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Isolation of *Trichomonas vaginalis* and *Gardnerella vaginalis* from high vaginal swabs among female students in federal polytechnic Idah, Kogi state, Nigeria

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ABSTRACT

Trichomonas vaginalis is a flagellated parasite, causing sexually transmitted infections such as pelvic inflammatory disease and adverse pregnancy outcomes in women. It is usually found with other sexually transmitted infections such as gonorrhea, syphilis and herpes simplex virus type II infections as a sensitive maker of high sexual behavior. *Gardnerella vaginalis* is a facultative anaerobic gram-variable rod that is involved in bacterial vaginosis in some women as a result of a disruption in the normal vaginal microflora. This study was carried out to isolate, identify and also to determine the antibiotic susceptibility of *T. vaginalis* and *G. vaginalis* in female students of Federal Polytechnic Idah, Kogi State. Vaginal swabs were collected randomly thirty (30) female students and examined microscopically by wet mount and culture techniques. *T. vaginalis* was identified in five (5) samples by wet mount while twenty five (25) samples were positive for *G. vaginalis*. Since *T. vaginalis* and *G. vaginalis* are common sexually transmitted infections, the screening and treatment of sex partners of infected women must be done as a public health measure to prevent re-infection and reduce infection rates and complications in males.

Keywords: *Trichomonas vaginalis*, *Gardnerella vaginalis*, sexually transmitted infections, vaginal microflora, Kogi State

ABSTRACT

Trichomonas vaginalis is a flagellated parasite, causing sexually transmitted infections mainly in women. It is usually found with other sexually transmitted infections such as Chlamydia, gonorrhea, syphilis and herpes simplex virus type II and is a sensitive maker of highly sexual behavior. It causes pelvic inflammatory disease and adverse pregnancy outcomes. *Gardnerella vaginalis* also is a facultative anaerobic gram-variable rod that is involved, together with many other bacteria, mostly anaerobic in bacterial vaginosis in some women as a result of a disruption in the normal vaginal microflora. Hence, this study is carried out to isolate, identify and antibiotic suscesptibility test of *G. vaginalis* by laboratory culture test. The study was conducted on 30 female students of the Federal Polytechnic Idah. Vaginal swabs were collected randomly and examined microscopically by wet mount and culture. Out of 30 patients *Trichomonas vaginalis* was identified in 5 patients by wet mount and 25 patients were culture positive for *Gardnerella vaginalis*. Culture was the gold standard method than wet mount examination. Since *T. vaginalis* and *G. vaginalis* are common sexually transmitted infection, the screening and treatment of sex partners of infected women must be done as a public health measure to prevent reinfection and reduce infection rates and complications in males.

INTRODUCTION

The adult human vagina is a complex biota containing a profusion of microorganisms. These can be either unicellular or acellular and are present everywhere in nature. They include bacteria, fungi, archaea, protists, some microscopic plants such as green algae and animals such as planktons and planarian [1]. Bacteria and yeast form normal flora of this ecosystem, which is normally found on the skin and every opening of the body such as mouth, ears, rectum and vagina. Even a neonate carries specific flora of his/her mother and soon develops own floral community. This flora persists till death of the individual. An adult human carries normal floral consisting of more than 200 bacterial species. Normally, these are harmless and are involved in benefiting their hosts. Yet some are parasitic in nature, living at the expense of their hosts, and some are even pathogenic, disease causing [1]. These pathogenic microbes, after getting a chance, invade their host and lead to opportunistic infection. These diseases caused by normal flora are termed as endogenous diseases [2]. Urinary tract is regularly flushed with sterile urine and its acidity, there it makes difficulty for microbes to access and establish here but anterior urethra is inhabited by relatively constant flora such as *Staphylococcus epidemidis*, *Enterococcus faecalis* and some Alpha-hemolytic streptococci [3]. Occasionally, some enteric bacteria like *E. coli*, *Proteus* and *Corynebacteria* may be found there. Vagina tract is colonized by *Corynebacteria*, *Staphylococci*, *Streptococci*, *E. coli* and Doderlein's bacillus (*Lactobacillus acidophilus*) after birth [4]. During reproductive life vaginal epithelial contains glycogen which is metabolized by *Lactobacillus acidophilus*. The lactic acid and other products of metabolism inhibit colonization of offending agents in that area and allow only *Lactobacillus acidophilus* and some other lactic acid producing species to grow [5]. The oral contraceptives, steroids and antibiotics disrupt either the normal flora or naturally acidic pH. Reduction in lactic acid leads toward high pH of vaginal epithelium which encourages other offending agents to grow and results in embarrassing vaginal odour (sometimes described as smelling "fishy"), abnormal discharge (often thin and white grey in colour) and discomfort (normally irritation or soreness in and around vagina). Vaginal discharge is a term given to the biological fluid contained within or expelled from vagina. Normally this discharge demonstrates various phases of menstrual cycle but it may also be due to vaginal infections. Thus vaginal infection is checked by the presence or absence of these offending microorganisms in a vaginal discharge. The major three kinds of vaginitis are vaginal candidiasis, bacterial vaginosis (BV) and Trichomoniasis. At a time a women may have any vaginal co-infections. Untreated vaginal infection may lead to any complication especially in pregnant women [6]. Antibiotic sensitivity is a term used to describe the susceptibility of bacteria to antibiotics. Antibiotic sensitivity testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection in vivo [7]. Testing for antibiotic sensitivity is often done by the Kirby-Buer method while other methods include the stokes method, E-test (also based on antibiotic diffusion) and Agar and Broth dilution methods (for minimum inhibitory concentration determination). Muller Hinton agar is most frequently used in this antibiotic susceptibility test. The common organisms implicated in bacterial vaginosis include *Gardnerella vaginalis*, *Mycoplasma hominis* and anaerobic bacteria such as *Peptostretococci*, *Pseudotella* spp. and *Mobiluncus* spp. [8]. Aerobic vaginitis is the accumulation of aerobic organisms such as *E. coli*, group B *Streptococci*, etc in the vagina [9]. Aerobic vaginitis like bacterial vaginosis, causes depletion of normal bacteria flora *Lactobacillus*. It is clinically characterized with red and inflamed sensation and dyspareunia [9]. It has been implicated in pregnancy complications, preterm delivery, preterm rupture of membrane and ascending chorioamnionitis [11]. Infection of the genitor-urinary tracts or reproductive tract infections are a major problem of women of sexual health. They are commonly seen in women of reproductive age and usually present with vaginal discharge [11]. The majority of these diseases are the four most common ones like gonorrhea, chlamydia, syphilis and trichomoniasis [12]. Bacterial vaginosis is the invasion of the vagina with anaerobeic bacteria organisms. It occurs when there is alteration of the vaginal ecology with depletion of the normal bacteria flora lactobacilli with overgrowth of anaerobic polymicrobial organisms [13]. It is the commonest form of vaginal infections in women of reproductive age and constitutes almost 40% of cases in women attending sexually transmitted disease clinic [14]. In pregnant women, it constitutes almost 30% of all cases [15]. Genital tract infections are one of the most common reasons why women seek gynecological advice. In the last few decades there has been an increase in these infections, vis-a-vis other sexually transmitted disease (STDs) [16]. Therefore this study evaluated the occurrence *T. vaginalis* and *G. vaginalis* in non-pregnant females, its antibiotic sensitivity and risk factors.

MATERIALS AND METHODS

Study Area

Idah an old river port town, lies on a sandstone cliff on the east bank of the River Niger, in the north central (middle belt) region of Nigeria. On the west bank of the Niger is Aganebode in the geografitical zone of the country, which serves as the capital and ancestral citu of all Wepprawanno (predominantly Etsako speaking) people and as the administrative headquarters of Etsako East Council. Modern Idah remains a major trading centre (palm produce, yams, cassava, rice nd fish) on the river Niger. Beside trade and farming the local population is engaged in making canoes, fishing nets and soap, hand crafts and cott on weaving are also significant. As was the case with most Africa

setting the Igala people adhere to the three main religions in Nigeria. Traditional African religion, Christianity religion in Igala land of which Idah remains the nucleus. Idah people are good observers of moral ethics such as respect for elders, decent dressing and table manners among others.

Samples Collection

A total of 30 High Vaginal Swab (HVS) samples were collected from different patients. Samples were collected mainly from different female students of Federal Polytechnic Idah, Kogi State. High vaginal samples were obtained from patients by using a sterile swab stick. The swab stick was inserted into the vagina and then used to collect the samples by gently rotating it around the site of the cervix. The samples were brought to microbiology laboratory of Federal Polytechnic Idah for further biological analysis, on ice pack.

Examination of Samples for *T. vaginalis*

Trichomonas vaginalis was identified by making wet mounts of vaginal swabs and viewing for viable organism under x10 and x40 objectives of the light microscope. Wet mount was also observed microscopically for evidence of epithelium clue cells.

Wet Mount

- A drop of normal saline was placed on a clean grease free slide
- The sample was emulsified on it and a cover slip was placed over it.
- Microscope observation was done under low power (10x) objective and later under high power (40x) objective.
- A positive wet mount was confirmed when pear shaped organisms with whip like motility were observed.

Isolation of *G. vaginalis*

Culture media used for the isolation of *G. vaginalis* were Nutrient Agar, Blood Agar and Chocolate Agar. The media were prepared under sterile condition and poured into sterile plates. The streaking of swab was done on the media prepared plates in sterile environment. Test plates were incubated at 37^{O}C for 24hours.

Preparation of Pure Culture

After 24 hours of incubation, the discrete colonies presumptive of *G. vaginalis* were picked and inoculated on fresh sterile media.

Morphological Identification

Gram Examination

The gram reaction was used to classify the isolates into gram positive and gram negative bacteria after examining the agar plates.

Biochemical Test

The biochemical test carried out are catalase test, oxidase test, urease test and indole test.

Catalase Test

A drop of 3% hydrogen peroxide was added to isolate following 48 hours growth on chocolate agar. The presence of gas bubbles indicated catalse production (positive reaction). No release of bubbles showed negative reaction (no catalase produced).

Oxidase Test

A filter paper impregnated with 2 – 3 drops of 1% tetramethyl-phenylene-diamine dehydrochloride (oxidase reagent) was placed in a petridish and a loopful of 48hours growth of *G. vaginalis* was smeared across the impregnant paper. A positive reaction was shown by the development of dark-purple colour within 10 seconds

Urease Test

G. vaginalis culture isolate was inoculated unto Christensen's agar slope and incubated aerobically at 37^{O}C and examined daily for 5 days.
The presence of a red colour indicated positive reaction

Indole Test

Kovac's colour reagent was added to 1ml of 48 hours *G. vaginalis* culture isolate. The presence of a red colour on the reagent layer on standing for 1 minute indicated indole production.

Antibiotic Susceptibility Testing

- Pure isolated colonies (3 – 5 colonies) were picked using a sterile wire loop and emulsified in 3 – 4ml of sterile physiological saline.
- The bacterial suspension was adjusted to 0.5 McFarland turbidity standards
- The diluted bacterial suspension was transferred to Mueller-Hinton agar plate using a sterile swab and streaked evenly over the surface of the medium in three directions, rotating the plate approximately 60^{O} to ensure even distribution
- With the petri-dish lid in place, it was allowed 3 – 5 minutes (no longer than 15 minutes) for the surface of the agar to dry

- After the inoculums were dried, antibiotic discs(Ciprofloxacin, Sparfloxacin and Pefloxacin) were applied on the surface of the inoculated plates using sterile forceps
- Within 30 minutes of applying the disc the plates were inverted and incubated aerobically at 35^{O}C for 24 hours.

Preparation of 0.5 McFarland Standard

(A) 1g = 100ml distilled water

(B) 1ml of concentrated H_2SO_4 + 99ml of distilled water = 100ml

0.5ml of (A) was added to 99.5ml of (B) and mixed properly to obtain 0.5 MacFarland.

RESULTS

Distributions of *T. vaginalis* and *G. vaginalis* in FPI Students

Out of 30 patients, *T. vaginalis* was identified in 5 patients by wet mount and 25 patients were culture positive for *G. vaginalis* (Table 1).

Table 1: Distribution of *T. vaginalis* and *G. vaginalis* among FPI Students

Organisms	Number screened	Number positive	Prevalence rate
T. vaginalis	30	5	16.6%
G. vaginalis	30	25	83.3%

Biochemical Tests for *G. vaginalis* Culture Isolates

The biochemical test of the culture positive sample of *G. vaginalis* s showed that all the isolates showed negative reaction catalase, oxidase, urease and indole (table 2).

Table 2: Biochemical Tests for *G. vaginalis* Culture Isolates

Tests	Results
Catalase	-ve
Oxidase	-ve
Urease	-ve
Indole	-ve
Key: -ve = Negative	

Sensitivity Test for *G. vaginalis* Culture Isolates (mm)

Sensitivity test for *G. vaginalis* isolates showed that *G. vaginalis* was sensitive to ciprofloxacin, sparfloxacin and pefloxacin (table 3).

Table 3: Sensitivity of *G. vaginalis* isolates to Ciprofloxacin. Sparfloxacin and Pefloxacin

Test Result	No of samples		
	Cipro	**Spar**	**Pef**
+	**07**	**06**	**08**
++	**10**	**12**	**11**
+++	**08**	**07**	**06**
Total	***25***	***25***	***25***

Key: Cipro (ciprofloxacin); Spar (sparfloxacin); Pef (pefloxacin); + = sensitive
++ = very sensitive; +++ = Highly sensitive

DISCUSSION

Vaginal discharge is a common health problem among women in the reproductive age. Whether asymptomatic or symptomatic, it is usually neglected by women making the diagnosis more difficult.The incidence of pathogens in vaginal discharge varies in different regions of the world [17]. Although precise data are difficult to obtain, the prevalence of genital infection can lead to considerable morbidity and mortality among neonates and their mothers [18]. High vaginal swabs were collected from 30 patients with symptomatic genital tract infection and abnormal vaginal discharge, swabs were examined microbiologically for causative pathogens (*Trichomonas vaginalis* and *Gardenerall vaginalis*). In this study, the distribution of *Gardenerella vaginalis* infections confirmed was 83.3% which is higher than *Trichomonas vaginalis* which is 16.6% (Table 1). However, the result is similar with that of [19], who reported a 68.7% prevalence of genital infection among women in Buea, Cameroon. *Gardnerella vaginalis* was

common among the study participants with the prevalence of 83.3% which is higher than the 15.2% reported by [20] and 17.6% reported by [21]. The prevalence of *Trichomonas vaginalis* was 16.6% which is higher than the 10.6% reported by [20]. These variations in prevalence are related to the differences in the type of techniques used in the isolation of the pathogens, and the presence or absence of symptoms in the study participants. Biochemical tests for *G. vaginalis* culture isolate (table 2). All the tests, catalase, oxidase, urease and indole shows negative reactions. Similar results were observed in one study by [22] in which biochemical test for *Gardnerella* culture isolate was negative for catalse, oxidase, urease and indole test. Table 3 showed sensitivity test for *G. vaginalis* culture isolates, *G. vaginalis* culture isolate were sensitive to ciprofloxacin, sparfloxacin and pefloxacin.

CONCLUSION

The results of this investigation revealed that *Gardnerella vaginalis* was high with the prevalence of 25 (83.3%), followed by *Trichomonas vaginalis* with the prevalence of 5 (16.6%). *G. vaginalis* infection recorded in this study demands that patients with symptom are investigated thoroughly and a routine screening of HVS (High Vaginal Swab) of female students should be carried out to prevent or cure the infections among female students. There should be an improved level of hygiene on the campus and its environs to reduce reproductive tract infection. Multiple sexual partners should be discouraged and avoided. The use of protective barrier e.g. condoms should be encouraged. Douching with antiseptics should be avoided. The general public should be educated on the danger in taking unprescribed drugs to discourage or stop indiscriminate use of antibiotics. Any forms or cases of genital tract infections should be promptly reported at the school's medical centre for laboratory investigation and proper treatment. Government should help to provide free testing centre to avoid complications that may arise from untreated cases.

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