal hygiene, multiple sex partners, low socio-economic status and underdevelopment are associated with high incidence of infection (Crosby *et al*., 2002). *T. vaginalis* infection is also associated with the presence of other sexually transmitted infections, including gonorrhoea, chlamydia, and sexually transmitted viruses. The infection increases the susceptibility to other viruses, including herpes, human papillomavirus (HPV) and human immunodeficiency virus (HIV) (Huppert, 2009)

The disease is caused by a pear-shaped protozoan, called *T. vaginalis* (Schwebke, 2002) and characterized in female patients by frothy-greenish-foul smelling vaginal discharge accompanied with vulvo-vaginal irritation, dysuria, lower abdominal pains, discomfort and psychosocial distress (Faro, 2004; Edrisian *et al*., 2006). These symptoms are usually aggravated during menses and pregnancy and other complications include cystitis, cervicitis and urethritis (Alcamo, 2000). Complications of *Trichomonas vaginalis* reported in pregnant women include: premature rupture of membrane, premature labour, slow labour, low birth weight, abortion and post hysterectomy infection, infertility and neoplastic transformation in cervical tissues (Uneke *et al.,* 2006; Perazzi *et al*., 2010; Soper, 2004).

According to Sobel (2005), trichomoniasis is also linked to increased mortality as well as predisposing factor to HIV infections and cervical cancers. Cervicitis due to trichomonasis is characterized by purulent discharges in the endocervical canal and induces early endocervical bleeding (Workowski and Berman, 2006). Studies reveal that *T. vaginalis* induces immune activation specifically Lymphocytes activation, replication and cytokine production leading to increased viral replication in HIV infected cells (Smith and Ramos, 2010). Symptomatic trichomoniasis is more common in women than in men but when parasitic organism is found in the anterior urethrea, external genitalia, prostrate, epididymis causes infertility in men (Smith and Ramos, 2010).

Factors such as poor personal hygiene, multiple sex partners, low socio-economic status and under development are documented to be associated with high incidence of infection (Crosby *et al.,* 2002).

Over 180million people are infected annually worldwide and 5 million in America (Bowden and Garnett, 2000; WHO, 2004). In Nigeria, it has been reported by previous workers that Sexually Transmitted Diseases (STDs) has been blamed on increase in poverty, unemployment and violence among women and children (Obiajuru, 2004; Ulogu *et al.*, 2007), among other factors sexual recklessness, lack of awareness, ignorance of the public health implications, poor sanitation and poor personal hygiene are other risk factors of trichomoniasis. Prevention of *T. vaginalis* infection has not been a priority due to lack of understanding of its public health implications and lack of resources (Schwebke and Burgess, 2004).

Among various pathogenic species of fungi, *Candida* is the most prominent cause of fungal infections (Sullivan *et al*., 1996). The genus *Candida* is composed of heterogeneous group of organisms and more than 17 different *Candida* species are implicated in human infections (Pfaller and Diekema, 2007). *C. albicans* is the most significant pathogenic species. Other *Candida* species include, C. *parapsilosis*, *C. dubliniesis* and *C. lusitania. C. albicans* is a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans (Ryan and Ray, 2004; Hedayati and Shafiei, 2010). Typically, *C. albicans* live as harmless commensals in the gastrointestinal and genitourinary tract and are found in over 80% of the human population without causing harmful effects, although overgrowth of the fungus results in candidiasis (Mardh *et al*., 2003). However, this disease usually occurs in immunocompromised individuals, such as HIV-infected victims, transplant recipients, chemotherapy patients, and low birth-weight babies (Kabir *et al*., 2012). The discharge appears to be like curded milk and deep erythma of vulva and vagina is often seen (Khan *et al*., 2009). Several factors can be associated with increased rate of vaginal colonization by *C. albicans*: these include pregnancy, use of high oestrogen content and oral contraceptives (Akah *et al*., 2010; Alli *et al*., 2011), uncontrolled diabetes mellitus (CDC, 2002; Alli *et al*., 2011), prolonged use of broad spectrum antibiotics (Mardh *et al*., 2002; Alli *et al*., 2011) which kill the good and beneficial bacteria, allowing yeast overgrowth, poor dietary habits and poor personal hygiene. Poorly supported risk factors include use of sponge, intrauterine devices (IUDS), diaphragms, condoms, orogenital sex, douching and intercourse (Mardh *et al*., 2002, Reed *et al*., 2003; Alli *et al*., 2011) and diet with high glucose content (De Leon *et al*., 2002; Akah *et al*., 2010; Alli *et al*., 2011). Indeed, evidence in favour of sexual transmission exists. For instance, infected partners commonly carry identical strains which orogenital transmission has been documented (Akah *et al*., 2010; Alli *et al*., 2011).

The incidence of the infection is almost in women. It always reoccurs during pregnancy as a result of the increased levels of estrogens and corticoids that reduce the vaginal defense mechanism against such opportunistic infections as *Candida* (Sobel, 1997). It has been estimated that up to 40% of pregnant women world-wide may have vaginal colonization by *Candida* species, a two-fold increase from the prevalence rate in non-pregnant women (Hay and Czeizel, 2007). Poor social economic status, inadequate knowledge, lack of diagnostic facilities and shortage of effective treatment all contribute to the high incidence of sexually transmitted and reproductive tract infections (Tyadal *et al*., 1992; Burrow and Bueshing, 1999).

The aim of this study was to investigate *T. vaginalis* and *C. albicans* infections among women of reproductive age in Ihiala town.

The specific objectives were to determine:

1. The prevalence of co-infection of *T. vaginalis* and *C. albicans* among women in Ihiala
2. The rate of co-infection of *T. vaginalis* and *C. albicans* by age, marital status, gravidity, pregnancy status, educational status, occupational groups and contraceptive usage among women in Ihiala
3. The treatment habits of *T. vaginalis* and *C. albicans* infections among women in Ihiala

2.0 MATERIALS AND METHODS

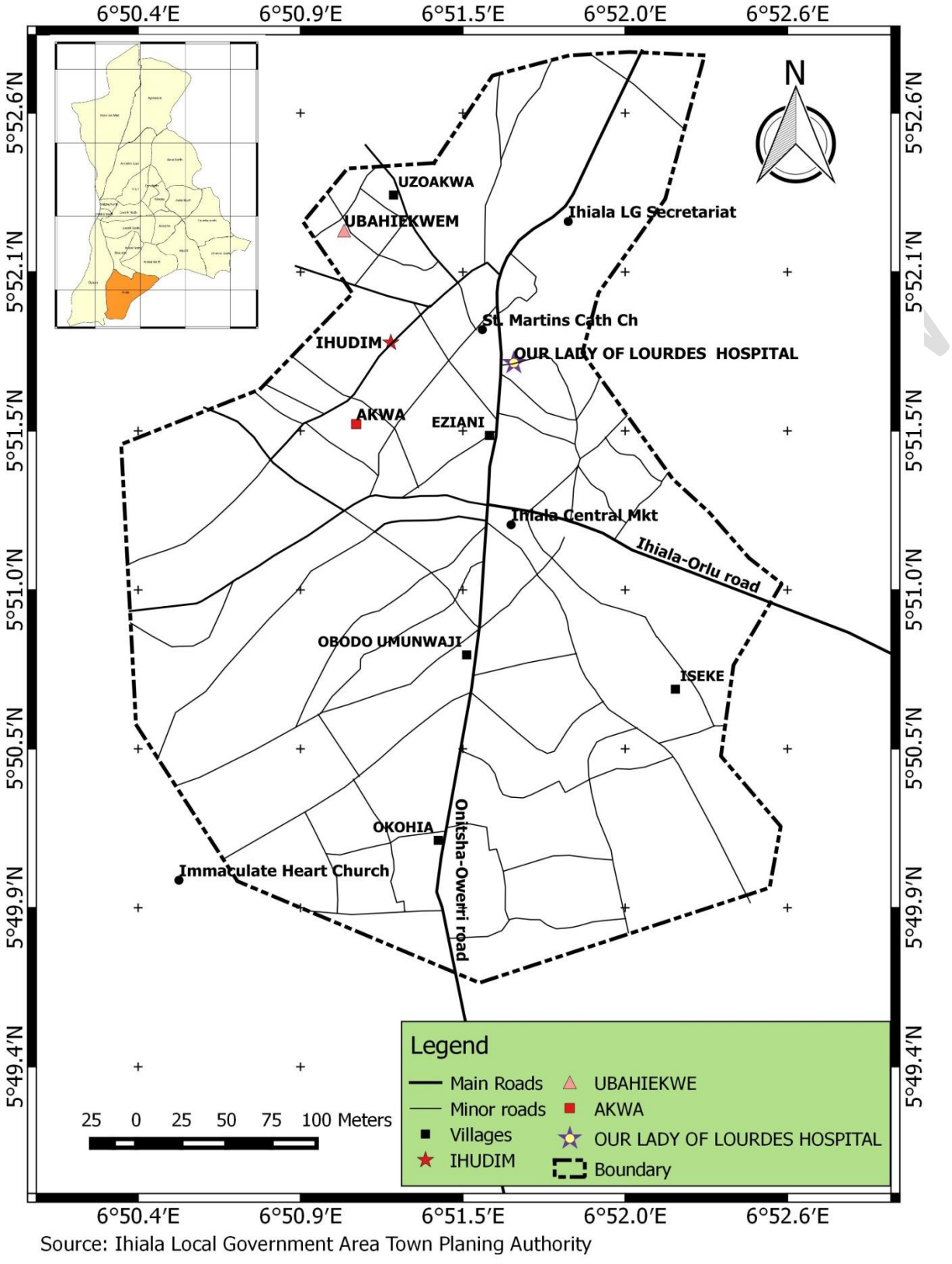
2.1 The Study Area

The study was carried out in Ihiala town in Ihiala Local Government Area, of Anambra State, Southeastern Nigeria. Ihiala has long served as the local administrative capital of the zone (The World Gazetteer, 2007). Ihiala is situated between Longitude 6^0.86' East and Latitude 5^0.85' North of the equator. It is situated within the tropical rainforest zone of Nigeria. It has about 7 months of wet season period (April to October) and 5 months of dry season (November to March) with a short period of harmattan season. The relative humidity of the area is about 70% in the dry season reaching 80% during the wet season (Microsoft encarta, 2009). The annual rainfall is between 2,250 to 2,500mm. The average daily temperature range of the area during the dry season is 27-33^0C and 21.1^0C-30^0C in wet season. The community is about 144m above the sea level. Ihiala has a population of 87,796 inhabitants (NPC, 2006). The town is an Igbo speaking community living peacefully with people of other ethnic groups in Nigeria. The inhabitants (both indigenes and non-

indigenes) are predominantly, farmers, traders, students and a few civil servants. They engage more in farming and trading.

Fig 1.Map of Anambra State showing Ihiala Local Government Area

Source: Anambra State Ministry of Lands and Survey (2012)



Source: Ihiala Local Government Area Town Planing Authority

Fig 2: Map of Ihiala town showing the villages where samples were collected

2.2 Study Design

The study was a cross-sectional survey of women of reproductive age in Ihiala town. Quantitative and qualitative methods were used to determine the prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among the women participants in Ihiala. The study was conducted for a period of three months starting from January to march 2015.

2.3 Advocacy Visit and Mobilization of Participants

A letter of introduction from the Head of Department of Parasitiology and Entomology, Nnamdi Azikiwe University Awka (Appendix I) was used to obtain permission from the following: the Chairman Ihiala Local Government Area, traditional head of Ihiala town, the chairmen of the four villages in the study area, the principal and ethical committee of the health institution of Our Lady of Lourdes Hospital, Ihiala and the management of Akwa Health Centre, Ihiala. Consent was also obtained from the women that participated in the study.

The participants were mobilized through town criers and public notices made in the churches within the villages. Health education on the health implications of infections with *T. viginalis* and *C. albicans* was given to the participants. They were also informed that every laboratory test will be handled confidentially and it will also be free of charge. Informed consent was obtained from each woman before enrolment. The study was conducted in line with the international guideline for experimentation (WMADH, 2000).

2.4 Study Population and Sample Size Determination

According to Nigerian Census Commission, the population of women from Ihiala was 15,008 women (NPC, 2006). A total of seven hundred and thirty-four (734) apparently healthy women without complains of infection were enrolled. The sample size (n) was determined using "Yaro Yamane" formula (Uzoagulu, 2011) for a finite population. The formula was given as:

$$n = \frac{N}{1 + N(e)^2}$$

Where:

n = the minimum sample size
N = the population size
e = level of significance or limit of tolerable error (0.05)
1 = unity (a constant)

2.5 Sampling Technique

The women were assembled at the Akwa Health Centre, Ihiala and at Our Lady of Lourdes Hospital complex, Ihiala, for examination. Convenience sampling in which each woman was attended to as she

arrived was used to study individual participants. Hospital examination rooms were used to provide each woman with the necessary privacy. Trained nurses and laboratory scientists assisted in swab collection and examination.

2.6 Specimen Collection

(a) Collection of Biodata of the Participants

A total of 734 structured questionnaires were used to obtain the biodata of the participants such as age, village, pregnancy status, occupation, marriage structure, gravidity, educational status and marital status.

(b) Collection of High Vaginal Swabs

Samples of vaginal discharge were carefully collected aseptically from the high vaginal area of the women using well labeled, sterile non-abrasive swab sticks through the assistance of two medical laboratory scientist in Our Lady of Lourdes Hospital laboratory, Ihiala and four nurses from Akwa Health Centre, Ihiala. The vaginal swabs were processed immediately after collection using hospital laboratory facility. Wet mounts were used to diagnose *T. vaginalis* while the swabs were cultured for *C. albicans.*

2.7 Parasitological examination of the specimens

(a) **Diagnosis of *T. vaginalis* (Wet – Mount Preparation)**

Each high vaginal swab from the women was mixed with a drop of sterile normal saline on a clean grease-free slide, covered with cover slip and examined with x10 objectives of the microscope. The presence of yeast cell, *T. vaginalis* and other deposits were noted. *T. vaginalis* was identified by the jerky movement, the flagella, axostyle and its oval shape. Ten percent (10%) Potassium hydroxide (KOH) was added to the wet preparation to increase the detection sensitivity of yeast cells, making the recognition of mycelia (pseudohyphae) much easier (Cheesbrough, 2000).

(b) **Diagnosis of *C. albicans* (Culture Method)**

Each high vaginal swab was inoculated on Sabouraud Dextrose Agar gel and incubated at the temperature of 37^0C for 48hrs. Suspected yeast colonies and positive colonies were gram stained and further sub-cultured for germ tube test (Cheesbrough, 2000).

Gram Stain:

Procedures: A small pinch of each yeast colony was smeared on a clean grease free slide, heat fixed and flooded with crystal violet solution for one minutes. The slide was washed with water and lugols iodine was applied for 30secs. The slide was washed again with water and decolourized rapidly with a few drops of acetone for two seconds. The slide was washed with water and counter stained with neutral red for two minutes. The stain was washed off with water, the slide air dried and examined under the microscope using oil immersion objectives. This helped to clarify the morphology of the yeast. *Candida albicans* were identified on the basis of the following features: thick-walled oval yeast cells with pseudomycelium.

Germ tube test (confirmation test for *C. albicans*)

Nutrient broth (0.5ml) was pipetted into a small test tube and a sterile wire loop was used to inoculate the tubes with the test colony from the Sabouraud Dextrose Agar slants. The tubes were put in a water bath and incubated at 37^0C for 3hrs. A drop of the culture was placed on a glass slide with a Pasteur pipette and covered with a cover slip. The preparation was examined using ×10 and ×40 objectives with the condenser iris diaphragm closed sufficiently to give good contrast. A drop of lactophenol cotton blue was also added to the preparation to stain the yeast cells. Sprouting yeast cells that were tube like out growths from the cells (known as germ tubes were looked for). This test served as confirmation test. The formula below was used to calculate the prevalence of infection of the participants.

$$\text{Prevalence} = \frac{\text{Number of subjects testing positive}}{\text{Number of subjects investigated}} \times 100$$

2.8 Data Analysis

The differences in the prevalence and co-infection of *T. vaginalis and C. albicans* in the various groups of the participants were tested using chi-square test. Chi-square statistics was computed to accompany each cross tabulation. The statistical package used was SPSS version 21.0.

3.0 RESULTS

Out of the 734 women examined for the prevalence and co-infection of *T. vaginalis* and *C. albicans*, 150 (20.4%) were positive to only *C. albicans*, 7(1.0%) women were positive to *T. vaginalis* and 4(0.5%) had co- infection of *T. vaginalis* and *C. albicans* among the women (Table 1). *C. albicans*

150 (20.4%) had the highest prevalence, followed by *T. vaginalis* 7(1.0%) and co- infection had the least prevalence 4(0.5%) among the women participants.

Table 1: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among the women participants from Ihiala.

No. Examined	No. infected with *C. albicans* (%)	No. infected with *T. Vaginalis* (%)	No. co-infected with *T. Vaginalis* and *C. albicans* (%)
734	**150 (20.4)**	**7 (1.0)**	**4 (0.5)**

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by age groups of the women are shown in Table 2. Of the 564 women in the age group 20-29 years, 2(0.4%) had only *T. vaginalis*, 96(17.0%) had *C. albicans* and 2(0.4%) had co-infection of the two pathogens. In the age group 30-39 years, 4(3.6%) of the 112 women examined had *T. vaginalis*, 32(28.6%) had *C. albicans*, and 2(1.8%) were co-infected with the parasites. Of the 58 women examined in the age group 40-49 years, 1(1.7%) was positive to only *T. vaginalis*, 22(37.9%) were positive to only *C. albicans* and none had co-infection of the parasites. The highest prevalence of *C. albicans* 22(37.9%) were recorded in women within the age bracket of 40-49 years while those within the age bracket of 20-29 years had the lowest prevalence 2(9.1%). The age bracket of 30-39 years had the highest prevalence of *T. vaginalis* only and co-infection 4(3.6) and 2(1.8), respectively. The age bracket of 40-49 recorded no co-infection (Table 2**).** The prevalence of *T. vaginalis* and *C. albicans* was significantly different ($P<0.05$) while their co-infection rates show a significant difference ($P>0.05$) among different age groups of the participants.

Table 2: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by age groups

Age Group (Years)	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
20-29	564	2 (0.4)	96 (17.0)	2 (0.4)
30-39	112	4 (3.6)	32 (28.6)	2 (1.8)
40-49	58	1 (1.7)	22 (37.9)	0
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

$X^2 = 3.876$, $df = 3$, $P<0.05$

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by location were shown in (Table 3). Of the 600 women from Eziani village, 102 (17.0%) had *C. albicans* and none had either *T. vaginalis* or co-infection of the parasites. Of the 48 women from Ubahiekwem, 3 (6.3%) had only *T. vaginalis*, 18 (37.5%) had *C. albicans* only, and 3 (6.3%) had co-infection of both pathogens. Of the 56 women from Akwa village, 3 (5.4%) had *T. vaginalis*, 23 (41.1%) had *C. albicans*, and 1 (1.8%) had co-infection of those parasites. Of the 30 women from Ihudim village, 1 (3.3%) had *T. vaginalis*, 7 (23.3%) had *C. albicans* and none had co-infection of the parasites. Ubahiekwem 3 (6.3%) had the highest prevalence of *T. vaginalis* while Ihudim village had the least 1 (3.3%) and Eziani village had no *T. vaginalis*. Akwa village had the highest prevalence of *C.albicans* 23 (41.1%), followed by Ubahiekwem 18 (37.5%), and least was among Eziani village, 102 (17.0%). The co-infection was observed only among Ubahiekwem and Akwa village 3 (6.3%) and 1 (1.8%) respectively. Eziani and Ihudim village had no co-infection. The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among villages showed a significant difference ($P<0.05$).

Table 3: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by location

Villages examined	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Eziani	600	0	102 (17.0)	0
Ubahiekwem	48	3 (6.3)	18 (37.5)	3 (6.3)
Akwa	56	3 (5.4)	13 (41.1)	1 (1.8)
Ihudim	30	1 (3.3)	7 (23.3)	0
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

$X^2 = 3.876$, df = 3, $P<0.05$

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by occupation of the participants was shown in Table 4. Of the 600 female students examined, none was positive with *T. vaginalis*, 102(17.0%) had *C. albicans* only while none had co-infection. Of the 18 civil servants examined, none had *T. vaginalis*, 3(16.7%) were positive to *C. albicans* and no co-infection was recorded. Among the 30 traders examined, 4(13.3%) were positive to *T. vaginalis* only, 10(33.3%) had *C. albicans* only, while 2(6.7%) had co-infection of the parasites. Among the 86 farmers examined, 3(3.5%) were positive to *T. vaginalis* only, 35(40.7%) were positive to *C. albicans* and 2(2.3%) had co-infection of both. Of all the four occupational groups that participated in the study,

the highest prevalence of *C. albicans* 102(17.0%), was observed among the students, followed by 3(16.7%) observed among the civil servants while the least prevalence 3(3.5%) was observed among farmers. The traders had the highest prevalence of *T. vaginalis* 4(13.3%) and co-infection of *T. vaginalis* and *C. albicans* 2(6.7%). The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among women from different occupational groups showed a significant difference (P<0.05).

Table 4: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by occupation

Occupation	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Students	600	0	102 (17.0)	0
Civil Servants	18	0	3 (16.7)	0
Trader	30	4 (13.3)	10 (33.3)	2 (6.7)
Farmers	86	3 (3.5)	35 (40.7)	2 (2.3)
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

X^2 = 29,169, df = 3 P<0.05

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by marital status were shown in Table 5. Among the 468 single women examined, 91(19.4%) were positive to only *C. albicans*, none was positive to *T. vaginalis* and there was no co-infection of the parasites among them. Among the 234 married women examined, 6(2.6%) had only *T. vaginalis* and 55(23.5%) had only *C. albicans* while 4(1.7%) had co-infection. Out of the 6 divorced women examined, 1(16.7%) had *C. albicans* and none had *T. vaginalis* or the co-infection of the parasites. Among the 26 widows examined, 1(3.8%) had *T. vaginalis* only, 3(11.5%) had *C. albicans* and none had co-infection. The married women had the highest prevalence of *C. albicans*, and the widows had the least 1(11.5%). Only the married women had co-infection of *T. vaginalis* and *C. albicans* 4(1.7%) among the marital groups. The prevalence of *T. vaginalis* and co-infection rates had significant difference (P<0.05) while the prevalence in *C. albicans* had no significant difference (P>0.05).

Table 5: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by marital status

Marital Status	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Singles	468	0	91 (19.4)	0

Married	234	6 (2.6)	55 (23.5)	4 (1.7)
Divorced	6	0	1 (16.7)	0
Widowed	26	1 (3.8)	3 (11.5)	0
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

$X^2 = 8.594$, df = 3 P<0.05

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by educational status of the participants were shown in Table 6. Among the 7 non-formal educational group examined, 4(57.1%) were positive to only *C. albicans*, 1(14.3%) was positive to *T. vaginalis* while 1(14.3%) had co-infection among them. Among the 42 primary educational group examined, 3(7.1%) had only *T. vaginalis*, 22(52.4%) had only *C. albicans* and 2(4.8%) had co-infection. Out of the 60 secondary educational group examined, 3(5.1%) had *T. vaginalis*, 25(41.7%) had *C. albicans* and 1(1.7%) had co-infection. Out of the 625 tertiary educational groups examined, none had *T. vaginalis*, 99(15.8%) had *C. albicans* while none had co-infection. The highest co-infection of 1(14.3%) was observed among the non-formal educational group and the least was observed among the secondary educational group, 1(1.7%)**.** The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates among the educational groups showed a significant difference (P<0.05).

Table 6: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by educational status

Educational Status	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Non-formal	7	1 (14.3)	4 (57.1)	1 (14.3)
Primary	42	3 (7.1)	22 (52.4)	2 (4.8)
Secondary	60	3 (5.1)	25 (41.7)	1 (1.7)
Tetiary	625	0	99 (15.8)	0
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

$X^2 = 42.983$, df = 3, P<0.05

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by marriage structure of the women participants were shown in Table 7. Of the 206 monogamous women examined, 5(2.4%) had *T. vaginalis* only, 44(21.4%) had *C. albicans*, and 3(1.5%) had co-infection of the parasites. Among the 28 polygamous women examined, 1(3.6%) had *T. vaginalis*, 11(39.3%) had *C. albicans* and 1(3.6%) had co-infection of the parasites**.** The women from polygamous families had higher infections of *T. vaginalis* 1 (3.6%) and *C. albicans* 11 (39.3%) than the women from monogamous families with 5 (2.4%) *T. vaginalis* infection and 44(21.4%) of *C. albicans*. The women from polygamous families had higher co-infection rate 1 (3.6%) of *T. vaginalis* and *C. albicans* than the women from monogamous families 3

(1.5%). The prevalence of *T. vaginalis* and co-infection rates were significantly difference (P<0.05) while the prevalence in *C. albicans* had no significant difference (P>0.05) in marital structure.

Table 7: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by marital structure

Marital Structure	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Monogamy	206	5 (2.4)	44 (21.4)	3 (14.3)
Polygamy	28	1 (3.6)	11 (39.3)	1 (3.6)

X^2 = 656, df = 1, P<0.05

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by pregnancy status of the women participants were shown in Table 8. Of the 44 pregnant women examined, 1(2.3%) had *T. vaginalis*, 19(43.2%) had *C. albicans*, and none had co-infection among them. Out of the 690 non-pregnant women examined, 131(18.98%) had *C. albicans*, 6(0.9%) had *T. vaginalis*, and 4(0.6%) had co-infection of the parasites. The pregnant women had higher infection of *T. vaginalis* 1(2.3%) than the non-pregnant women 6(0.9%). There was no co-infection of *T. vaginalis and C. albicans* among the pregnant women. The prevalence of *T. vaginalis* and co-infection rates showed no significant difference (P>0.05) while the prevalence in *C. albicans* showed significant difference (P<0.05).

Table 8: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by pregnancy status

Pregnancy Status	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Pregnant	44	1 (2.3)	19 (43.2)	0
Non-Pregnant	690	6 (0.9)	131 (18.98)	4 (0.5)
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

X^2 = 256, df = 3, P<0.05

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates according to the gravidity of the women participants were shown in Table 9. Of the 458 nulligravida examined, 1(0.2%) had *T. vaginalis*, 74(16.2%) had *C. albicans*, none had co-infection among them. Out of the 70 primigravida examined, 2(2.9%) had *T. vaginalis*, 9(12.9%) had *C. albicans* and 1(1.4%) had co-infection of the parasites. Out of the 206 multigravida women examined, 4(1.9%) had *T. vaginalis*, 67(32.5%) had *C. albicans*, and 3(1.5%) had co-infection among them. *T. vaginalis* infection was highest among primigravida 2(2.9%) and least 1(0.2%) among the nulligravida. *C. albicans* infection was highest among

the multigravida 67(32.5%) and the least 9(12.9%) among the primigravida. Co-infection of the parasites was not observed among the nulligravida. The multigravida had a higher rate of co-infection 3(1.5%) than the primigravida 1(1.4%). The prevalence of *C. albicans* and co-infection rates showed significant difference (P>0.05) while the prevalence in *T. vaginalis* showed no significant difference (P<0.05).

Table 9: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by gravidity

Gravidity No. examined	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Nulligravidae	458	1 (0.2)	4 (57.1)	0
Primigravidae	70	2 (2.9)	9 (12.9)	1 (1.4)
Multigravidae	206	4 (1.9)	67 (32.5)	3 (1.5)
Total	**734**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

X^2 = 6.675, df = 3, P<0.05

The prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates according to the Clinical manifestation of the women participants were shown in Table 10. Of the 248 symptomatic participants examined, 2(0.8) showed *T. vaginalis*, 62(25.0) showed *C. albicans* and 2(0.8) had co-infection among them. Out of the 486(66.2) asymptomatic participants examined, 5 (1.0) showed *T. vaginalis*, 40(8.2) showed *C. albicans* and 2(0.4) showed co-infection of the parasites. Symptomatic women had 62(25.0%) higher prevalence of *C. albicans* compared to asymptomatic women 40(8.2%). Asymptomatic women 5 (1.0%) had higher prevalence of *T. vaginalis* than the symptomatic women. The co-infection occurred more among the symptomatic women 2(0.8) than asymptomatic women 2(0.4). The prevalence of *C. albicans* showed significant difference (P>0.05) while the prevalence in *T. vaginalis* and co-infection rates showed no significant difference (P<0.05).

Table 10: Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates by clinical manifestation among the women participants.

Clinical Manifestation	No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Symptomatic	248 (33.8)	2 (0.8)	81 (32.7)	2 (0.8)
Asymptomatic	486 (66.2)	5 (1.0)	69 (14.2)	2 (0.4)
Total	**734 (100)**	**7 (0.95)**	**150 (20.4)**	**4 (0.5)**

X^2 =0.473, df=2, P<0.05

From the results obtained, women using Metronidazole always, had a higher prevalence of C. albicans infection 122 (23.5%), no co-infection of the two parasites and had the least T. vaginalis 1(0.2%) while women who do not take Metonidazole always had the lowest prevalence of C. albicans infection

28(13.1%), had highest co- infection 4(1.9%) and highest T. vaginalis 6(2.8%) (Table 11). There was no statistical difference between them (P>0.05).

Out of 534 women examined under self-medication had the higher prevalence of C. albicans infection 138 (25.8%), T. vaginalis 6(1.1%) and co-infection 3(0.6%) while 200 women examined without self-medication had C. albicans infection 12 (6.0%), T. vaginalis 200(0.5%) and co-infection 1(0.5%) (Table 11).

Of the 186 women examined under family planning showed the highest prevalence C. albicans infection 122 (65.6%), T. vaginalis 5(2.7%) and co-infection 2(1.1%) while 548 women examined with no family planning showed C. albicans infection 28 (5.1%), T. vaginalis 2(0.4%) and co-infection 2(0.4%) (Table 11).

Table 11: **Prevalence of *T. vaginalis* and *C. albicans* and their co-infection rates according to treatment habit of the women participants**.

Treatment Habit		No. Examined	No. infected with *T. vaginalis (%)*	No. infected with *C. albicans (%)*	No. co-infected with *T. vaginalis* and *C.albicans* (%)
Use of Metronidazole	Yes	520	1 (0.2)	122 (23.5)	0
	No	214	6 (2.8)	28 (13.1)	4 (1.9)
Self-medication of antibiotics	Yes	534	6 (1.1)	138 (25.80)	3 (0.6)
	No	200	1 (0.5)	12 (6.0)	1 (0.5)
Use of Contraceptives	Yes	186	5 (2.7)	122 (65.6)	2 (1.1)
	No	548	2 (0.4)	28 (5.1)	2 (0.4)

$X^2 = 4.800$, df = 3, P<0.05

4.0 DISCUSSION

This study showed that out of the 734 women examined, 150 (20.4%) had *C. albicans*, 7 (1.0%) had *T. vaginalis* while 4 (0.5%) had co-infection of *T. vaginalis* and *C. albicans*. Also the prevalence of candidiasis is relatively higher than trichomoniasis. The high prevalence of *C. albicans* (20.4%) reported in this study could be attributed to the fact that *C. albicans* is a frequent colonizer and responsible for most cases of vulvovaginitis (Singh, 2003; Akah *et al*., 2010; Donbraye-Emmanuel *et al*., 2010; Alli *et al*., 2011). The result is higher than what was reported by previous researchers, Choudhry *et al*., 2010; Konje *et al.,* 1991; Nwokedi and Aniyam, 2003; Cronje *et al*., 1994; Di Bartolomeo *et al*., 2002, who recorded 2.0%, 2.20%, 12.0%, 2.6%, and 17.8% respectively in their studies. However this is lower than the findings of Khan *et al*., (2009); Muvunyi and Hernandez, (2009); Usanga *et al*. (2010) and Adeoye and Akande (2007) who reported the prevalence rate of 28.0%, 52.5%, 21.3% and 33.6% respectively. It is

also lower than the 40.0%, reported by Oyewole *et al*. (2010), among non-HIV- infected women in Sagamu, Ogun State, Nigeria. It is also lower than 29.7% reported by Hedayati and Shafiei (2010) in their study and the 78.0% reported by Rizvi and Luby (2004) among Nepalese women, 70.0% reported by Nwankwo *et al*. (2010) among females of reproductive age in Kano, Nigeria, and the 65.4% reported by Donbraye-Emmanuel *et al*. (2010). Many other studies reported higher prevalence of candidiasis among women of reproductive age (Rao *et al*., 2004; Nwadioha *et al*., 2010; Murta *et al*., 2000). The high prevalence 150(20.4%) of *C. albicans* observed in this study could be that candidiasis is spreading as a result of its invasive colonization of human host either due to health condition or due to sexual activities. This agrees with Horn *et al*., (2009) who reported that high incidence of *C. albicans* could be associated with severe immunosuppression or illness, and sexual activities of the woman. *Candida* species are part of the lower genital tract flora in 20-50% of healthy asymptomatic women and may spread due to sexual activities or immunosuppression (McClelland *et al.,* 2009; Akah *et al.,* 2010).

High prevalence of *C. albicans* was recorded among age groups 20-29 years (17.0%), 30-39 years (28.6%) and 40-49 years (55.6%). This disagrees with the findings of previous studies by Konje *et al.* (1991) who observed that the infections were almost uniformly distributed in all age groups studied. This result did not support the findings of Engberts *et al.* (2006) who reported the prevalence of *C. albicans* to be significantly higher in cohort of 30 years Old Dutch women and lower in the cohorts of 45-50, 55 and 60 years Old Dutch women. However, the result disagrees with Adad *et al.* (2001), who reported that infection by *C. albicans* were most frequent among younger patients, especially those under 20 years. Also, Murta *et al.* (2005) reported that the frequency of *Candida* species is a less common feature among ages between 40 years and 49 years. The highest prevalence rate of Candidiasis was found amongst women in age 40-49 years and could be due to higher oestrogen content of the vaginal epithelial (Sobel, 1997). Also, the age range of between 20-49 years constitute the sexually active period of most women. This conforms to the findings of previous studies by Pfaller and Diekema (2007) who pointed out that, although *C. albicans* infection is not a classical sexually transmitted disease, it is known to be transmitted, in some cases, through sexual intercourse especially among sexually active person and sex workers. Ononge *et al (*2005) also reported that the high prevalence rate found in the younger age was attributed to the group being the most sexually active age range which further strengthens the belief that sexual activity could contribute to a large extent, the spread of the disease.

The low prevalence rate of trichomoniasis (1.0%) recorded in this study is similar to the result of Aboyeji and Nwabuisi (2003) who reported 4.7%. Also, it is in tandem with low prevalence of (2.3%) and (3.3%)

reported by Orji (2015) and Gundiri and Okwuosa (2005). Although, estimated global prevalence of *T. vaginalis* is 8.1% for women in Africa. It is estimated that 2-50% of the population has the infection (WHO, 1992). Various studies in other countries have also shown a low prevalence of trichomoniasis among reproductive age women. Kalantari *et al.* (2014) recorded prevalence rate of 0.2% in Babol in Iran, Nsagha *et al.* (2015) reported 1.2%, Shutter *et al.* (1998) and Franklin and Monif (2000) recorded the prevalence of 46.9% and 36.1% respectively. However some studies have reported higher prevalence rates, Lopez-monteon (2013), Jombo *et al.* (2006) and Alo *et al*. (2012) recorded prevalence of 23.4%, 24.1% and 24% respectively. This result also disagrees with Ulogu *et al*. (2007) who observed a prevalence rate of 21.53% among women in Nnewi community. Low prevalence of *T. vaginalis* in Ihiala could be attributed to multiple factors such as awareness of people towards the spread of human immuno-deficiency virus (HIV) due to public campaign by World Health Organization (WHO) and other agencies in Nigeria and abroad. This has led to a change in attitude of people towards unsafe sex and increase in the use of condoms. The use of condom has helped in reducing organisms especially *T. vaginalis* that affects the vaginal mucosa in women. This is in concordance with the findings of Evans *et al*. (1995). It is also likely to be associated with the frequent use of Metronidazole (Flagyl) tablets, which is taken very often even without doctor's prescription for any intestinal problems. Many people take the drugs in any case of diarrhoea or stomach arch, indirectly treating trichomoniasis without knowing.

The co-infection rate of trichomoniasis and candidiasis in this study was 0.5% with only 4 participants infected. This is similar to the findings of Adeoye and Akande (2007) who reported co-infection rate of 0.6% of *T. vaginalis* and *C. albicans* in Lagos metropolis. Olorode *et al*. (2014) reported co-infection rate of 3.2% which is slightly higher. However, higher rate of co-infection of trichomoniasis and candidiasis has been reported by other studies. Lopez-monteon (2013) reported co-infection rate of 14.28% in central Veracruz Mexico while Alo *et al.* (2012) recorded 22% co-infection rate in Abakaliki, Ebonyi State. Low co-infection rate of the parasites observed in this study could be attributed to improved life style, especially in personal hygiene and literacy in reproductive health, as well as the use of Metronidazole in treatment of other protozoal infections.

Also, co-infection of trichomoniasis and candidiasis were recorded among younger women peaking at 3.6% and 1.8% in age group 30-39. This study attributed the high increase rate of the co-infection among the age group 30-39 to be more sexually active. This is in conformity with the study of Donbraye *et al*. (2010) where higher prevalence of co-infection of trichomoniasis and candidiasis was reported among young age group.

5.0 CONCLUSION AND RECOMMENDATION

The study has revealed that infections caused by *T. vaginalis* and *C. albicans* and their co-infections exist among women of reproductive age in Ihiala. The data generated will provide baseline information for the formulation of policies and control strategies for the control of the infections. In addition, it will be useful in educating the women of reproductive age to be more conscious of their reproductive health. It is therefore suggested that mass diagnosis and treatment coupled with health education will help to eradicate the infections in Ihiala town.

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