

Bacterial Vaginosis, *Candida albicans* and *Trichomonas vaginalis* Infection in Antenatal and Gynaecological Patients in Ghana.

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Abstract

Context: The syndromic approach to STI case management depends largely on the patients' presenting symptoms and clinical findings. For a more effective application of this management tool it is important to know how symptoms and clinical findings relate to specific pathogens in a particular geographical area.

Objectives: To determine the prevalence and epidemiology of *G. vaginalis*, *T. Vaginalis* and *C. albicans* in antenatal and gynaecological patients, and the association between presenting symptoms and specific pathogens.

Design: Prospective observational survey.

Settings: Korle-bu Teaching Hospital, Accra and Noguchi Memorial Institute for Medical Research (NMIMR), Accra.

Subjects: 517 consecutive newly registered patients at antenatal and Gynaecology clinics

Methods: Laboratory examination of vaginal swabs and serum samples.

Main Outcome Measures: Pathogen detection was conducted for all patients and the results were compared with reports from other institutions.

Results: The prevalence of infection with *G. vaginalis* was 1.2%, *C. albicans* 34.2%, and *T. vaginalis* 2.7%.

Conclusions: The absence of association between the presenting symptoms, clinical findings and specific pathogens has implications for the syndromic approach to STI case management. The low prevalence of *G. vaginalis* and *T. vaginalis* in our survey calls for further research in primary health institutions in rural areas to compare the rates.

Key Words: Sexually Transmitted Infections, Syndromic Approach

Introduction

Sexually transmitted infections (STIs) are a major health problem worldwide. The reported prevalence in developing countries is much higher and it is ranked among the top ten most important health problems for which adults seek help in outpatient clinics.^{1,2} In most African countries, including Ghana, STIs are not notifiable and most prevalence studies have been based on patients attending either family planning or antenatal clinics in urban areas and who may not be representative of the general population. Some STIs of the female genital tract have major health consequences that include pregnancy wastage, neonatal morbidity, pelvic inflammatory disease, infertility and ectopic pregnancy. It is estimated that approximately 67% of infertility is due to tubal blockage from STIs and other organisms.² This study was undertaken to determine the prevalence and epidemiology of Bacterial vaginosis (BV), *Candida albicans* and *Trichomonas vaginalis* in women attending the antenatal and gynaecology clinics at the Korle-Bu Teaching Hospital and the association between presenting symptoms and specific pathogens.

Bacterial vaginosis is caused by multiple organisms and available data indicates that four categories of vaginal bacteria are independently associated with this clinical condition, the most predominant among them being *Gardnerella vaginalis*.³ Other organisms include

Bacteroides spp., *Mobiluncus spp.*, and *Mycoplasma hominis*. *G. vaginalis* has been implicated in obstetric, neonatal and gynaecological infections and in general clinical practice a diagnosis of bacterial vaginosis is usually made after other conditions that are associated with vaginal discharge, such as trichomoniasis, vulvovaginal candidiasis, or cervicitis have been excluded even though these conditions can without doubt co-exist with bacterial vaginosis.

Vulvovaginal candidiasis is estimated to be the leading cause of colpitis worldwide,⁴ and previous reports have indicated that some 75% of women will have the infection during their lifetime, approximately half of them will contract a second infection,⁵ and 5% of the infections will be recurrent with frequent, and sometimes refractory episodes.⁴ The chronic form has been linked with an increased titre of autoantibodies to the ovary, thymocytes; T cell and B cell lines, and it is postulated that there are one or more cross-reactive antigens in the ovarian follicle, T. lymphocytes and *Candida*.⁵

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Vaginal candidiasis is the cause of 40-50% of leucorrhoea and Sobel observed that 80-90% of the acute infection is usually due to *C. albicans* with *Candida (Torulopsi) glabrata* being the second most common species causing approximately 5-15% of cases of vaginal candidiasis.⁶ From the therapeutic point of view, vaginitis caused by non-albicans *Candida spp.* are often more resistant to conventional therapy and Hurley & co-workers have documented that 45% of their pregnant patients required more than one treatment.⁴ *Trichomonas vaginalis* is the cause of about 30-35% of cases of vulvovaginitis with a male to female infection ratio of 1:13, and up to 40% of females in STD clinic with trichomonal infection have been noted to have gonorrhoea.⁵

Patients and Methods

The study was carried out between November 2001 and December 2002. Clinical samples were collected at the gynaecological and antenatal clinics of the Korle-Bu Teaching Hospital, a 1,600 bed hospital affiliated to the University of Ghana Medical School in Accra, the capital city of Ghana. It is the largest hospital in the country. Two hundred and seventy four newly registered regular antenatal patients and two hundred and thirty three gynaecological patients whose subjective symptoms were abdominal pain, genital bleeding, vaginal discharge, and dysuria and who gave informed consent were enrolled consecutively into the study. Three patients declined participation. A standardised questionnaire was administered by trained STI nurse/counselors. Among others the age, ethnic origin, marital status, educational background and obstetric history of each participant were recorded.

After a routine general examination a bivalve vaginal speculum was passed. No antiseptic lotions or creams were used for lubrication and where necessary the vaginal speculum was moistened with sterile water. The vaginal walls and cervix were inspected and the presence of vaginal discharge recorded. Two sterile plastic swabs tipped with alginate wool in single peel pouch (Medical Wire & Equipment Co. Ltd., Corsham, Wilts. England) were inserted simultaneously to take specimen from the posterior vaginal fornix or any other site where discharge had collected. These swabs were examined at NMIMR for *Trichomonas vaginalis* and *Candida albicans* in a wet-mount preparation and cultured on Sabouraud's medium for *Candida albicans*. The other swab was transported in Amies transport medium for *Gardnerella vaginalis* isolation.

Pathogen Identification:

Gardnerella vaginalis: High vaginal swabs transported to the laboratory in Amies transport medium were inoculated onto Columbia selective agar plates and

incubated in 5% CO₂ at 37° C for 48 hours. After incubation the inoculated plates were examined for typical colonial appearance. Identification was done by oxidase, catalase, and carbohydrate utilization tests.

Trichomonas vaginalis: A drop of high vaginal swab suspended in 1ml of normal saline was placed onto a clean slide and the wet-mount preparation was examined with a microscope for typical characteristics of *Trichomonas vaginalis* i.e. ovoid globular, pear-shape flagella with four free anterior flagella and one extra posterior flagellum attached to an undulating membrane, extending along the length of its body and with a typical jerky motility.

Candida albicans:

1. Wet-mount examination; Drops of vaginal swab saline suspension were placed onto a clean slide, covered with a cover slip and examined with x 40 objective lens for yeast cells.
2. Culture; High vaginal swabs transported to the laboratory in tubes containing 0.5 milliliters of normal saline were inoculated onto Sabouraud's dextrose agar plates and incubated aerobically at 37°C for 18-24 hours. After incubation the inoculated plates were examined for opaque, white to creamy colonies.
3. Germ tube test; Presumptive colonies of *C. albicans* were inoculated into a test tube containing 500µl of human plasma and incubated at 37°C for 4 hours. After incubation a drop of the suspension was placed onto a slide and examined under low power magnification for the characteristic germ tube that confirms the true identification of *C. albicans*.

Women who were infected with any of the pathogens were located and given treatment. The Ethical Review of Research committee of the University of Ghana approved the study.

Statistical analysis:

The frequency data were analyzed by chi-square or Fisher's exact tests using EPI-Info 2002 software (CDC, Atlanta, USA). *P* value <0.05 was considered statistically significant.

Results:

Laboratory results were available for all the 517 women recruited into the study. The age groups and marital status are shown in table 1. The age and marital status of 17 patients were not recorded. The mean age was 29.6 (SD=6.9). Over 78 percent (n=400) of patients were aged thirty-five years and below with 5.2 percent (n=28) above 41 years. Sixty point nine percent (n=315) of participants had secondary school education while 12 percent (n=60) had no formal education. Aside from 15 patients who did not provide information about parity,

59.8% (n=300) of participants had one or more children. Eighty one point six percent (n=182) of the gynaecological patients had at least one of the four specific presenting symptoms. Ten gynaecological patients were excluded because of missing information. Eleven point two percent (n=25) presented with vaginal bleeding as against 49.8% (n=111) that complained of vaginal discharge. Lower abdominal pain was the complaint of 58.7% (n=131) patients while 9.4% (n=20) complained of dysuria.

Table 1
Age Groups and Marital Status of Subjects

	Married	Divorced/ Widowed	Not married	Total
16-20yrs	24		9	33
21-25	104		20	124
26-30	133	5	6	144
31-35	90	5	5	100
36-40	62	5	4	71
41+	21	7		28
Total	432	22	44	500

Table 2:
Prevalence of Pathogens by Pregnancy Status.

	Pregnant (%)	Non-pregnant (%)	Total Prevalence (%)
<i>Gardnerella vaginalis</i>	4/294 (1.4)	2/223 (0.9)	6/517 (1.2)
<i>Candida albicans</i>	117/294 (39.8)	60/223 (26.9)	177/517 (34.2)
<i>Trichomonas vaginalis</i>	10/294 (3.4)	4/223 (1.8)	14/517 (2.7)

C. albicans positive only 84 (47.5 percent) complained of vaginal discharge and 63.1 percent (n=53) of the complaints were from pregnant women. (Table 4) Four patients who tested positive for *C. albicans* and one who tested positive for *T. vaginalis* also tested positive for the Human Immunodeficiency Virus (HIV). All the six cases of multiple infections occurred in pregnant women as follows; two cases of *C. trachomatis* and *T. vaginalis*, two of *C. trachomatis* and *C. albicans*, one each of *C. albicans* and *T. vaginalis*, and *C. albicans* and *G. vaginalis*.

Discussion

Candidiasis and bacterial vaginosis are reproductive tract infections that are not usually sexually transmitted. A survey of STIs in Commercial Sex Workers in Accra, Ghana, showed that 18.9 percent (68/359) of patients seen at the STI clinic were infected with

In terms of association between presenting symptoms and clinical observation, 82.5 percent (n=184) of patients who complained of vaginal discharge actually had the discharge on clinical examination, while 59.0 percent (n=161) of those who didn't report vaginal discharge showed the discharge during examination (chi-square=27.4; p<0.01). Eight point six percent (n=43) of patients were using the combined oral contraceptive pill. (Data not shown). Table 2 shows the prevalence of the various infective agents in the study population. *Candida albicans* was isolated from 39.8 percent of pregnant women as against 26.9 percent in gynaecological patients (chi-square 9.4, p<0.01). *G. vaginalis* was significantly associated with low abdominal pain (Fisher's exact test; p=0.039). Regarding the association with the four presenting symptoms, lower abdominal pain was significantly associated with vaginal discharge ($\chi^2=17.6$; p<0.01) and with dysuria ($\chi^2=11.4$; p<0.01).

There was no infection with either *G. vaginalis* or *T. vaginalis* in any of the patients aged between 16-20 years (Table3). However the infection rate of 39.4 percent with *C. albicans* in this age group was not significantly different from most of the other age groups. Out of the 177 (34.2 percent) patients who were

C. albicans (Ghana AIDS Control Programme-Personal communication). Odds FC and co- workers isolated *Candida* from 30-40 percent of women during pregnancy and found the infection to be particularly virulent during the last trimester. ⁷ In our study *C. albicans* was the commonest pathogen (34.2 percent), a prevalence rate that is similar to what has been reported from random surveys in other antenatal clinics in Africa (35 percent in Gambia, 23 percent in South Africa, 33 percent in Nigeria and 37 percent in Swaziland). ⁸ The pathogen was isolated from 39.8 percent of our pregnant women as against 26.9 percent in non-pregnant women (p<0.01). The increased susceptibility to vaginal candidiasis during pregnancy has been attributed to the general decrease in resistance and the higher levels of estrogen during pregnancy. ⁹ For candida to be invasive, it first has to recognize and adhere to specific receptors on the cell membranes of vaginal epithelium. Estrogen

Table 3:
Age Distribution and Prevalence of BV, *C. albicans*, and *T. Vaginalis*

Age group (yr)	No of patients (%)	G. vaginalis (%)	C. albicans (%)	T. vaginalis (%)	Total no of infections (%)
16-20	33	0(0)	13(39.4)	0 (0)	13
21-25	126	2(1.6)	47(37.3)	6(4.8)	55
26-30	144	1(0.7)	53(36.8)	2(1.4)	56
31-35	100	2(2)	40(40.0)	3(3.0)	45
36-40	71	1 (1.4)	18(25.4)	2(2.8)	21
41+	28	0 (0)	6(21.4)	1(3.6)	7
Total	502	6 (1.2)	177(35.3)	14(2.8)	197(38.1)

*15 patients without age data are excluded in the analysis.

enhances candida adherence by increasing surface exposure of the glycoprotein receptors for blastopore.⁹ For one reason or another, *Candida albicans* is better able to do this than any of the other *candida species* (*C. Glabrata*, *C. tropicalis*, *C. pseudotropicalis*, and *C. krusei*),⁹ and this may explain its higher frequency in clinical settings. Patient variability in candida adherence to vaginal epithelium may also account for the variation in individual susceptibility to candida infection. The postulated role of estrogen in the pathogenesis of vaginal candidiasis may explain why it is uncommon during hypoestrogenic periods and the use of high dose oral contraceptives and pregnancy have been noted to cause higher symptomatic infection. These factors together with the higher content of glycogen in vaginal environment produce excellent conditions for increased candida growth and virulence during pregnancy.⁹

Table 4:
Candia albicans Infection and Patients' Complaint of Vaginal Discharge.

	<i>C. albicans</i> present	<i>C. albicans</i> absent	Total
Vaginal discharge -Yes	84 (37.7%)	139 (62.3%)	223
Vaginal discharge - No	93 (31.6)	201 (68.4)	294
Total	177 (34.2)	340 (65.8)	517

Our study did not show a statistically significant association between combined oral contraceptive use and infection with any of the three pathogens (Data not shown). In contrast Calzolari and co-workers observed a protective effect against bacterial vaginosis in both oral

contraceptive and condom users and a significant increase of BV among IUD users.¹⁰ In chronic recurrent cases, laboratory discrimination of *Candida* infection using the Germ tube test may be necessary to identify non-albicans candida species resistant to conventional therapy. In particular some clinical features of the vaginal discharge were significantly associated with the presence of *C. albicans* (discharge adherent to vaginal wall, chi-square 30.8; p<0.01, curd-like discharge, chi-square 41.7; p<0.01, whitish discharge, chi-square 5.7; p<0.05, creamy discharge, chi-square 19.5; p<0.01).

Fifty two point five percent (n=93) of our candida positive patients were asymptomatic. By contrast previous authors have reported approximately 20 percent asymptomatic candida infection in the vaginal samples of women in their reproductive years with a dramatic decrease in the rate after menopause.⁷ Apart from the discomfort it may cause to the parturient vaginal candidiasis may infect the neonate in up to 50 percent of cases,¹¹ with a high incidence of oral thrush that occasionally may be so severe as to interfere with breastfeeding.

Gardnerella vaginalis (*haemophilus vaginalis*) was first suggested as a sexually transmitted pathogen by Gardner & Dukes when they isolated it from the urethra of 70-90 percent of the male partners of infected females.¹² The reported prevalence of infection with *G. vaginalis* has varied depending on the population studied and rates of between 5-37 percent have been reported.^{13,14} In Nairobi the pathogen was isolated from 75 of 100 patients complaining of vaginal discharge. The prevalence of *G. vaginalis* in this study was 1.2 percent and there was an appreciable association between isolation of the organism and the patient's complaint of low abdominal pain (Fisher's exact test p=0.039). *G. vaginalis* is an important entity in the aetiology of bacterial vaginosis, a polymicrobial group of Gram negative and anaerobic bacteria including

Gardnerella vaginalis and *Mobiluncus Bacteroides*, *Prevotella*, *Peptostreptococcus*, *Porphyromonas* and *Micoplasma spp.* Previously regarded as a harmless condition, BV is associated with obstetric and gynecology complications such as chorioamnionitis, premature rupture of membranes, pre-term labor and delivery, low birth weight, postpartum endometritis, post cesarean and post-hysterectomy wound infection, post-abortion pelvic inflammatory disease as well as increased prevalence of HIV infection.¹⁶ Although our study did not suggest a significant association between HIV and *G. vaginalis*, the associated complications make prompt and effective treatment of the condition particularly in pregnancy mandatory.

Trichomoniasis, an infection with a flagellated protozoa, *Trichomonas vaginalis*, is a common sexually transmitted infection. The prevalence of infection with *T. vaginalis* has been found to correlate with the general level of sexual activity in specific population groups. In the United States, infection rates of 5 percent was found in women attending family planning clinics and among married female employees. In contrast, 13-25 percent of women attending gynecology clinics, 50-75 percent of prostitutes and 7-32 percent of women in STI Clinics in the same study were found to have trichomoniasis.¹⁷ In a study conducted in Ghana, 11.2 percent (40/358) of prostitutes were infected with *T. Vaginalis*. (Ghana AIDS control program-Personal communication). The prevalence of trichomonas infection in this study was 2.7 percent. This is consistent with the reported findings

in many countries that trichomoniasis in women attending gynecology clinics has become less commonly seen,¹⁸ an observation that may in part be due to the widespread availability of effective treatment. It is worthy of note that in Sweden, a 5.4 percent prevalence of trichomoniasis was reported in 1980 and over a period of seven years the rate in the same community had dropped to 0.7 percent.¹⁸

That all the multiple infections occurred in pregnant women may in part be due to the increased susceptibility to vaginal candida infection during pregnancy. This study has described the current status of *G. vaginalis*, *C. albicans*, and *T. vaginalis* in gynaecological and antenatal patients who attended a tertiary institution in Ghana. The low prevalence of *G. vaginalis* and *T. vaginalis* may have been the result of self-medication and requires further research in primary health institutions in rural areas to compare the rates. The absence of association between the presenting symptoms, clinical findings and specific pathogens has implications for the syndromic approach to STI case management.

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