



Microflora Imbalance Related Vaginal Infections among Female Senior High School Students in Navrongo, Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EE, YFM, SA, GAM and JOK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors YFM and SA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Vaginal infections affect most females during their lifetime, with approximately 50% having two or more episodes of vaginal candidiasis. The study was conducted to assess the incidence of *Candida*, bacteria and Lactic acid bacteria deficiency related vaginal infections among female senior high school students. Seventy-two high vaginal swabs were obtained from consenting students for laboratory analysis and a structured questionnaire administered to assess symptoms, risk factors and demographic information. Methods employed in laboratory analysis included wet mount, whiff test, clue cell test, pH test, Gram staining and culture. The results revealed 40% (29) of participants were infected with *Candida* while 14% (10) were diagnosed with bacterial vaginosis. Lactic acid bacteria which are supposed to be a normal flora in a healthy vagina were isolated in only 29% (21) of the participant who were between the ages of 14 to 22 years. For symptoms, 100% (72) of the participants had vaginal discharge, 68% (49), 63% (45) and 18% (13) had itching,

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irritation and burning sensation respectively. Among the risk factors, sexual activity recorded the highest 76% (55), 61% (44) douched and 32% (23) were on antibiotics. The incidence of *Candida* vaginitis especially was high in the study population and this may be due to few participants having Lactic acid bacteria isolated from their vagina and this may also explain the incidence of bacterial vaginosis.

Keywords: *Candida*; bacteria; lactic acid bacteria; vaginal infections; students.

1. INTRODUCTION

Vaginal infection is one of the most common reasons for women to seek medical attention. It usually presents as inflammation accompanied with itching, burning, irritation, discharge and discomfort in symptomatic cases [1,2]. Most cases of vaginal infections are caused by overgrowth of *Candida albicans* (85%–90%) and other non-*albicans* species. It is the second most common cause of vaginitis with approximately 40- 50% of women having two or more episodes their life time [3,4]. Bacterial vaginosis (BV) on the other hand is an imbalance of the vaginal bacterial microbiota, its etiology is still unknown and about 50% of women with BV are asymptomatic [5,6]. The importance of bacterial vaginosis are its associated gynecological and obstetric complications [7]. Generally, vaginal infections especially bacterial vaginosis syndrome also increase the risk of contracting sexually transmitted disease such as HIV, herpes simplex virus 2, gonorrhoea, *Trichomonas vaginalis*, and *Chlamydia trachomatis* [8,9].

The health of a woman's vagina depends on the amount and type of microorganism present and it is characterized by fungi; *Candida albicans*, and bacteria like *Lactobacillus* species [10]. Bacteria of the genus *Lactobacillus* are the primary colonizing bacteria of the vagina of 96% of healthy individual [11]. *Lactobacilli* ensure long-term colonization of the vagina through adherence to vaginal epithelial cells, blocking the adherence of bacterial pathogens to the vaginal epithelium [12]. It is generally accepted that this is primarily achieved through lactic acid production and antagonistic mechanisms which consist of hydrogen peroxide (broad-spectrum antimicrobial) and bacteriocin (target specific antimicrobials) production [13]. The objective of the study was to assess the incidence of *Candida*, bacteria and Lactic acid bacteria deficiency related vaginal infections among female senior high students.

2. MATERIALS AND METHODS

2.1 Sample Collection and Questionnaire Administration

The study was conducted among females in a senior high school in Navrongo in the Upper East of Ghana where there is a cosmopolitan mix of students from all across the country. Seventy-two high vaginal swab samples were obtained from 72 consented volunteering students. The girls were prior educated on how to take a good high vaginal swab. A structured questionnaire was also administered to assess symptoms, risk factors and demographic information. The swabs were transported for laboratory analysis.

2.2 Laboratory Analysis

2.2.1 Culture

Two types of agar media were utilised; Sabouraud Dextrose and de Man Rogosa Sharpe agar and they were prepared using the manufacturer's instructions. To identify discrete colonies of *Candida spp*, each swab was inoculated onto Sabouraud Dextrose agar plates, streaked and incubated aerobically at 35°C to 37°C for 18-24 hours. Similarly, de Man Rogosa Sharpe (MRS) media was used to isolate *Lactobacilli* and other Lactic acid bacteria [14].

2.2.2 Gram staining

A smear was prepared by rolling the swab on a clean glass slide. The slide was heat fixed by passing over a Bunsen burner flame for three times. The film was flooded with crystal violet for one minute and was washed with clean water and again flooded with Gram's iodine for 30 seconds. The film was decolorized with acetone-alcohol and washed immediately with water and counter stained with neutral red for 30 seconds. The film was washed with clean water and allowed to air dry, then examined under the microscope with x100 objective lens and oil immersion. Upon examination of the stained

preparation, the yeast cells were seen as large violet oval cells indicating Gram positive. In addition, Gram negative reactions were also recorded.

2.2.3 Vaginal pH

The swab stick was rubbed on the Pink litmus paper to check the acidity or alkalinity. Pink and blue colour signified acidic and alkaline pH respectively. To control bacteria and growth of other microorganisms, a healthy vagina is slightly acidic with pH of 3.8-4.2, whereas a pH greater than 4.5 signifies probable bacterial vaginosis.

2.2.4 Whiff test

Two drops of Potassium hydroxide (KOH) was added to a microscope slide containing a smear of the vaginal discharge. A characteristic fishy odour was considered a positive whiff test [5].

2.2.5 Clue cell test

The presence of clue cells was determined by placing a drop of normal Saline on the slide containing vaginal discharge smear. Clue cells are epithelial cells that are coated with bacteria. If present, clue cells were visualized under the microscope with x10 and x40 objective lens [5].

2.2.6 Wet mount preparation

Two drop of sterile normal saline was placed into the swab tube and the bottom of the tube tapped gently for few seconds. A drop of the saline mixture was placed on a glass slide and covered with a cover slip. The edges were pressed down to seal the chamber against evaporation. The preparation was examined under the microscope using x10 and x40 objective lens. Upon examination, oval budding cells (*Candida sp*), epithelial cells, Clue cells (Epithelial cells coated with bacteria) were seen and noted.

3. RESULTS

The laboratory results (Table 1) revealed that out of 72 female students between the ages of 14 years to 22 years, 29 (40%) were infected with *Candida* and 10 (14%) with Bacteria Vaginosis (BV) based on applied criteria [5]. In turn, normal vagina flora, Lactic acid bacteria (LAB) were isolated from only 21 samples representing 29% of the total number. The presence of epithelial cells coated with bacteria (clue cells) which is

usually indicative of bacterial vaginosis and was identified in 10 (14%) participants. This was further confirmed by the results of whiff test which recorded the same proportion of infections among the participants. pH was not a strong indicator of infection in our study; both alkaline (50%) and acidic (50%) pH were recorded in analysed swabs. An alkaline vagina however, is predisposed to colonisation by potential pathogenic microorganism.

Table 1. Test results of culture, wet mount, pH and whiff test

Type of test	Type of isolate	Number of samples n=72 (%)
SDA media culture		
	<i>Candida</i>	29 (40)
	Gram positive rods	8 (11)
	Gram negative rods	10 (14)
MRS media culture		
	Lactic acid bacteria	21 (29)
Wet mount		
	<i>Candida</i>	19 (26)
	Clue cells	10 (14)
	Epithelial cells	54 (75)
Whiff test		
	Positive	10 (14)
	Negative	62 (86)
pH		
	Alkaline	36 (50)
	Acidic	36 (50)

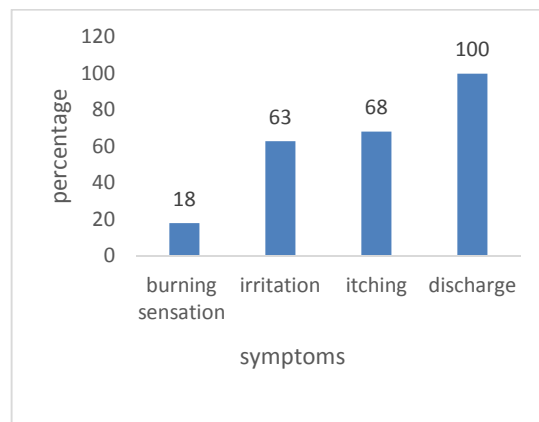


Fig. 1. Symptoms associated with vaginal infections

Table 2. Association of lactic acid bacteria with *Candida* and bacteria related vaginal infection

S/n	Age (yrs)	pH	Whiff test	Clue cells	Discharge	<i>Candida</i>	Lactic acid bacteria	Bacteria	Gram stain
1	16	Al	Neg	Neg	Cream	None	Seen	None	+rods
12	21	Al	Neg	Neg	White	None	Seen	None	+rods
13	19	Ac	Neg	Neg	White	None	Seen	None	+rods
15	17	Al	Neg	Neg	Cream	Seen	Few	None	+rods
16	15	Al	Neg	Neg	Cream	None	Seen	None	+rods
19	15	Al	Pos	Pos	White	Seen	Few	Seen	-+rods
23	17	Al	Pos	Pos	White	Seen	Few	Seen	-+rods
30	14	Al	Neg	Neg	White	Few	Seen	None	+rods
33	18	Ac	Neg	Neg	Cream	None	Seen	None	+rods
39	17	Al	Neg	Neg	Cream	None	Seen	None	+rods
43	16	Al	Neg	Neg	Cream	Seen	Few	None	+rods
44	20	Al	Pos	Pos	Yellow	Few	Seen	Few	+rods
46	16	Al	Pos	Pos	Cream	Seen	Seen	Seen	+rods
48	18	Al	Pos	Pos	Yellow	Seen	Seen	Seen	+rods
53	18	Ac	Neg	Neg	White	None	Seen	None	+rods
55	18	Ac	Neg	Neg	White	None	Seen	None	+rods
62	20	Ac	Neg	Neg	Cream	None	Seen	None	+rods
63	18	Ac	Pos	Pos	Yellow	Seen	Seen	Seen	-+rods
64	22	Ac	Neg	Neg	White	None	Seen	None	+rods
69	18	Ac	Neg	Neg	White	None	Seen	None	+rods
71	16	Ac	Neg	Neg	Cream	Seen	Seen	None	+rods

Key: Neg = Negative, Pos = Positive, Ac = Acidic, Al = Alkaline, + = Positive, - = Negative

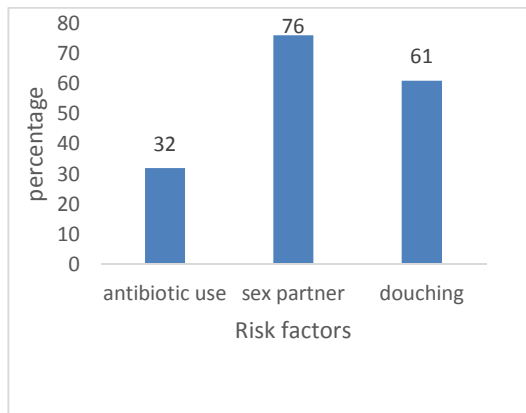


Fig. 2. Risk factors of vaginal infections

The association of lactic acid bacteria (LAB) with pH and vaginal infection was not clear in our study (Table 2). In 57% of cases (12/21), LAB were isolated from alkaline vagina conditions with or without the accompanying infections under study. In 33.3% (7/21) where the vagina was acidic and lactic acid bacteria were isolated, none of pathogens tested in the study was identified.

Among the symptoms, vaginal discharge was the commonest (100%), while burning sensation was the least (18%). Both symptoms usually represent either the onset and the progressive or the advance form of the infection respectively. Sexual activity (76%) was also recorded as the highest risk factor in our study whereas douching (61%) was second ranked and antibiotic use (31%) was the least.

4. DISCUSSION

The low number of Lactic acid bacteria isolated gives an indication of the infection rate among the female students in the school. This may also imply that most students suffered vaginal infections due to this underlying factor. The microflora of the vagina has been reported to be characterized by *Candida albicans*, *Lactobacillus* species and other bacteria like *Gardnerella vaginalis*. However, the issue of what the normal flora is composed of is confusing and complicated since some symptomatic individuals may have these isolated whereas asymptomatics may not be colonised by the expected flora [10,15]. The depletion of *Lactobacilli* or other Lactic acid bacteria and subsequent overgrowth of other microorganisms lead to higher risk of vaginal infections. Other studies have reported that *Lactobacillus* colonized as few as 10% of

sexually active non pregnant women participants in Iraq, [2] while another study conducted back in 1999 isolated these protective microbes from 71% of their female subjects of a similar category [16].

The incidence rate of *Candida* (40%) in the present study is high in comparison with a similar study in the same region which reported 25.5% [17]. Another study [15] also recorded 31% intermittent candidiasis in analysed cohort during characterization of vaginal microflora. The high incidence of *Candida* in the present study may be as a result of lactic acid bacteria deficiency (71%) that was observed among our study population, in addition to the high incidence of risk factors that predispose an individual to vaginal infections. The incidence rate of 14% of bacterial vaginosis (BV) in our work was however within the range reported by studies from Europe and the USA which found prevalence between 4.9% and 36.0% [18]. Another study [10] also reported 7% which is a lower incidence rate as compared to our study where as another [15] reported 35% intermittent BV in their study.

In this study, Lactic acid bacteria were isolated in some alkaline vagina, however report by Hay, [8] indicates that as pH rises from 4.5 to 7, these organisms reduce in number, eventually disappear and then other commensals are able to increase in number and become opportunistic. In some participants, even though Lactic acid bacteria were present, either *Candida* or bacteria or both were also isolated. In such cases, the presence of these organisms may not be necessarily indicative of a severe condition but occurring in a good balance. On the other hand, this may also infer replacement by some non-hydrogen peroxide producing strains of *Lactobacilli* and the subsequent reduction in the number of the normal strains [19,20].

Healthy individuals may have some small amount of normal discharge, however copious, malodorous and discoloured discharge may be indicative of an underlying infection. All the participants in our study reported some form of vaginal discharge which has been identified as one of the most common symptoms. However, only between 14-40% of participants were confirmed to be infected in the present study. Other symptoms such as irritation and itching were recorded 63% and 68% of participants respectively, making the second and third highest occurrence among our subjects. Previous research reported rather itching as the

most common symptom especially for *Candida* infection [16,21] while BV is usually characterised by high vaginal pH (>4.5) which causes a change in its environment. Bacteria may then function as a mono-etiological agent to infect and in symptomatic cases it is associated with a homogenous discharge and no inflammatory response [9,22]. It has been also reported that the vaginal flora is highly susceptible when it is predisposed to risk factors such as contraceptives, vaginal medications or douching, antibiotics, sexual intercourse, and stress [3]. The high records of various risk factors in the study which ranged between 32% and 78% for antibiotic use, douching and sexual activity may explain the incidence rates of the vaginal infections that were recorded in the study.

5. CONCLUSION

The incidence of *Candida* infection (40%) was high among these high school girls and bacterial vaginosis was also identified in 14%. Lactic acid bacteria which serve as protective bacteria and gatekeepers of the vagina were isolated from only 29% of our participants. The occurrence of symptoms and risk factors of vaginal infections was high and it is recommended that health talk on vaginal infections should be periodically organised to educate the girls.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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