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Prevalence of Vaginal Infection among Female University Students: A Case Study of Achievers University, Owo, Ondo State, Nigeria

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Abstract

Prevalence of vaginal infection in both self-reported symptomatic and asymptomatic female students of Achievers University, Owo, was investigated. The aim of this study was to determine the prevalence rate and forms of vaginitis among female students of Achievers University, with the aim of selecting an appropriate antibiotic treatment and prevention. Vaginal infection (vaginitis) is a condition that involves inflammation or infection of the vulva and the vagina. Fifty high vaginal swabs (HVS) were collected, cultured and their susceptibility to various antibiotics was determined. The study was carried out over a three months period on female students within the age range of 15 to 28 years. Information was obtained as regards, marital status, antiseptic use during bath, front to back or back to front wiping style after defecating, numbers of users of toilets and sexual relationships (safe sexual practices, multiple sex partners, abstinence or monogamous relationship). Out of 50 samples examined, 40 (80%) had one form of microbial organism or the other, ranging from bacteria to fungi; bacteria, making up 33 of the isolate while 7 were of fungal origin. Ten (10) patients representing 20% had none. The frequency of isolation of organism was E. coli 16 (46.0%) and Staphylococcus aureus 17 (42.3%). Almost all of the patients who practiced douching with soap had more than 60% of the symptoms of vaginal itching, odor and discharge. This shows that there is a significant effect of the douching method on soap indications for BV. The most effective antibiotic against E.coli isolates was Gentamycin 10 (62.5%), Erythromycin 37.5%, while Streptomycin 1 (6.3%) was the least effective. Staphylococcus aureus isolate was most sensitive to Gentamycin 9 (52.8%) whereas they were resistant to Cotrimoxazole and Nalidixic acid 0 (0%) each. Routine high vaginal swab (HVS) examination for women is advised. Women need to stop the practice of douching especially with soap and other preferred methods. Restriction in the indiscriminate use of and abuse of antibiotics to forestall resistance is also advised.

Key words: Vaginosis, douching, prevalence, high vaginal swab.

INTRODUCTION

Female vaginal infection (vaginosis or vaginitis) is a clinical condition characterized by a shift in vaginal flora away from Lactobacillus species toward more diverse bacterial species, including facultative anaerobes. It is a biological state that includes localized swelling and reddening or contamination of the **female genital organ and also the canal**. It is a **standard** medical **grievance** amongst **ladies** of **procreative** age. The altered microbiome causes a rise in vaginal pH and symptoms that range from none to very bothersome. Vaginosis and Vaginitis are broad terms indicating any disease process of the vagina caused by or leading to infection, inflammation, or changes in the normal vaginal flora. The difference between vaginitis and vaginosis is the presence (vaginitis) or absence (vaginosis) of inflammation. The most common symptoms of vaginitis/vaginosis are vaginal discharge, odor, itching, burning, or pain. The most common causes of vaginitis/vaginosis are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomoniasis. Often, the causative conditions and microbial flora of vaginitis **are** divided into nonspecific and specific causes. Specific vaginitis is contamination related and can be venereal in nature while nonspecific vaginitis constitutes non-infectious processes such as skin lesions, foreign contaminants or chemical irritants. Other causes of vaginitis can be stratified by age and are generally associated with postmenopausal vaginal atrophy or foreign bodies in the pediatric population. Lichen planus, lichen sclerosus, psoriasis, and contact/allergic dermatitis

may also cause vaginitis. Future health implications of vaginitis include, but are not limited to, increased susceptibility to other sexually transmitted infections and preterm birth. For the purpose of uniformity in this study we will be using the term “vaginitis”.

A female of reproductive age may have any aggregate of vaginal contaminants at one time. Emerging prodromes vary with the contamination even though there are well known signs that each one vaginitis infections have and it **should** be noted that affected **ladies may** be **symptomless** (Nyirjesy, 2008). Fifty to 75 percent of women with BV are asymptomatic, symptomatic women typically present with vaginal discharge and/or vaginal odor. The discharge is off-white, thin, and homogeneous; the odor is an unpleasant "fishy smell" that may be more noticeable after sexual intercourse and during menses. The presence of these symptoms suggests mixed vaginitis (symptoms due to two pathogens) (Klenbanoff *et al.,* 2004; Yen *et al.,* 2003). Earlier studies by Olartan (2006) reported a prevalence of 10% asymptomatic vaginitis in female students' population of a Nigeria University. Anh *et al.,* (1996) reported a prevalence of 20.12% asymptomatic vaginitis infection among women attending maternal and child health and family planning clinic in Hanoi, Vietnam. Some researchers have previously reported that vaginitis is more common among younger women in Kenya (Bukusi *et al.,* 2006), while others like Morris *et al.,* (2003) and Jones *et al.,* (2007) found that risk of vaginitis increases with age which is a proxy for cumulative sexual activity, especially, women between 15-20 years of age in United Kingdom and that there is a high and prevalence of 27% of vaginitis in Peruvian women from social economically deprived populations, respectively. In a study also conducted by both Joesoef *et al.,* (2001) and Morris *et al.*, (2001) it was noted that vaginitis was the most common cause of vaginal discharge in women of childbearing age, accounting for 40 to 50 % of cases in the United States and the prevalence of vaginitis was 29 % in the general population of women aged 14 to 49 years and 50 % in African-American women. This included both symptomatic and asymptomatic infection. Worldwide, vaginitis is common among women of reproductive age, with variations according to the population studied (Kenyon *et al.,* 2013).

The aims of this study are**:** (i) determine the prevalence of vaginitis among the female students of Achievers University, Owo; (ii) investigate microorganisms responsible for symptomatic and asymptomatic vaginitis and (iii) determine the antibiotic susceptibility pattern of the isolates to common antibiotic agents.

METHODOLOGY

Study Population

The study comprised of 50 female students of Achievers University, Owo, Ondo State, within the age range of 15-28 years. All eligible students were interviewed and verbal informed consent was obtained. Demographic data regarding age, marital status, and antiseptic usage during baths, front to back or back to front wiping style after defecating, numbers of users of toilets in their respective hostels and sexual relationship (safe sexual practices, multiple sex partners, abstinence or monogamous relationship) was obtained. Female students were excluded if they were placed on antibiotics or were undergoing treatment with symptomatic genital tract infection.

Collection of Samples

A sterile swab stick was used to collect the vaginal swabs. Self-collected vaginal samples were obtained thrice weekly. Participants were instructed to insert the vaginal swab 1-2 inches into the vaginal, 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 seconds, and roll each swab across a slide and allow the material to air-dry. The swab sticks were immediately replaced in its casing and labeled appropriately.

Each specimen was refrigerated at 4^{o} C as soon as it was collected. Samples were collected between a three month periods of May to July 2015.

Sample Processing

Swabs were collected and used to inoculate pre-prepared agar using the spread plate method and inoculated at different temperatures depending on the organisms (37^{o}C for 24hrs for bacteria, 28^{o}C for fungi and 48^{o}C for protozoans).

Microscopy (Structural Identification)

After incubation, the colonies were isolated and viewed under the microscope for physical appearances. Morphological different colonies from the cultured plates were sub-cultured repeatedly onto nutrient agar slants by streaking and incubated at 37^{o}C for 24 - 48 hrs.

Biochemical tests

The following biochemical tests as described by Cheesebrough (2002) were carried out in order to further identify the isolates; catalase test, oxidase test, citrate test, methyl-red test, Voges-proskauer test, indole test, sugar fermentation test, coagulase test, gram reaction.

Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined by agar diffusion technique as described by Baker and Breach (1980) using antibiotics disc after pure colony sub-culturing. The isolates were inoculated on Muller Hinton agar plates, streaked in different directions and allowed to set at room temperature. Antibiotic discs were placed on the set agar plates, allowed to equilibrate at room temperature for 15 minutes and finally incubated at 37^{o}C for 24h.

Thereafter, the plates were observed for obvious zones of inhibition using CLSI, 2006 standard. The following antibiotic discs were used; Gentamycin (10µg), Nalidixic acid (25µg), Cotrimoxazole (25µg), Streptomycin (10µg), Tetracycline (25µg), Ampicillin (25µg), Chloramphenicol (10µg), Penicillin (10µg), Erythromycin (5µg).

RESULTS

Fifty female undergraduates of Achievers University, Owo who lives in the hostel were examined for vaginitis. Students were predominantly aged 15 to 28 years of age. Samples were collected based on three age group brackets of 15 - 18, 19 - 22 and 23 - 28. Table 1 show the morphological and biochemical characteristics of the bacterial isolates from vaginal swab cultures of samples after growth on Nutrient, Chocolate, MacConkey, and Blood agar plates. *Staphylococcus aureus* had a convex, smooth and light-yellow colony with an entire edge on nutrient agar plates and appeared as gram-positive cocci in clusters. *Staphylococcus aureus* was negative in urease, motility, oxidase, indole, Voges-Proskauer tests and unable to ferment arabinose sugar while it was positive in the methyl red test, catalase, hemolysis, citrate, nitrate and coagulase tests, including the various sugar fermenting tests with glucose, sucrose, lactose, maltose, and mannitol.

Escherichia coli, on the other hand had convex, rough and dark red colony with an entire edge on MacConkey agar plates. They appeared as Gram-negative straight rods on stained preparations. Biochemical tests showed that *Escherichia coli* was majorly negative in catalase (though some strains showed a late slightly positive reaction), coagulase, urease, oxidase, citrate and Voges-Proskauer tests while in motility, indole, nitrate and methyl red tests were positive. Sugar fermentation showed it was positive to glucose, lactose, arabinose and mannitol but was unable to ferment sucrose and maltose.

The results of demographic data stratified by the douching methods and a number of sexual partners were compared with the different age groups as shown in Table 2. A total of 21 out of the 50 students douched with soap, 29 with antiseptics while 24 douched with ordinary water. Out of 14 students examined within the age group of 15 - 18; 5 had just one (1) sex partner, zero with two (2) sex partners while nine (9) had no sex partner. Also, out of 20 and 16 female students examined within the age of 19-22, 23-28 respectively; 17 and 15 had one sex partner respectively.

The clinical indications for vaginitis as observed in this study are shown in Table 3. A large number of the students presented with one form of symptom or the other. 9 presented with vaginal odour, 8 had vaginal discharge, and 10 with itching. Of this numbers, 7(77.8%) douched with soap, 6(60%) with antiseptics and 7(87.5%) douched with water. This result indicates that there was a significant effect ($P \geq 0.5$) of douching on the various indications for vaginitis with higher values recorded for douching with water. The result of the microscopic examination of the samples of the vaginal swabs is reported in Table 4. The result revealed that 25 (50%) samples contained significant epithelial cells, 35(70%) contained pus cells, while 16(32%) 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 33 samples had significant bacterial growth ($<10^5$ cfu/ml).

Table 6 also shows the frequency of occurrence of the bacterial isolates from vaginal swab samples. A total of 16(40%) of *E. coli* was isolated out of a total of 50 isolates. *Staphylococcus aureus* occurred in a total of 17(42.5%), while yeast cells were isolated from 50 of the vaginal swab samples representing 17.5% of the total organisms isolated.

Table 7 shows the relationship between the different douching methods and the number of microbial counts recorded per method. The antibiotics sensitivity pattern of the bacterial isolates as shown in Table 8 revealed Erythromycin 6(37.5%) and Gentamycin 10(62.5%) were the most effective antibiotics for *E. coli* isolates. *Staphylococcus aureus* isolated were most sensitive to Gentamycin 9(52.9%) followed by Streptomycin 3(17.6%). All the *S. aureus* isolates showed 100% resistance to Nalidixic acid and Cotrimoxazole.

DISCUSSION

The high number of bacterial growth observed suggested an infectious process by organisms such as *E. coli* and *S. aureus*. According to Smith *et al.,* (1999) and Veeh *et al*., (2003*), Staphylococcus aureus* (which colonizes female vaginal mucosa (Schlievert *et al.,* 2007)) is one of the most common causes of infection, the incidence of which has been steadily increasing. In the work of Gilbert *et al.,* (2002), vaginal microorganisms associated with vaginal infection and hence bacterial infections were found to be mainly group B *Streptococci, S, aureus and E. coli*. Earlier studies by Olartan *et al.,* 2006 reported a prevalence of 10% asymptomatic genital tract infection in female students' population of Nigeria University. Bukusi *et al.,* (2006) had previously reported that bacterial infection is more common among younger women, while others like Morris *et al.,* (2001) and Jones *et al.* (2007) found that risk of bacterial infection increases with age which is a proxy for cumulative sexual activity, especially, women between 15-20 years of age. The high prevalence observed could be possibly due to an increase in sexual activity among the University age group (Olartan *et al.,* 2006). Out of the 50 female

students sampled, 42(84%) of them admitted having only one sexual partner, while 2(10%) had multiple sexual partners each. In a treatment trial by Bradshaw *et al.,* (2006), it was found that bacterial infection recurrence was 3 times more common among women who remained with their regular sexual partner and was significantly less likely among women who changed sexual partners. In this study, the female students admitted using either soap or antiseptics, thus increasing the level of bacterial infection symptoms. According to Roberta *et al.,* (2002), most women reported douching for symptoms or hygiene, both of which elevated bacterial infection and vaginal colonization by organisms.

The most effective chemotherapeutic agents observed against *E. coli* in this study were Gentamycin 62.5% and Erythromycin 37.5%. Least activity was noted against Streptomycin, for *E. coli*, with a sensitivity of 2(6.3%) and a resistance of 81%. The most effective chemotherapeutic agent against *S. aureus* in this work was Streptomycin 17.6% followed by Penicillin 11.8%, Erythromycin 11.8% and Chloramphenicol 11.8% respectively. The organism was completely resistant to Cotrimoxazole and Nalidixic acid 0(0%) in each. This result is very similar to the studied where bracket of the females was between 15-18 years with a mean age of 22 years.

CONCLUSION

The results obtained from this study revealed a high prevalent rate of vaginal infection among the students. 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 an organism or the other in the genital tract leading to bacterial infection. This research also revealed that contraceptive use can cause changes in the vaginal environment allowing pathogens to proliferate. The implication of the high prevalence rate of asymptomatic female students with vaginal infection found in this study could also be due to the students not going for a regular medical checkup, which can lead to serious infertility problems when bacteria from the vagina travel into the uterus causing serious damage to the fallopian tubes. Regular checkups are therefore necessary for every woman whether sexually active or not.

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Table 1: Structural and biochemical presentation of the bacterial isolates

Sample	Shape	Edge	Colour	Gram-staining	Hemolysis	Methyl red	VP	Urease	Motility	Oxidase	Citrate	Indole	Catalase	Coagulase	Nitrate	Glucose	Sucrose	Mannitol	Lactose	Maltose	Arabinose	Probable Organism
F10, F18, F23, F20, F17	Rod	Rough	Dark-red	-ve	-	+	-	-	+	-	-	+	-/+	-	+	+	-	+	+	-	+	*E. coli*
F2, F6, F9, F11, F31	Rod	Rough	Dark-red	-ve	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	*E. coli*
F3, F41, F25, F27, F22	cocci	Smooth	Light-yellow	+ve	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	-	*Staph aureus*
F4, F13, F19 F29, F46	Rod	Rough	Dark-red	-ve	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	*E. coli*
F5, F7, F16, F24, F47	Rod	Rough	Dark-red	-ve	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	*E. coli*
F48, F44, F39 F33, F36	Rod	Rough	Dark-red	-ve	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	*E. coli*
F28, F26, F37 F40, F8	cocci	Smooth	Light-yellow	+ve	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	-	*Staph aureus*
F42, F50, F38 F34, F30	cocci	Smooth	Light-yellow	+ve	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	-	*Staph aureus*
F45, F21, F14 F35, F43	cocci	Smooth	Light-yellow	+ve	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	-	*Staph aureus*
F32, F12, F15, F29, F49	Rod	Rough	Dark-red	-ve	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	*E. coli*

Table 2: Demographic Data by Douching methods and Number of sex partners

Age Range	No of virginal swabs	No of sex partners			Douching with antiseptic soap	Douching with antiseptic	Douching with water
		None	1	2			
15 - 18	14	9	5	0	4	2	8
19 - 22	20	1	17	2	9	1	10
23 - 28	16	0	15	1	8	26	6
Total	**50**	**10**	**37**	**3**	**21**	**29**	**24**

Table 3: Indications for Bacterial infection

Indications		Total no of patients	Douching with soap (%)	Douching with antiseptic (%)	Douching With water
Odour	1(11.1)	9	7(77.8)	1(11.1)	1(11.1)
Itching	2(20)	10	6(60)	2(20)	2(20)
Yellow Discharge	1(12.5)	9	7(87.5)	1(12.5)	1(12.5)
No Symptoms		20	1(11.1)	1(11.1)	18

Table 4: Microscopic examination of virginal infection swab samples

	Significant sample	scanty sample	No sample
Epithelial cells (%)	25(50)	12(24)	13 (26)
Pus cells (%)	35(70)	10(20)	5 (10)
Yeasts (%)	16(32)	11(22)	23 (46)

Significant sample = $\geq$ 5Hpf (High power field)
Scanty sample = $\leq$ 5Hpf

Table 5: Distribution of microorganisms isolated from Virginal infection

Age Group	No of virginal with swabs examined	No of sample with significant bacterial	No of samples with growth scanty	No of samples with yeast growth
15 - 18	14	8	2	3
19 - 22	20	14	0	1
23 - 28	16	11	1	3
Total	50	33	3	7

Significant = > 10^{5}/(cfu/ml)
Scanty = < 10^{5} (cfu/ml)

Table 6: Occurrence of microorganism isolated from virginal scab samples.

Organism	Frequency %	Total swab sample
E. coli	16(40)	50
S. aureus	17(42.5)	50
C. albicans	7(17.5)	50

Table 7: Effect of Douching on the microbial count of virginal swabs

Methods	No of patients	No of microbial count isolated
Douching with soap	21 (42)	21 (42)
Douching with antiseptics	5 (5)	13 (38.5)
Douching with water	24 (48)	20. (83.3)
Total	50	43

Table 8: Antibiotic sensitivity pattern of bacterial isolates

Antibiotics	Percentage (%) of sensitivity of isolates to various antibiotics					
	E. coli (n=16)			*S. aureus* (n=17)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Gentamycin	10(62.5)	1(6.3)	5(31.2)	9(52.9)	2(11.8)	6(35.3)
Nalidixic acid	29(12.5)	2(12.5)	12(75)	0(0)	0(0)	17(100)
Cotrimoxazole	4(25)	1(6.3)	11(68)	0(0)	0(0)	17(100)
Streptomycin	1(6.3)	2(12.5)	13(81.3)	3(17.6)	1(5.9)	13(76.5)
Tetracycline	2(12.5)	0(0)	1(87.5)	0(0)	1(5.9)	16(94.1)
Ampicillin	3(18.8)	2(12.5)	11(68.8)	1(5.9)	6(35.3)	10(58.8)
Chloramphenicol	5(31.2)	2(12.5)	9(56.3)	2(11.8)	5(29)	10(58.8)
Penicillin	5(31.2)	1(6.3)	10(62.5)	2(11.8)	1(5.9)	14(84.4)
Erythromycin	6(37.5)	2(12.5)	8(50)	2(11.8)	2(11.8)	13(76.5)

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 above limits