

Prevalence Of Bacterial Vaginosis Among Female Students Of Michael Okpara University Of Agriculture, Umudike, Abia State, Nigeria

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Abstract: The prevalence of bacterial vaginosis in both self reported symptomatic and asymptomatic female students of the Michael Okpara University of Agriculture, Umudike, was investigated. Two hundred high vaginal swabs were collected, cultured and their susceptibility to various antibiotics was determined. Out of 200 samples examined, 148 (74%) had one form of microbial organism or the other, ranging from bacteria to fungi; bacteria making up to 104 of the isolates while 44 isolates were of fungal infection. Fifty two (52) patients representing 26% had none. The frequency of isolation of organism was *E. coli* 68 (46.0%), Yeast, 44 (29.7%) and *Staphylococcus aureus* 36 (24.3%). Almost all the patients who practiced douching with soap and antiseptics had more than 90% of the symptoms of vaginal itching, dour and discharge. This shows that there is significant effect of douching method on the various indications for BV. The low sensitivity (28.1%), low positive predictive value and high specificity (65%), using vaginal discharge as a gold standard, pointed more to bacterial vaginosis. The most effective antibiotic against *E. coli* isolates was ciprofloxacin, 52 (76.5%) and tarvid, (77.9%), while tetracycline 2 (3.0%) was the least effective. *Staphylococcus aureus* isolate was most sensitive to ciprofloxacin 33 (91.7%) whereas they were resistant to cotrimoxazole and nalidixic acid, 0 (0%) each. This study emphasizes the need for routine HVS examination among the female students, the need to stop the practice of douching especially with antiseptics and also on the importance of restriction of indiscriminate use and abuse of antibiotics to forestall resistance.

Keywords: Prevalence, Bacteria Vaginosis and Fungi and Sensitivity.

I. Introduction

Bacterial vaginosis (BV) is the most common cause of vaginal discharge among women in reproductive age with a prevalence of 9-37%, depending on the population studied (Goldberg et al., 1996). The prevalence of vaginal infections, particularly BV, is high in many countries in Sub-Saharan Africa. For example, 20% to 50% of women of reproductive age are affected in Zimbabwe (van De Wijgert et al., 2000). It is characterized by a disorder of the vaginal ecosystem characterized by a change in the vaginal flora from the normally predominant lactobacillus to one dominated by sialidase enzyme-producing organisms.

The vagina is a complicated environment containing a number of microbial species in variable quantities and relative proportions (Mumtaz, et al., 2008). A complex and intricate balance of microorganisms maintains the normal vaginal flora and changes with a multitude of events in the patient's life (Cook, et al., 2001). The dominant microbial species is *Lactobacillus*, which maintains the generally acidic vaginal pH (Khan and Khan, 2004).

The presence in the vagina of other bacteria such as *Gardnerella vaginalis*, group B streptococci and *Escherichia coli* termed commensal bacteria (Laren and Monif, 2001) is not synonymous with infection (Cook, et al., 2001). According to Hammill (1989), the incidence of *E. coli* in the vagina of normal, pre-menopausal, non-pregnant, asymptomatic women is about 21%. However, vaginal *E. coli* may also cause symptomatic infections such as vaginitis or tubo-ovarian abscess and is associated with life-threatening neonatal sepsis (Percival-Smith, 1983).

According to Larsen and Monif (2001), *G. vaginalis* was isolated from the vaginal samples obtained from 150 of 446 women who visited a student health center and who were free of clinically overt disease. During the past several decades, the many published survey of vaginal flora specimens obtained from asymptomatic women have clearly shown that *Candida albicans* may be present without the typical symptoms of yeast vaginitis (Glover and Larsen, 1998). Bacteria that are normal constituents of the vaginal flora of the host

have the potential to cause symptoms of disease, but they apparently require some alteration in the microenvironment to do so (Larsen and Monif, 2001).

Other microorganisms such as *Neisseria gonorrhoeae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Listeria monocytogenes* and *Trichomonas vaginalis* are not ordinarily part of the flora of the female genital tract, but they bring the potential for disease to the vaginal/endocervical area by virtue of their inherent biological properties, (Larsen and Monif, 2001). Although the presence of these properties do not guarantee that disease will occur.

The frequent cause of vaginal discharge is an infection or colonization with different microorganisms (Mylonas and Friese, 2007). Vaginitis, whether infectious or not, poses one of the most common problems that lead women to seek out an obstetrician or gynecologist (Adad et al., 2001; Mumtaz et al., 2008), in approximately 10 million office visits annually (Kent, 1991; Donder et al., 2002). The tradition of diagnosis of vaginitis which involves patient's symptoms, clinical findings observed during vaginal examination, and laboratory analysis of vaginal fluid and treatment can be elusive, leading to lack of relief from the symptoms (Schaaf, et al., 1990; Bornstein et al., 2001).

Although some pathologic conditions causing vaginitis are well defined like bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis yet, 7-72% of women with vaginitis may remain undiagnosed and such forms of abnormal vaginal flora neither considered as normal, nor can be called bacterial vaginosis have been termed as 'intermediate flora' and its management probably differ from that of bacterial vaginosis (Vigneswaran and McDonald, 1994; Mumtaz et al., 2008).

Data on asymptomatic lower genital tract infections among females are sparse. In a prospective study of genital tract infections in a family planning clinic (among the women with both signs and symptoms), *Trichomonas vaginalis*, *Candida albicans* and bacterial vaginosis were equally prevalent (Riordan et al., 1980). Also *Neisseria gonorrhoeae* was isolated from 4% of women with, and 1% of those without symptoms. Klufio et al., (1995) reported the presence of *Candida* in 48 women, *Trichomonas vaginalis* in 39 and bacterial vaginosis in 48 in the study of prevalence of vaginal infections (with bacterial vaginosis, *Trichomonas vaginalis* and *Candida albicans*) among pregnant women at the Port Moresby General Hospital Antenatal clinic.

In a study of bacterial vaginosis and lower genital tract infections in women attending out-patient clinic at a tertiary institution serving a developing community, Kharsany et al., (1999) detected vaginal infections in a total of 104 women out of 208 examined, endocervical infections alone in 18 and concurrent vagino-endocervical infection in 41, bacterial vaginosis was diagnosed in 73 women. *Trichomonas vaginalis* was detected significantly more often in women attending the sexually transmitted disease (STD) and antenatal clinics. Also Microorganisms such as *Gardnerella vaginalis*, *Mycoplasma hominis*, anaerobes and curved Gram negative rods were found in significant higher number of women with bacterial vaginosis. In a study to investigate the possible role of microorganisms in vulvovaginitis among 50 virgin girls; Mahdi and Maysoun (2001) reported *Staphylococcus aureus* (10%) *Enterococcus faecalis*(10%) and *Esherichia coli* (8%) as the most common pathogens. Eggs of *E. vermicularis* and trophozoites of *T. vaginalis* were recovered at a rate of 4% and 6% respectively. However, *Candida* was diagnosed at a rate of 8%. Non specific vulvovaginitis was exceedingly common (28%). Akerele et al., (2002) investigated the prevalence of asymptomatic genital infection among pregnant women in Benin City, Nigeria. They reported a significant microbial growth in 300 samples, giving a prevalence rate of 60% for asymptomatic genital infection. *Candida albicans* (65%), *Staphylococcus aureus* (51.8%) and enterobacteriaceae (*E. coli*, *Klebsiella* species) were predominately isolated, followed by *Trichomonas vaginalis* and *Neisseria gonorrhoea*. Most of the isolated bacteria were susceptible to ciprofloxacin, ceftazidime, cotrimoxazole, norfloxacin and augmentin. All the isolates, except *Streptococcus faecalis* were resistant to ampicillin. In a related study by Anh et al., (2003) to determine the prevalence of lower genital tract infection (LGTI) with *Candida* sp. among 1000 symptomatic and asymptomatic women attending maternal and child health family planning clinic in Hanoi, Vietnam, the overall prevalence of *Candida* sp. was 11.1%, *T. vaginalis* (13.1%), non gonococcal infection was found; the prevalence of *Chlamydia trachomatis* was 4.4% and of bacterial vaginosis (3.5%). They reported that prevalence of these infections was quite similar, both in the asymptomatic and the symptomatic group.

AIMS AND OBJECTIVES

This study therefore was aimed at:

- determining the prevalence of BV among women self-presenting with vaginal discharge at Michael Okpara University Umudike attending the institution's Medical Centre,
- investigating the microorganisms responsible for the prevalence of symptomatic and asymptomatic bacterial vaginosis,
- determining the antibiotic susceptibility pattern of the isolates to commonly used antimicrobial agents.

II. Materials And Methods

STUDY POPULATION

The study was comprised of 200 female students of the Michael Okpara University of Agriculture, Umudike, Abia State aged between 17-29 years old. All potential eligible patients were interviewed and informed consent was obtained. Demographic data regarding their age, genital tract infections, douching history and methods used and usage of antibiotics were collected. Clinical data relating to vaginal discharge, vaginal odour and vaginal itching or their absence were collected. The female students were excluded if they were placed on antibiotics or are already undergoing treatment with any symptomatic pelvic inflammatory disease (PID) or any other genital tract infection for at least 2 weeks prior to the study. Ethical committee of the University's Medical Centre gave approval for the work to be done in the Centre.

SAMPLE COLLECTION

A sterile swab stick was used to collect the vaginal swabs. Self-collected vaginal samples were obtained twice weekly. Participants were instructed to insert the vaginal swab 1–2 inches into the vagina, twist the swab to collect material on all sides of the cotton tip, wipe in several full circles on the vaginal wall, keep in the vagina for 20 sec, and then roll each swab across a slide and allow the material to air-dry. Each sample was labeled with a serial number and kept at a temperature of 4°C for onward transmission to the laboratory not more than 2h after collection for analysis. Samples were collected between March to July 2010.

MICROBIOLOGICAL ANALYSIS

Swabs were rinsed in 9ml of a quarter Ringer's solution in a test tube to make a dilution of 10^{-1} . Serial dilution was carried out by transferring one ml from 10^{-1} dilution into a fresh tube of 9ml of Ringer's solution to make 10^{-2} dilution. The samples were inoculated on blood, MacConkey's agar and chocolate agar according to standard protocol as described by Cheesbrough (2000) after which the Petri dishes were incubated at 37°C for 24h, while the Chocolate agar media were anaerobically incubated. Duplicate plates of plate count agar (PCA) were inoculated from each dilution and incubated aerobically at 37°C for 24h. Colony counts yielding bacterial growth of 10^5 cfu/ml or more of pure isolates per ml were recorded as showing significant growth.

Morphologically different colonies from the plate count were subcultured repeatedly into nutrient agar slants by streaking and incubated at 37°C for 24-48h.

BIOCHEMICAL CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES

The bacterial colonies were examined for appearance based on shape, elevation, edge and pigmentation. The bacterial isolates were characterized according to the methods described by Cheesbrough (2000) in which the colonial characteristics on Plate Count Agar (PCA) and MacConkey agar, Gram reaction and biochemical tests such as catalase, coagulase, indole, citrate utilization, urease activity, oxidase, methyl red, Voges-Proskauer and motility were conducted.

Gram Reaction

The Gram reaction was used to classify the isolates into gram positive and gram negative bacteria after examining the agar plates. A thin smear of young bacterial culture (18-24 hours old) was made on a clean grease free glass slide, it was allowed to air dry, then heat fixed by passing it through a Bunsen burner flames about 3 times. The heat-fixed smear was then covered with crystal violet stain for 30-60 seconds. The stain was quickly washed off with clean water. The water was tipped off and the smear was covered with Lugol's iodine for 30-60 seconds. The iodine was washed with clean water. The smear was decolorized rapidly for about 20 seconds with 95% ethanol. The smear was quickly washed with clean water and then covered with dilute carbol fuchsin for 30 seconds. The stain was washed off with clean water and the slide was allowed to dry at room temperature.

The gram stained slide was examined first with x40 objective lens to check for the staining and distribution of the gram stained bacteria, then with oil immersion objective lens (x100) to look for the bacteria. Gram positive bacteria appeared purple while gram negative appeared red or pink.

Motility Test

This test was used to detect motile bacteria using the 'hanging drop' method. A little petroleum jelly was placed round at the centre of a clean grease free cover slip. Then a drop of normal saline was placed in the ring. The bacteria culture was emulsified on the saline placed at the centre of the cover slip. Then, the slide was carefully placed on the cover-slip and quickly inverted (with the cover-slip sticking at the glass slide by means of the petroleum jelly). The slide was viewed using x40 objective lens at reduced illumination for maximum contrast. A directional movement of the organism is a positive test.

Catalase Test

A sterile wooden spatula containing a good growth of the organism was dipped into 2-3ml hydrogen peroxide contained in a test tube. The pressure of gas bubbles indicates a positive reaction (catalase produced). No release of bubbles showed negative reaction (no catalase produced).

Coagulase Test

The slide method described by Cheesbrough (2000) was used for the test. A drop of normal saline was placed on each end of a slide. An 18-24 hours old culture of test organism was emulsified in each of the drops to make two thick suspensions. Thereafter, a drop of human plasma was added to one of the suspensions. The mixture was stirred for about 5sec. Clumping of the organism within 10sec. is a positive test. No plasma was added to the second suspension which is the control to differentiate any granular appearance of the organism from true coagulase clumping.

2.4.5 Urease Test:

The nutrient agar incorporated with urea and phenol red indicator was inoculated with the suspected colony. The culture was incubated at 37⁰C for 24 h. A positive urease test was shown by a change in colour of the agar from pale yellow to red.

Oxidase Test

Two drops of freshly prepared oxidase reagent (1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride) were made on a clean filter paper in a Petri dish. Then, a sterile wooden stick was used to collect the test organism and smeared on the portion of filter paper dampened with oxidase reagent. The appearance of blue-purple colour within 10seconds indicates a positive reaction.

Indole Test

An aliquot of 1.5% sterile peptone water in a test tube was inoculated with the test bacterium culture and incubated for 48hours at 30⁰C. Kovac's reagent, 0.5ml, was added. A red colour indicated positive indole test.

Citrate Utilization

Simmon's citrate agar was prepared based on manufacturer's instruction, sterilized, poured and allowed to solidify at an angle of 45⁰C. The test organism was streaked on the surface of the agar slant and then incubated for about 5days at 37⁰C. A change in the colour of agar medium from green to blue following growth of the organism on the slant indicated a positive test, while the absence of growth and colour change indicated a negative test.

Methyl Red Test

Two (2) ml of sterile glucose phosphate peptone water was inoculated with the bacterial isolates and incubated at 37⁰C for 48hours. 3-5drops of methyl red indicator was added, mixed and read immediately, a bright red colour indicated a positive test indicating acidity, while yellow colour indicated a negative test.

Voges-Proskauer Test

Two (2) ml of sterile glucose phosphate peptone water was inoculated with the bacteria culture and incubated at 37⁰C for 48 h. Then, 1ml of 40% potassium hydroxide was added followed by 3ml of 5% alcoholic alpha-naphthol. The test tube was mixed very well and observed for colour change. A pink-red colour within 2-5 minutes shows a positive test.

Sugar Fermentation Tests

A 5ml of 1.5% peptone water was added with 5ml of 0.2% phenol red indicator and sterilized by autoclaving at 121⁰ C for 15 minutes. Thereafter, 0.1ml of 10% of sterile sugar to be tested was added to the medium aseptically; the sugar was sterilized separately by filtration. The test organism was inoculated into the growth medium and a sterile inverted Durham tubes introduced into each test tube and incubated for 24 h at 37⁰C. The tubes were observed for acid and gas production. Acid production gave a red to yellow colour change, while gas production was identified by air displacement in the inverted Durham tubes.

Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined by the agar diffusion technique as described by Baker and Breach (1980) and antibiotic discs (Abtek Biologicals Ltd). A sterile cotton swab dipped into the broth culture of isolate was streaked evenly all over the surface of Mueller Hinton Agar and antibiotic disk was placed aseptically on the inoculated plates using sterile forceps. The plates were then incubated for 24hours at 37⁰C. Isolates were considered as sensitive or resistant to an antibiotic according to the diameter of inhibition zone interpretative chart (Clinical Laboratory Standards Institute 2006).

Determination of Minimum Inhibitory Concentrations (MIC)

Ampicillin was used as the commonly used antibiotic against E. coli for the determination of MIC. This was done using the paper disc method (Oloke, 2000). Sterile paper discs were dipped into different concentrations of antibiotics prepared. Sterile paper disc was dipped into water as control. The soaked discs were each layered on Mueller Hinton Agar plates already seeded with an 18 h. broth culture of the isolate in

duplicate. Each plate was incubated at 37°C for 24 h. This was examined for zones of inhibition. The lowest concentration of antibiotic which inhibited growth was taken as the MIC.

Abtek Laboratories UK has the following Antibiotic Discs below:

Ampicillin(10µg)	Ampicillin(25µg)
Chloramphenicol (10µg)	Gentamycin (25µg)
Cloxacillin (5µg)	Tetracycline (25µg)
Erythromycin (5µg)	Colistin(25µg)
Gentamycin (10µg)	Streptomycin (25µg)
Penicillin (1iu)	Nalidixic acid (25µg)
Streptomycin (10µg)	Nitrofurantoin (200µg)
Tetracycline (10µg)	Cotrimoxazole (25µg)

III. Results

Two hundred female undergraduates of Michael Okpara University of Agriculture, Umudike who attended the University's medical centre were examined for bacterial vaginosis. Patients were predominantly aged 17 to 29 years of age. Samples were collected based on three age group brackets of 17-20, 21-24 and 25-29 years with a mean age of 23 years. The results of demographic data stratified by the douching methods, and number of sexual partners were compared with the different age groups as shown in Table 1.

A total of 97 out of the 200 student patients douched with soap, 28 with antiseptics while 75 douched with ordinary water. Out of 69 patients examined within the age group 17-20, 46 had one (1) sex partner, 8 with 2 sex partners while 15 had no partners.

The clinical indications for bacterial vaginosis as observed in this study are shown in Table 2. A large number of the students (71: (35.5%) out of the 200 presented with one form of symptom or the other. Twenty three (23) presented with vaginal odour, (20) had vaginal discharge, and 28 with itching. Of this numbers, 44(65.2%) douched with soap, 22 (30.4%) with antiseptics and 5 (3%) douched with water. This result indicates that there was a significant effect ($P \geq 0.5$) of douching on the various indications for bacterial vaginosis with higher values recorded for douching with antiseptics.

Table 3 also show the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPP) of symptoms associated with BV using vaginal discharge as the gold standard. The sensitivity was calculated as 28.2%, specificity as 65.0%, PPV as 22.0% and NPP as 72.0%.

The result of the microscopic examination of the samples of the vaginal swabs is reported in Table 4. The results revealed that 98 (49.0%) samples contained significant epithelial cells, 102 (51.0%) contained pus cells, while 24 (12.0%) samples showed the presence of yeast cells. Fewer numbers of samples from the patients showed scanty epithelial cells.

The distribution of microbial load from the samples is indicated in Table 5. A total of 50 samples had significant bacterial growth ($\geq 10^5$ cfu/ml) while 54 samples had scanty growth ($< 10^5$ cfu/ml).

Table 6 show the morphological and biochemical characteristics of the bacterial isolates from vaginal swab cultures of samples after growth on chocolate, MacConkey, Blood, and Nutrient agar. *Staphylococcus aureus* had a convex, smooth and light yellow colony with entire edge on Nutrient agar plates and appeared as gram positive cocci in clusters. *Staphylococcus aureus* was negative in urease, motility, oxidase, indole, citrate, Voges Proskauer and methyl red tests while it was positive in the catalase and coagulase tests, including the various sugar fermenting tests with glucose, sucrose, lactose, maltose, and mannitol. *Escherichia coli* on the other hand, had convex, smooth and dark red colony with entire edge on MacConkey agar plates. They appeared as Gram negative straight rods on stained preparations. Biochemical tests showed that *Escherichia coli* was negative in catalase, coagulase, urease, oxidase and Voges Proskauer tests while in motility, indole and methyl red tests were positive. Sugar fermentation showed it was positive to glucose, lactose and mannitol but was unable to ferment sucrose and maltose.

Yeast isolates from the samples in Table 7 had colonial morphology of moderate, convex or oval creamy colony with entire edge. Gram reaction and cellular characteristics on Sabouraud agar shows budded gram positive cocci in stained preparations.

Table 6 shows the frequency of occurrence of the bacterial isolates from vaginal swab samples. A total of 68(65.4%) of *E. coli* was isolated out of a total of 148 isolates. *Staphylococcus aureus* occurred in a total of 36(34.6%), while yeast cells were isolated from 44 of the vaginal swab samples representing 29.7% of the total organisms isolated.

Table 7 shows the relationship between the different douching methods and the number of microbial counts recorded per method. Out of the 200 samples examined, 97 (48.5%) douched with soap, 28 (14.0%) douched with antiseptics while 75 (37.5%) with water. The microbial count of samples from those that douched with soap ranged from 10^5 - 10^7 cfu/ml. Those that douched with antiseptics were 24 (85.7%). Those who

douched with water had 50 (66.7%) microbial counts isolated (table 7). This result shows that douching by the patients had a significant effect ($p \geq 0.05$) on the number of microbial counts.

The antibiotic sensitivity pattern of the bacterial isolates as shown in table 8 revealed ciprofloxacin 52 (76.5%) and tarivid 53 (77.9%) were the most effective antibiotics for E. coli isolates, followed by gentamycin 49 (72.1%). Tetracycline was the least effective antibiotics for E. coli with resistance rate as high as 97.1% and a sensitivity of 3% ,followed by penicillins with resistance rate of 89.7%. Staphylococcus aureus isolated were most sensitive to ciprofloxacin 33 (91.7%) followed by tarivid 34 (88.9%) whereas it was resistant to cotrimoxazole and nalidixic acid with sensitivity of 0(0%) for each. All the S. aureus isolates showed 100% resistance to nalidixic acid and cotrimoxazole.

Table 1: Demographic Data Stratified by Douching Methods and Number of Sex Partners

Age range	No of vaginal swabs examined	No of partners	of sex	Douching with antiseptic soap (%)	Douching with antiseptics (%)	Douching with water (%)
		None	1	2		
17-20	69	15	46	8	31(44.9)	9(13.0)
21-24	78	10	59	9	37(47.4)	11(14.1)
25-29	53	9	39	5	29(54.7)	8(15.1)
Total	200	34	144	22	97	28
						75

Table 2: Indications for Bacterial Vaginosis

Indications	Total No. of patients with symptoms	Douching with soap (%)	Douching with antiseptics (%)	Douching with water (%)
Odour	23	15(65.2)	7(30.4)	1(4.3)
Itching	28	17(60.8)	9(32.1)	2(7.1)
Yellowish Discharge	20	12(60.0)	6(30.0)	2(10)
No symptoms		53	6	70

Table 3: Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Symptoms Associated with Bacterial Vaginosis

Test	No. of participants with the following results (N = 200)				Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	TP	FP	TN	FN				
Vaginal discharge	20	71	129	51	28.2	65.0	22.0	72.0

Key:

- TP = True Positive
- FP = False Positive
- TN = True Negative
- FN = False Negative
- PPV = Positive Predictive Value
- NPV = Negative Predictive Value

Table 4: Microscopic Examination of High vaginal swab samples (HVS)

	Significant Sample	Scanty Sample	No Sample
Epithelial Cells (%)	98 (49.0)	56 (28.0)	46 (23.0)
Pus Cells (%)	102 (51.0)	53 (26.5)	45 (22.5)
Yeasts (%)	24 (12.0)	20 (10.0)	156 (78.0)

Key:

- Significant sample = ≥ 5 Hpf (High power field)
- Scanty sample = ≤ 5 Hpf

Table 5: Distribution of Microorganisms isolated from HVS Samples

Age group	No. of vaginal swabs examined	No. of samples with significant bacterial growth	No. of samples with scanty growth	No. of samples with yeast growth (%)
17-20	69	17	14	14 (31.8)
21-24	78	23	26	17 (38.6)
25-30	53	10	14	13 (29.6)
TOTAL	200	50	54	44

Note:

Significant growth = $\geq 10^5$ (cfu/ml)

Scanty growth = < 10⁵ (cfu/ml)

Table 6: Occurrence of Microorganisms isolated from Vaginal Swab Samples

Organism	Frequency (%)	Total swab samples
E. coli	68 (46.0)	200
S. aureus	36 (24.3)	200
C. albicans	44 (29.7)	200
Total	148 (100)	

Table 7: Effect of Douching on the Microbial Count of Vaginal Swabs

Methods	Douching	No. of Patients	No. of Microbial count Isolated (%)
Douching with soap		97	74 (76.3)
Douching with antiseptics		28	24 (85.7)
Douching with water		75	50 (66.7)
TOTAL		200	148

Table 8: Antibiotic sensitivity pattern of bacterial isolates

Antibiotics	Percentage (%) of sensitivity of isolates to various antibiotics					
	E. coli (n=68)			S. aureus (n=36)		
	Sensitive	Intermediate	Resistant.	Sensitive	Intermediate	Resistant.
Gentamycin	49(72.1)	5(7.4)	14(20.9)	17(47.2)	7(19.4)	12(33.3)
Colistin	12(18.2)	11(16.2)	45(66.2)	8(21.1)	4(11.1)	24(66.7)
Nalidixic acid	14(21.1)	6(8.8)	48(70.6)	0(0)	0(0)	36(100)
Nitrofurantoin	16(24.2)	8(11.8)	44(64.7)	6(15.8)	2(5.6)	28(77.8)
Cotrimoxazole	20(30.3)	1(1.5)	47(69.1)	0(0)	0(0)	36(100)
Streptomycin	6(9.1)	2(2.9)	60(88.2)	2(5.3)	1(2.8)	33(91.7)
Tetracycline	2(3.0)	0(0)	66(97.1)	0(0)	1(2.6)	35(97.2)
Ampicillin	3(4.5)	8(11.8)	57(83.8)	1(2.6)	7(19.4)	28(77.8)
Chloramphenicol	5(7.4)	11(16.2)	52(76.5)	9(25)	4(11.1)	23(63.9)
Ciprofloxacin	52(76.5)	12(17.7)	4(5.9)	33(91.7)	2(5.5)	1(2.8)
Tarivid	53(77.9)	12(17.7)	3(4.4)	32(88.9)	4(11.1)	0(0)
Augument	10(14.7)	19(27.9)	39(57.4)	3(8.3)	3(8.3)	30(83.3)
Penicillins	4(5.8)	3(4.4)	61(89.7)	2(5.6)	1(2.6)	33(91.7)
Erythromycin	8(11.8)	4(5.9)	56(82.4)	2(5.6)	2(5.5)	33(91.7)

Key:

Sensitive: a zone within 3mm radius of that of the positive control.

Resistant: a zone of not more than 2mm radius.

Intermediate: a zone falling between the above limits.

IV. Discussion

The high number of bacterial growth observed in this study suggested an infectious process by organisms such as E. coli and S. aureus which causes a variety of suppurative (pus forming) infections and toxinoses in humans

This study revealed that 148 (74%) of the 200 female students had both symptomatic and asymptomatic lower genital tract infection .This finding suggests increased risk of infection of the female students. Earlier studies by Olartan (2006) reported a prevalence of 10% asymptomatic genital tract infection in female students' population of Nigerian University. Anh et al., (2003) reported a prevalence of 20.12% asymptomatic Bacterial vaginosis infection among women attending maternal and child health and family planning clinic in Hanoi, Vietnam. Some researchers have previously reported that BV is more common among younger women (Bukusi et al., 2006), while others like Morris et al., (2001) and Jones et al., (2007) found that risk of BV increases with age which is a proxy for cumulative sexual activity, especially, women between 15-20 years of age. BV has been associated with a variety of sexual behavior-related characteristics including young age at coitarche, life time number of sex partners, a recent history of multiple sex partners and a recent history of a new sex partner (Hay and Ugwumadu 2009). This result is very similar to the one in this study where the age bracket of the females was between 17-29 years with a mean age of 23 years. Bornstein et al., (2001) and Shobeiri et al., (2006) found out that out of 117 women with documented clinical information, the common symptom found was vaginal discharge in 39.3% followed by itching 31.6%. They further said this

number was consistent with vaginal infections. The high prevalence observed could be possibly due to increase in sexual activity among the university age group (Olartan, 2006). In this work, it was found out that out of 200 female students, 144 (72%) of them admitted having one sexual partner, while 22 (11.0%) had 2 sexual partners each. Further support for sexual transmission of BV comes from a treatment trial by Bradshaw et al., (2006), that found BV recurrence was 3 times more common among women who remained with their regular sexual partner after treatment and was significantly less likely among women who changed sexual partners. It may also be attributed to the difference in socio-economic and hygienic level of people. Some studies have found a relationship between BV and high-risk behaviours associated with sexually transmitted infections (STIs) such as early sexual debut and multiple sex partners (Larsson, et al., 2005). According to Olartan (2006), asymptomatic BV occurred relatively more frequently in females as compared with males and it is a major criterion of urinary tract infection (UTI). Fleury (1981) reported that BV is the leading cause of vaginal symptoms such as vaginal discharge and pruritus in developed communities of Rwanda. The most frequent symptom reported, according to Geisler et al., (2003) among 296 women for routine first time visit in a clinic but diagnosed to have BV was vaginal discharge (46%), with vaginal odour (28%), and genital itching (15%) This agrees with our findings in this work where 71 (35.5%) out of 200 had various degrees of vaginal odour, itching and vaginal discharge.

According to Roberta et al., (2002), most women reported douching for symptoms or hygiene, both of which elevated BV and vaginal colonization by organisms. In this work, the female students admitted using either soap or antiseptics and hence, increased the level of BV symptoms, but deferred by the fact that vaginal discharge was the least in our findings. In the work of Roberta et al., (2002), he reported that women with vaginal symptoms were found to douche and this led to elevated bacterial vaginosis. According to Ness et al., (2002) and Holzman et al., (2001), women douche following the development of BV in response to abnormal symptoms; however, in cross-sectional analyses, douching for reasons not associated with symptoms remains associated with BV. The research conducted in this work show a significant effect of douching method on the various vaginal symptoms for bacterial vaginosis. This is similar with the results of Roberta et al., (2003) in which they evaluated the relationship between risk factors such as vaginal symptoms and racial factors among black women and white women as it relates to douching, and was found out that BV increased among black women.

In the works of Muvunyi et al., (2009), there was low sensitivity and low positive predictive value (PPV) while specificity was high using vaginal discharge as a gold standard. This was similar to the findings of this research with low sensitivity (28.1), low PPV of 22.0% ($P > 0.05\%$) and high specificity of 65% for BV, also using vaginal discharge as a gold standard.

According to Smith et al., (1999) and Veeh et al., (2003), *Staphylococcus aureus* is one of the most common causes of infection, incidence of which has been steadily increasing. The vaginal mucosa of females is colonized by this organism (Schlievert et al., 2007). In the work of Gilbert et al., (2002), vaginal microorganisms associated with vaginal infection and hence BV were found to be mainly group B Streptococci, *S. aureus* and *E. coli*. McDonald et al., (1994) found *E. coli* to be important pathogens associated with mid-trimester pregnancy losses, alongside the classic bacterial vaginosis organisms. *Staphylococcus aureus* is one of the most persistent pathogen of humans and has remained one of the most common causes of infection, incidence of which has been steadily increasing (Smith et al., 1999; Butt, 2001). Olusanya and Olutiola (1984) isolated *S. aureus* (25%) from majority of healthy female students, closely followed by *E. coli* (21%). Omer and Ahmed (1992) found *S. aureus* as the commonest case in asymptomatic lower genital tract infection and hence, bacterial vaginosis followed by *E. coli*. This result is similar with the microorganisms isolated in this work but in contrast with the 65.4% and 34.5% for *E. coli* and *S. aureus* respectively in this study. This contradicts with the work of Peipert et al., (2003) where other organisms were isolated such as *Gardnerella vaginalis*, *Mycoplasma* sp. and anaerobes, but agrees with the results of Olartan (2006) who also found *E. coli* to be more prevalent. The author also isolated *S. aureus* among other micro organisms. Anh et al., (2003) reported the presence of *Candida* spp. in asymptomatic lower genital tract infection, and hence, bacterial vaginosis. Mahdi and Maysoun (2001) reported the isolation of *S. aureus* (10%) and *E. coli* (8%) as part of vaginal microorganisms associated with BV. This work varied slightly with previous work who reported a high prevalence of *S. aureus* (Akerele, 2001) and *Candida* Spp. in the vaginal flora (Mumtaz, et al., 2008). The absence of *Neisseria gonorrhoeae* in this work is consistent with the findings of Anh et al., (1996) which could be due to antibiotic usage.

The vaginal flora of adult females contains lactobacilli responsible for maintaining the vaginal pH and preventing the overgrowth of potential pathogens. This helps in reducing the frequency of infections at such age group. Antibiotics like broad-spectrum penicillins or tetracyclines can kill or suppress helpful bacteria in the genital tract, allowing resistant organisms to grow unchecked (Lowy, 1998).

Tariq et al., (2006) reported that the presence of members of faecal flora in the vagina was attributed to unhygienic bowel practice in the past. Martin et al., (1999) and Sewankambo, et al., (1997) reported that various studies across the world show that women with bacterial vaginosis (BV) are more likely to be co-infected with herpes simplex virus type-2 (HSV-2), *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and HIV. This deviated

from the result obtained in this study because HIV screening was not part of the main study. High co-infection rates with other STIs raise the possibility that BV may either increase susceptibility to STI or share a common pathway with other STIs as reported by Moodley et al., (2002). This is different from the results of this work. *Candida* spp. was the most common cause of vulvovaginitis among female children (Mandi and Mayssoon, 2001). It has been isolated from vaginal swabs in 0% to 22% of asymptomatic adults (Linder and Planteina, 1978, Mahdi and Mayssoon, 2001), 35% of adolescents aged 11 to 15 years and 26% of apparently healthy children (Mahdi and Mayssoon, 2001). Another study also indicates that there was no difference between the sexually active and non-active adolescents (Shafer et al., 1985). In Basrah, *Candida* spp. has been recovered at a rate of 13.9% and 31.1% in non-pregnant and pregnant women respectively (Mahdi and Al – Hamdani, 1998). Various douching methods were seen to be practiced by the students, highest among which were those who douched with soap, 97 (48.5%) with 74 (76.3%) organisms isolated. 28 (14%) students douched with antiseptics with 24(85.7%) organisms isolated. 75 (37.5%) douched with water with 50 (66.7%) organisms isolated. This is similar with the findings of Holzman et al., (2001) who found that vaginal douching within 2 months and above was associated with an increased prevalence of bacterial vaginosis. Fonck et al., (2001) found that, in female sex workers in Nairobi, Kenya, douching in general and douching with soap and water were both significantly associated with BV, with a significant trend for increased frequency of douching and higher prevalence of BV. The most effective chemotherapeutic agents observed against *E. coli* in this study were ciprofloxacin 52 (76.5%) and tarivid 52 (77.9%). Least activity was noted against tetracycline, for *E. coli*, with a sensitivity of 2 (3.0%) and a resistance of 66(97.1%) followed by penicillins and ampicillins. This is the same thing for *S. aureus* with resistance of 35(97.2%) for tetracycline and 33(91.7%) for penicillins. This contradicts with the work of Akhtar et al., (1997) and Mumtaz et al., (2002) who reported a 98.64% effectiveness for imipenem and 93.6% for vancomycin against *S. aureus* infection but agrees with the less effectiveness of penicillins, tetracycline and sulphonamides. The most effective chemotherapeutic agent against *S aureus* in this work were ciprofloxacin 33 (91.7%) followed by tarivid 32 (88.9%). The organism was completely resistant to cotrimoxazole and nalidixic acid 0 (0%) in each. The level of antibiotic resistance observed in this study especially penicillins and ampicillins could be attributed to the possession of the enzyme β -lactamase by these organisms leading to resistance against the main stay antibiotics for managing *S. aureus* and *E. coli* infections (Akhtar et al., 1997 and Mumtaz et al., 2008).

V. Conclusion

The high prevalence of asymptomatic bacterial vaginosis infection recorded in this study demands that patients with gynecological symptoms are investigated thoroughly and a routine screening of HVS of female students for asymptomatic lower genital tract infection be instituted as part of health care for females.

From the study, it was also found that almost all the students practiced one form of douching or the other, either with antiseptics or soap which was found to contribute to the proliferation of one form of organism or the other in the genital tract leading to BV.

VI. Recommendations

While improved level of hygiene is likely to assist in reducing asymptomatic lower genital tract infection, and hence bacterial vaginosis, the general public should be educated on the danger in taking unprescribed drugs and therefore stop indiscriminate use of antibiotics. Over congestion of facilities especially the conveniences should be avoided in order to reduce the spread of this bacterial and yeast infections among the female students. The use of condoms or total abstinence should be encouraged among the female undergraduates. This will help in reducing sexually transmitted infections including BV.

The females should always clean from forward to backwards after using the toilet so as not to transfer the micro organism from the bowel to the vagina. This will help reduce the high rate of asymptomatic bacterial vaginosis infection among university female students.

The female students should be made to be aware of the dangers of douching, especially with antiseptics as they have, over time, believed it's a normal and a healthy form of maintaining hygiene within the genital region.

Reporting of any form of symptoms of lower genital tract infections such as burning vaginal sensations, yellow, creamy or whitish vaginal discharge with foul or offensive, fishy odour and itching, should be promptly reported at the school's medical centre for laboratory investigation and proper treatment. This is to avoid complications that may arise from untreated cases.

Multiple sexual partners should be discouraged and the use of protective barriers such as condoms or the female diaphragms preached.

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